An analysis of ingested microplastics found in offshore Atlantic cod *(Gadus morhua)* and inshore capelin *(Mallotus villosus)* using scientific and citizen science methods

by

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Abstract

Analyzing plastic ingestion rates in fish and other marine organisms is an effective tool to understand the impacts of marine plastic pollution worldwide. As more and more marine organisms ingest plastic pollution, more attention has focused on the ability of local citizens to locate and identify plastics in their food fish. In this study, I expanded the list of species examined for plastic ingestion by adding inshore capelin *(Mallotus villosus)* and offshore Atlantic cod *(Gadus morhua)* from Northwest Atlantic Fisheries Organization Division 3 of Newfoundland, Canada. The frequency of occurrence of plastic ingestion in Atlantic cod and capelin was 1.1%, and 0, respectively. I also examined the success rate of citizens locating and identifying ingested microplastics in fish without the use of scientific tools. I found that citizen scientists can be successfully organised to monitor microplastic in fish.

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Lastly, without my family’s love and emotional support, I would have never made it to the end.

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List of Abbreviations

%FO ..... Frequency of Occurrence

ABS ..... Cellulose Acetate

BPA ..... Bisphenol A

CBM ..... Community Based Monitoring

CFER ..... Centre for Fisheries Ecosystems Research

CLEAR ..... Civic Laboratory for Environmental Action Research

DFO ..... Department of Fisheries and Ocean Canada

DI ..... Deionized

GI ..... Gastrointestinal

KOH ..... Potassium hydroxide

LXHXW ..... LengthXHeightXWidth

µg ..... Microgram

µm ..... Micrometer

MEOPAR ..... Marine Environmental Observation Prediction and Response Network

MI ..... Marine Institute

NAFO ..... Northwest Atlantic Fisheries Organization

NP ..... Nonylphenol

PA ..... Polyurethane

PBT ..... Polybutylene Terephthalate

PC ..... Polypropylene

PCBs ..... Polychlorinated Biphenyls

PE ..... Polyethylene

PET ..... Polycarbonate

PMMA ..... Acrylonitrile Butadiene Styrene

PP ..... Polyvinylchloride

POPs..... Persistent Organic Pollutants

PS ..... Polyamide

PVC..... Polystyrene

PU ..... Poly(Methyl Methacrylate)

SSHRC..... Social Science and Humanities Research Council

UNEP .....United Nations Environment Programme

Co-Author Statement

This research is part of a broader Marine Environmental Observation Prediction and Response Network (MEOPAR) project with Irving Shipbuilding, and the Social Science and Humanities Research Council (SSHRC) Insight Development Grant #430-2015-00413 headed by Dr Max Liboiron in Geography. Dr Max Liboiron was the PI and Dr Charles Mather was the co-I. The two journal articles included in this thesis, chapters two and three, were initially developed and written by the candidate with targeted journals in mind. After ideation sessions with co-advisors, the first draft of each paper was written by the candidate and then revised based on the comments of co-supervisors. Chapter two will contribute to other baseline plastic ingestion studies that have been conducted by CLEAR members for a province-wide, multi-species study. Authors will include; Jessica Melvin, Jackie Saturno, France Liboiron, Justine Ammendolia, Max Liboiron, Charles Mather and Emily Wells. The co-authored baseline paper will be submitted to Marine Pollution Bulletin. Chapter three will be submitted to the Citizen Science: Theory and Practice and the candidate will be the first author and co-authorship will include, Dr Max Liboiron and Dr Charles Mather.

1.1. Introduction

In Newfoundland, Canada, Atlantic cod (*Gadus morhua*) is an important food source for recreational fish harvesters (Lowitt, 2013) and has limited economic value for a small number of commercial fish harvesters (DFO, 2016a). Capelin (*Mallotus villosus*) is a fish species that is also widely consumed locally by Newfoundlanders and exported as a commercial commodity (DFO, 2011). The sustainability of these two species, which play roles for both personal consumption and commercial gain could be threatened by marine plastics, widely considered to be one of the most important environmental challenges facing the oceans.

Marine plastics have affected the world's oceans and can persist in the ocean for thousands of years (Derraik, 2002; Edyvane et al., 2004; Barnes et al., 2009; Ogata et al., 2009; Obbard, 2104). Large plastics, known as macroplastics (>5 mm), have been studied extensively (Derraik, 2002; Barnes and Milner, 2005; Moore, 2008; Barnes et al., 2009; Barnes et al., 2010; Browne et al., 2015). However, more recently attention has turned to microplastics (<5-1 mm). Microplastics have raised concerns because they are present across trophic levels and are accessible for ingestion by very small marine organisms (Farrell and Nelson, 2013; Setälä et al., 2014), potentially affecting trophic interactions, including humans consuming these organisms (Di Beneditto and Awabdi, 2014; Phillips and Bonner, 2014; Neves et al., 2015; Rochman et al., 2015; Romeo et al., 2015; Rummel et al., 2015; Liboiron et al., 2016; Miranda and de Carvalho-Souza, 2016). One of the key concerns is the transfer of toxic chemicals associated with plastics from marine organisms to humans, and the related health risks (Mato et al., 2001; Rochman et al., 2013; Koelmans et al., 2016; Sussarellu et al., 2016). This is especially relevant for communities that rely on fish for food or for commercial gain.

Research examining the frequency of occurrence of marine plastics in fish caught for food and commercial sale in Newfoundland and Labrador has been limited to the vicinity of St. John's, the provincial capital and the province's largest city. Liboiron et al. (2016), found a frequency of occurrence of 2.4% microplastics ingested in the gastrointestinal (GI) tracts of Atlantic cod caught inshore at Petty Harbour and St. Phillips Harbour during the fall 2015 food fisheries. Of the 205 of cod collected, 5 had ingested 7 pieces of plastic between them (range 0–2 plastics/fish). The present study draws on a larger sample size of Atlantic cod and includes cod caught in offshore waters off Newfoundland. This study also analyses a second fish species, capelin, for the frequency of marine plastics. In this way, the current study will build on limited existing research on marine plastics ingested by fish in Newfoundland with the aim of providing new knowledge on this problem.

Second, the thesis was to determine if citizen scientists could detect and identify microplastics in food fish without the use of a laboratory, and to determine which tools or protocols will enable citizens to participate in monitoring efforts for microplastics using citizen science. Citizen science is scientific research conducted, in whole or in part, by amateur or nonprofessional scientists who collect data and bring awareness and information to communities about a project or problem (Bonney et al., 2009). Citizen science is important because marine plastics are now distributed widely, making it extremely difficult for one, or even several agencies to monitor all marine species for ingested plastic destined for human consumption.

As Zettler et al. (2017) argue, community involvement using citizen science monitoring programs is an important way of improving our understanding of marine plastic ingestion by fish.

1.2. Research Objectives

There were two primary objectives for this study: 1) establish a baseline for the frequency of occurrence of ingested marine plastics by offshore Atlantic cod and inshore capelin in the waters of Newfoundland, Canada; 2) determine if citizen science methods can be effective in identifying marine plastics in cod.

Research Collaboration and Design

Every year the Department of Fisheries and Oceans (DFO) and the Fisheries and Marine Institute of Memorial University of Newfoundland (MI) conduct offshore surveys in Northwest Atlantic Fisheries Organization (NAFO) division 3, to evaluate the status of fish stocks. These surveys provide the basis for scientific advice concerning conservation outcomes related to various fishery management options. I contacted both DFO and MI and organized meetings to discuss the potential for a research collaboration, as these existing surveys would potentially provide a ready source of data for my research.

In the initial stages of my research, several meetings took place with DFO and MI to determine the best fish to sample for this project. Through these meetings the decision was made to sample inshore capelin and offshore Atlantic cod. These species were chosen for two reasons. First, there is no data for plastic ingestion in capelin and only one study for plastic ingestion for Atlantic cod, which relied on fish caught inshore. Second, both Atlantic cod and capelin are important to Newfoundlanders as food sources and commodities exported for commercial gain.

My efforts to establish a research collaboration with DFO and MI were successful. DFO agreed to provide me with inshore capelin samples caught in 2015 by commercial harvesters in NAFO 3Ps, 3L, and (3K). DFO also provided Atlantic cod samples collected in the 2016 spring survey. Another source for Atlantic cod samples came from the MI 2016 spring survey in the offshore waters of Newfoundland. The survey sites for Atlantic cod were determined by DFO and MI. Both organizations allowed me to collect Atlantic cod samples in excess of those required for their stock evaluation surveys. Formalizing the plastic sampling and laboratory protocols also took place during the meetings between DFO and MI.

The second component of this project focused on the relationship between marine plastics and citizen science. Through an established relationship between the Civic Laboratory for Environmental Action Research based at Memorial University, and *Fishing for Success*, a non-governmental organization based at Petty Harbour, an opportunity presented itself to test citizen science marine plastic kits to determine if citizens could detect and identify marine plastic ingested by food fish. The collaboration with *Fishing for Success* also helped to improve the design of the kits and presented opportunities to test the kits with citizen scientists in Newfoundland.

1.3. Research Questions

The primary research questions addressed in this thesis are:

- What is the frequency of occurrence for ingested marine plastic by capelin *(Mallotus villosus)* caught in, Lawn, Burin Peninsula; Port de Grave, Conception Bay; Hickman Hr., Trinity Bay; Lumsden, Bonavista Bay; Comfort Cove, Notre Dame Bay; La Scie, White Bay and Leading Tickles, Notre Dame Bay of Newfoundland, Canada?
- What is the frequency of occurrence for ingested marine plastic by Atlantic cod *(Gadus morhua)* surveyed by the Department of Fisheries and Ocean Canada and the Marine Institute in St. Pierre Bank, Burgeo Bank and the Grand Banks?
- What is the detection and identification rates of microplastics found in Atlantic cod across four different methods: Method 1: 10% Potassium hydroxide (KOH) and visual analysis using a dissecting microscope with a laboratory; Method 2: visual analysis with dissecting microscope with a laboratory; Method 3: citizen science dissecting kit in a laboratory; and Method 4: citizen science dissecting kit in the field.

1.4. Literature Review

Introduction to Plastic

Plastic is a synthetic organic polymer derived from the polymerisation of monomers extracted primarily from oil and gas (Derraik, 2002). To enhance plastic performance and appearance, it also contains a wide variety of additives, such as fillers, plasticisers, flame retardants, UV and thermal stabilisers, and antimicrobial and colouring agents (American Chemical Council, 2015). Plastic is designed to be lightweight, flexible, inexpensive and corrosion-resistant (Thompson et al., 2009). The qualities of plastic have meant that it has been produced in enormous volumes: in 2013 alone, 299 million tons of plastic were produced globally, a 4% increase from 2012 (Gourmelon, 2015). There is another quality to plastic that has made it such a serious environmental problem. The longevity of plastic is estimated to be hundreds to thousands of years but is likely to be far longer in deep sea and non-surface polar environments (Barnes et al., 2009).

Plastic Pollution in the Marine Environment

Globally, the primary source of plastics to the ocean is likely to be improperly managed plastic waste on land (Jambeck et al., 2015). In 2010 alone, researchers estimated that between 4.8 and 12.7 million metric tonnes of plastic entered the oceans through improper waste management (Jambeck et al., 2015). The accumulation of microplastic is found to vary depending on factors such as proximity to urban settlements and shore use (Ryan et al., 2009). With more densely populated or industrial areas, significant inputs of plastic from land-based sources end up on coastlines and the seabed (Pruter, 1987; Gregory, 1991).

Plastic pollution in the marine environment has attracted much attention from both scientists and the public, as marine plastics can severely contaminate and affect the ocean's ecosystems (UNEP, 2005; Cole et al., 2011). Due to mass production of plastics in the last 60 years, microplastics (<5 mm) have been distributed throughout the world's oceans from the Arctic (Zarfl and Matthies, 2010; Obbard, 2014) to the Antarctic (Eriksson and Burton, 2003) and from the ocean's surface (Eriksen

et al., 2014) to the deep sea (Pham et al., 2014). During 24 expeditions surveying marine plastics between 2007-2013, it was estimated that 5.25 trillion particles weighing 268,940 tonnes are floating in the world's oceans across all five subtropical gyres (Eriksen et al., 2014).

Once plastic enters the water, it begins the process of degradation and fragmentation (Barnes et al., 2009; Andrady, 2011). Degradation and fragmentation of larger plastics called macroplastics (>5 mm) occurs through various chemical and physical processes, such as sunlight, oxygen, wave action and feeding fish (Barnes et al., 2009; Andrady, 2011). Over time, as these plastics begin to break down into smaller particles called secondary microplastics (<5-1 mm) they are spread over trophic levels of the marine food web and are accessible for ingestion by a wide range of organisms (Farrell and Nelson, 2013; Setälä et al., 2014). Another source of microplastics in the marine environment comes from primary plastics. Primary plastics are manufactured either for indirect use as precursors ("nurdles" or virgin resin pellets), that are melted down to produce polymer consumer products or for direct use, such as in cosmetics, scrubs, and abrasives. Until recently, facial cleansers, hand soaps, and toothpaste that are used by millions of people worldwide, particularly in developed countries, contain PS (Polystyrene) and PE (Polyethylene) particles (10 µm to 500 µm) that directly enter sewage systems and adjacent coastal environments (Zitko and Hanlon, 1991; Gregory, 1996; Fendall and Sewell, 2009; Tanaka and Takada, 2016). In 2015 many countries recognised and responded to the growing concerns regarding microplastics, and bans were enacted to eliminate the PS and PE particles from facial cleansers (Microbead-Free Waters Act of 2015; UNEP, 2015). In 2015, The Government of Canada proposed to include microbeads on the List of Toxic Substances under the Canadian Environmental Protection Act, 1999 (Environment Canada, 2015). Starting July 1, 2018, the Federal Canadian Government will ban the sale of shower gels, toothpaste and facial scrubs containing plastic microbeads (Canada Gazette, 2016).

Marine Organisms Ingesting Plastic

Marine organisms have been known to ingest plastic (Laist, 1987; Thompson et al., 2004; Rochman et al., 2015) or become entangled (Laist, 1987) which can cause harm or even death (Bjorndal et al., 1994; Derraik, 2002; Tomás et al., 2002). Since the 1960s, microplastics have been identified in the guts of numerous species such as seabirds, plankton, bivalves, crustaceans and fish (Moore et al., 2001; Tourinho et al., 2010; Cole et al., 2011; Von Moos et al., 2012; Choy and Drazen, 2013; Foekema et al., 2013; Jantz et al., 2013; Provencher et al., 2014; Setälä et al., 2014; Van Cauwenberghe and Janssen, 2014; Lusher, 2015; Liboiron et al., 2016). As research has expanded to a broader range of marine organisms, plastic ingestion has now been documented for 233 examined for marine species including, all species of marine turtles, 36% of seals, 59% of whales, and 59% of seabirds, as well as 92 species of fish and 6 species of invertebrates (Kuhn et al., 2015; Wilcox et al., 2015).

Recent surveys have found plastic in marine organisms that are also destined for human consumption (Choy and Drazen, 2013; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014). Plastic found in seafood is cause for alarm, given the knowledge that marine plastics carry a cocktail of contaminants (Teuten et al., 2009). Chemically, plastics recovered from the marine environment have been shown to carry an assemblage of industrial chemicals, both absorbed from the ambient

seawater as well as the ingredients from the plastic itself (Ogata et al., 2009; Lithner et al., 2011; Holmes et al., 2012). Marine contaminants include polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons, petroleum hydrocarbons, organochlorine pesticides (2, 2'-bis (p-chlorophenyl)-1, 1, 1-trichloroethane, hexachlorinated hexanes), polybrominated diphenyl ethers, alkylphenols and bisphenol A, at concentrations from sub ng to µg (Teuten et al., 2009). These contaminants can be transferred to human body tissue upon consumption of fish that have consumed plastic (Koelmans et al., 2014; Rochman et al., 2014; Teuten et al., 2009) causing a number of health concerns, such as 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). Communities that rely heavily on seafood for protein, and consumers who buy contaminated fish, could potentially be impacted by consuming seafood that has ingested marine plastics.

Global Plastic and Citizen Science

A key problem is monitoring marine plastic pollution that it is widely dispersed through the world's oceans. With limited human resources and costly ship time, collecting sufficient scientific data on plastic marine pollution can be a significant challenge. Citizen science - which involves monitoring programs where participants, who do not necessarily have science degrees, collect, categorise, transcribe, and analyse data (Booney et al., 2014) - provides a potential solution to the problem of monitoring marine plastics. Citizen scientists have gathered information, data, and samples in a wide range of contexts for scientific studies (Bonney et al., 2009; Bravo et al., 2009; Ogata et al., 2009; Ribic et al., 2010; Hidalgo-Ruz and Thiel, 2013; Eastman et al., 2014; Smith and Edgar, 2014; Hidalgo-Ruz and Thiel, 2015). Plastic in the marine environment has become a significant focus for citizen science, due to the full range of media coverage on the subject found on the Internet and in books, magazines, newspapers, and documentaries. Not surprisingly, there has been growing interest globally in the potential of citizen science or community science to assist in monitoring pollution (Conrad and Hickey, 2011).

Although many see the promise of citizen science for marine pollution monitoring, the collection of microplastics using citizen scientists can be a challenge due to the identification and sorting of plastic. For example, Hidalgo-Ruz and Thiel (2013), enlisted school children (8-16 years old), teachers and scientific advisors to assist in examining the abundance and occurrence of marine plastic on a Chilean beach. They were instructed to identify systematically, classify, process, count and enter plastic marine debris in a dataset. Scientists then recounted and classified the plastic to validate the data. They found the most common mistakes were identifying plastics, such as not recognising Styrofoam as plastic, and incorrectly entering data into the dataset. Recommendations to correctly identify plastics included a field guide that illustrates the diverse types of small plastic debris (rigid fragments, pieces of plastic bags, Styrofoam and mono-filaments), and to explain the differences between small plastic debris and other small objects in beach samplings, such as shells and other natural debris.

Using citizen scientists is not only limited to one-time beach cleanups, but can be extended to more rigorous studies, such as quantitative comparisons between sites, or overtime at the same sites. Smith and Edgar (2014), with the assistance of >300 underwater volunteers (divers) identified, counted and when possible removed marine debris from 120 different sites, such as estuarine settings adjacent to major population centres, to offshore islands within marine parks in Australia. Citizen

science was only possible by developing standardised protocols and providing training to the volunteers. Their findings identified damaging interactions between marine debris and marine biota at specific locations, emphasising the need for management intervention to ensure habitat sustainability. The data collected not only provided crucial baseline data for a long-term citizen science monitoring program, but it also delivered data that will continue to inform and assist with management decisions at both statewide and local levels. The success of this study reinforces the importance of citizen science and the contribution assessing conservation issues that require broad-scale data collections. While using citizen science to collect data can present problems, it is nonetheless invaluable because it may be the only source of data on widely dispersed marine pollution. Using citizen scientists to monitor marine species for ingested plastic destined for human consumption could expand research that would otherwise be impossible (Zettler et al., 2017).

Marine Plastic Monitoring in Newfoundland Canada

Atlantic cod has cultural and economic significance to the people of Newfoundland, and today still serves as an important food source (Lowitt, 2013; Schrank and Roy, 2013). However, only one published study (Liboiron at al., 2016) has investigated the ingestion rates of plastic in fish in Newfoundland.

The significance of the Liboiron et al. (2016) was that it involved citizen scientists and it engaged closely with local communities. The study used citizen scientists to gather GI tracts for biomonitoring and analysis for fish caught for human consumption and their findings were reported to the community during a town meeting before publication of the findings. Other studies have used citizen scientists to gather information, data, and samples for general scientific studies (Bonney et al., 2009; Bravo et al., 2009; Ogata et al., 2009; Ribic et al., 2010; Hidalgo-Ruz and Thiel, 2013; Eastman et al., 2014; Smith and Edgar, 2014; Hidalgo-Ruz and Thiel, 2015). However, Liboiron et al. (2016) were the first to use citizen scientists to gather GI tracts for biomonitoring and analysis for fish caught for human consumption.

While collecting data during the Liboiron et al. (2016) study, a team member collected GI tracts from fish harvesters during the fall food fisheries in September 2015 at popular fishing wharves, such as Petty Harbour and St. Phillips Harbour, both within the vicinity of St. John's Newfoundland. During these collection times, members interacted with the community, explained that the fish's GI tract would be examined for plastic, and gave them information on retrieving the data from an online database of results (harvesters were given a unique number to identify their fish).

After the calculated results, the CLEAR lab organised a town meeting in Petty Harbour to discuss the findings. The town meeting allowed for questions and concerns regarding plastic found in the public's food fish. Not surprisingly, citizens attending was nervous to find out what the % of the frequency of occurrence (%FO) of ingested plastic was for Atlantic cod. The meeting allowed for feedback regarding the results that aligned with their understandings of plastics and fish in the area. The public also had concerns about consuming fish such as mackerel and capelin and suggested in the future examining these species for ingested plastic.

Due to the low %FO of plastic ingested by Atlantic cod, human health was not of high concern. However, an outcome of the meeting was that biomonitoring of species caught through recreational and commercial fisheries should be ongoing.

Biomonitoring will allow for measuring any changes in plastic ingestion in fish while providing plastic monitoring within the human food web. The research presented here is in part, a continuation that was requested from that town meeting.

After attending the town meeting, it was important that my objectives were in-line with community involvement. The objectives included taking the information from the town meeting that was presented in Liboiron et al. (2016) and examine capelin along with offshore Atlantic cod while working with local non-profit and government agencies. This also included finding a citizen science method that would enable citizens to locate and detect plastic in their food fish.

Structure of Thesis

Chapter 2

Chapter 2 is intended to be an extension of previous work for ingested marine plastics in Newfoundland, Canada and to answer the research questions concerning the %FO of ingested plastic in offshore Atlantic cod, while establishing a baseline for plastic ingestion for capelin. The research presented here will complement the established plastic ingestion data for inshore Atlantic cod off the east coast (Avalon Peninsula) of Newfoundland while also addressing questions regarding plastic ingestion for the two species (location, feeding behaviours, sex, and plastic polymer/density).

Chapter 3

Chapter 3 presents data on compared methods which locate and identify plastic in the GI tracts of fish using visual analysis. These methods ranged from a chemical laboratory to citizen scientists using standard household tools. The purpose of this study was to determine if citizen scientists can locate and identify ingested plastic without the use of scientific tools and limited marine plastic training. The chapter includes a spotter's guide, dissecting diagram and instructions to guide either scientists or citizen scientists to detect plastics in fish.

These chapters were written to be read independently. However, they both address the issue of marine plastic pollution in Newfoundland, while testing and answering the presented research questions. Both Chapter 2 and 3 are baseline studies that are intended to be a starting point for further research.

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Chapter 2

Baseline study of ingested microplastic found in offshore Atlantic cod *(Gadus morhua)* and inshore capelin *(Mallotus villosus)*, Newfoundland, Canada

2.1. Abstract

Marine plastics have affected all the world's oceans and can persist in the ocean for thousands of years. They can be ingested by a wide range of marine organisms, posing a potential health threat to human health. Plastics recovered from the marine environment have been shown to carry an assemblage of industrial chemicals, both absorbed from the ambient seawater as well as the ingredients from the plastic itself. Through a research collaboration with the Department of Fisheries and Ocean and the Marine Institute of Newfoundland, Canada, I sampled 350 inshore capelin *(Mallotus villosus)*, and 460 offshore Atlantic cod *(Gadus morhua)* for ingested microplastics in the Northwest Atlantic Fisheries Organization Division 3. Capelin and Atlantic cod are consumed locally and are also sold as an export commodity. Given the significance of Atlantic cod and capelin to the region, this study aimed to quantify the frequency of occurrence in these two species. Using visual analysis and Raman Micro-Spectroscopy this study found that capelin had a frequency of occurrence of ingested plastic of zero, and Atlantic cod had a frequency of occurrence of 1.1%. This study was the first in-depth examination of microplastic ingestion by offshore cod and inshore capelin and serves as a baseline for monitoring the impacts of microplastic pollution on human food fisheries in this area.

2.2. Introduction

Marine plastics are widely distributed across all the Earth's oceans and can be found in various parts of the water column (Derraik, 2002; Edyvane et al., 2004; Thompson et al., 2004; Barnes et al., 2009; Ogata et al., 2009; Law et al., 2010; Claessens et al., 2011; Collignon et al., 2012; Obbard, 2014). Large plastics, known as macroplastics (>5 mm), have been studied extensively, but in recent years microplastics (<5-1 mm) have raised concerns due to their effects on the marine environment, organisms, and the food web (Andrady, 2011; Farrell and Nelson, 2013; Setälä et al., 2014). Low-density microplastics float at the surface of the ocean because they are less dense than salt water. These low-density microplastics will remain at the surface (Derraik, 2002), but can sink when attached to organisms through biofouling (Thompson et al., 2004; Barnes et al., 2009). In this way, low-density plastics can be transported from the surface layers into the deeper water column, and then onto the seabed (Colton and Knapp, 1974; Law et al., 2010; Claessens et al., 2011; Collignon et al., 2012; Obbard et al., 2014), making them accessible for ingestion by a wide range of marine organisms (Browne et al., 2008; Farrell and Nelson, 2013; Setälä et al., 2014).

The toxic effects of persistent organic pollutants (POPs) and other chemicals that concentrate in plastics are a concern for ocean ecosystems and human health (Mato et al., 2001; Rochman et al., 2013; Koelmans et al., 2016; Sussarellu et al., 2016). Plastics recovered from the marine environment have been shown to carry an assemblage of industrial chemicals, both absorbed from the ambient seawater as well as the ingredients from the plastic itself (Ogata et al., 2009; Lithner et al., 2011;

Holmes et al., 2012). When ingested, marine plastics can produce a biological response through both physical and chemical mechanisms of toxicity (Gregory, 2009; Von Moos et al., 2012; Browne et al., 2013; Rochman et al., 2013, 2015), causing physical damage leading to inflammation and lacerations of the gastrointestinal (GI) tract and cellular necrosis (Rochman et al., 2015). The transfer of chemicals from plastic to organisms could potentially be dangerous to humans who consume seafood (Rochman et al., 2013, 2014). The knowledge that chemicals from plastics in fish can be potentially transferred to humans has motivated research on marine plastic ingestion rates in the North Atlantic Ocean and, the Newfoundland region.

There has been a limited amount of studies examining plastic ingestion by Atlantic cod *(Gadus morhua)* in the North Atlantic oceans. Collectively, these studies found a mean frequency of occurrence (%FO) of 4.1% (Foekema et al., 2013; Bråte et al., 2016; Rummel et al., 2016; Liboiron et al., 2016). Foekema et al. (2013) examined both inshore and offshore cod in the North Sea. They reported zero ingested plastic out of the thirteen samples caught offshore, and a %FO of 14.9% for marine plastics ingested by inshore cod (n=69). Bråte et al. (2016) examined 302 Atlantic cod samples from Norwegian waters and found a %FO of 3%. However, the samples from Bergen City Harbour, their most human populated test site, Atlantic cod (n=8) were found to have a %FO of 27%. Rummel et al. (2015), examined offshore cod from both the North Sea and Baltic Sea. In the North Sea, seven samples were examined and zero were found to have ingested plastic. In the Baltic Sea, one cod out of 80 samples was found to have ingested plastic (%FO of 1%).

In Newfoundland there has only been a single study for plastic ingestion in Atlantic cod. Liboiron et al. (2016) found a low %FO (2.4%) of marine plastics ingested by inshore cod destined for human consumption. To date, there has not been a single study that has examined capelin *(Mallotus villosus)* for ingested marine plastic. Relative to the widespread distribution of marine plastics there has been limited research for fish ingesting plastic, particularly those destined for human consumption in the North Atlantic. There is a need for additional studies, and this study fills that gap by examining offshore Atlantic cod and inshore capelin. Both species are important in Newfoundland because they are consumed locally and are exported for commercial gain (Department of Fisheries and Oceans, 2011, 2014, Figure 2.1). This study established a baseline for long-term monitoring of plastic ingestion for inshore capelin and offshore Atlantic cod in the NAFO subdivisions 3Ps, 3L, 3N, 3O, and 3K (Figure 2.2.).

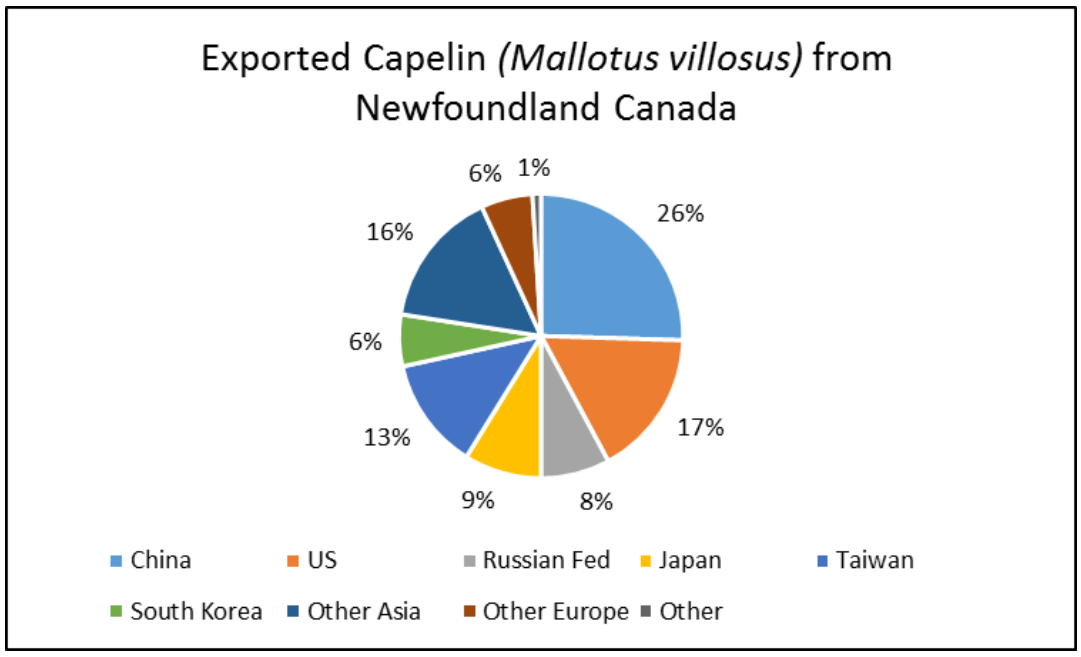

Figure 2.1. The percent of capelin (Mallotus villosus) exported from Newfoundland, Canada for 2009 in Northwest Atlantic Fisheries Organization (NAFO) subdivisions 4R, 4S, and 4T and 2J, 3L, 3K and 3Ps (Department of Fisheries and Oceans, 2011).

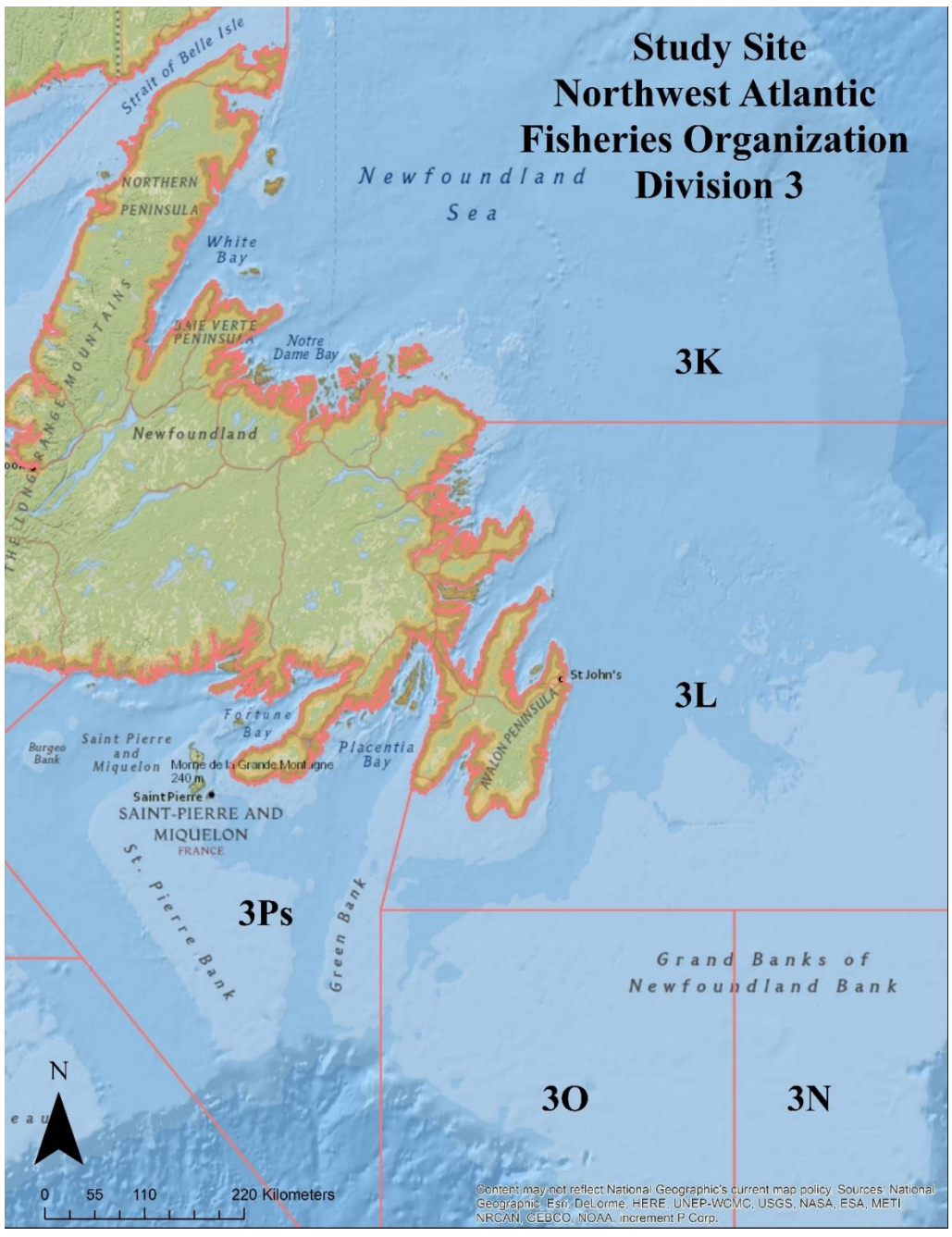

Figure 2.2. Map of Northwest Atlantic Fisheries Organization (NAFO) subdivisions. The locations for Atlantic cod and capelin surveys are as follows, St. Pierre Bank (3Ps), Southeast Shoal (3N and 3O), and Eastern Newfoundland and Labrador (3L), and capelin, (3Ps, 3L, and Eastern Newfoundland and Labrador (3K)) (Department of Fisheries and Oceans, 2012).

This paper begins with a description of the study site and behaviours for both capelin and Atlantic cod that place them in specific locations in the water column. Department of Fisheries and Ocean (DFO) provided intact capelin from Newfoundland, Canada that had been caught by commercial harvesters in the summer of 2015. For Atlantic cod, gastrointestinal (GI) tracts were collected in the spring of 2016 during annual surveys conducted by the DFO and the Marine Institute (MI) of St. John's, Newfoundland, Canada. Both Atlantic cod and capelin samples were analysed in the lab according to standard methods (van Franeker et al., 2004), and the types and characteristics of ingested plastics were analysed visually and processed using Raman micro-spectroscopy. The collected data for both Atlantic cod and capelin were used to determine the %FO for locations. The data for Atlantic cod included the water depth and temperature within the geographical locations. The sex, length of fish and stomach contents for Atlantic cod and capelin were collected and recorded.

2.3. Contextualizing the Study Site and Species Behaviours

2.3.1. Study Site

The Northwest Atlantic Fisheries Organization (NAFO) was established in 1979 to provide scientific advice to member states with the premise of ensuring the conservation and management of fish stocks in the region (NAFO, 2016). The Convention Area covered by NAFO (6,551,289 km^2) encompasses a significant part of the Atlantic Ocean and includes the 200-mile exclusive economic zone of Coastal States jurisdiction where vessels from the USA, Canada, St. Pierre et Miquelon and Greenland can fish (Fig. 1). Atlantic cod and capelin are known to migrate among NAFO management subdivisions 3Ps, 3N, 3O, 3L and 3K (Department of Fisheries and Oceans, 2016; Figure 2.2).

Newfoundland fisheries

The northwest Atlantic marine ecosystem off Newfoundland and Labrador, Canada went through extreme changes in the early 1990s due to a climatic regime shift and centuries of commercial fishing exploitation (Rose, 2004; Buren et al., 2014). The capelin stock suffered a significant biomass decline in 1991 - from which it has not fully recovered (Frank et al., 1996; Department of Fisheries and Oceans, 2010, 2011). Subsequently, Atlantic cod was devastated by overfishing and the changing distribution and availability of capelin, its primary food source (Gough, 2007, p. 6 and 417). In the early 1990s, due to low biomass levels of Atlantic cod, several NAFO subdivisions were closed to commercial fishing. The closures were as follows: 2J, 3K, and 3L (1992); 3Ps, 4R, 4S, and 3Pn (1993); and, 3N and 3O (1994) (NAFO, 2013), and this study considers two of these zones (3L, 3Ps).

Similarly, fishing for capelin was also restricted and did not reopen until 1997 for NAFO divisions 3K, 3L and 4R (Department of Fisheries and Oceans, 2015b), two of which are studied here (3L, 3K). However, on 19 May 1997, a limited cod fishery opened in NAFO subdivisions 4R, 4S, 3N, and 3Ps. The abundance of capelin has remained low since the initial collapse and may have contributed to the lack of rebound in the Atlantic cod stock (Rose and O'Driscoll, 2002). However, until capelin and cod regain healthy biomass, a moratorium will remain in effect except for NAFO divisions 3Ps, 3K, 3L and 4R for Atlantic cod and 3K, 3L and 4R for capelin (Department of Fisheries and Ocean, 2016).

This study was able to draw samples from ongoing surveys. Every year DFO and the Fisheries and Marine Institute of Memorial University of Newfoundland (MI) conduct offshore surveys in Northwest Atlantic Fisheries Organization (NAFO) division 3, to evaluate the status of the stocks. These surveys provide the basis for scientific advice concerning conservation outcomes related to various fishery management options. I contacted both DFO and MI and organized meetings to discuss research collaboration, as these existing surveys would easily enable data collection for my research.

In the initial stages of my research, several meetings took place with DFO and MI to determine the best fish to sample for this project. Through these meetings, the decision was made to sample inshore capelin and offshore Atlantic cod. These species were chosen for several reasons. First, there is no data for plastic ingestion in capelin and at the time only one study for plastic ingestion for Atlantic cod, which relied on fish caught inshore. Second, both Atlantic cod and capelin are important to Newfoundlanders as a food source and exportation. DFO and MI agreed to provide samples of Atlantic cod samples collected in the 2016 spring surveys, and DFO provided inshore capelin caught in 2015 from commercial harvesters. The survey sites for Atlantic cod were predetermined by both DFO and MI. Both organizations allowed me to collect samples once their required samples had been met for the stock evaluation surveys. Regarding capelin, DFO collected these samples in 2015 from commercial harvesters, and again the sites were predetermined by DFO. Formalising the plastic sampling and laboratory protocols also took place during the meetings between DFO and MI.

2.3.2. Behavioural aspects

It is essential to examine the dispersal dynamics, life cycle, breeding behaviours and ecological importance of these two highly migratory species to see how their behaviours and geographies impact their potential exposure to plastics.

Capelin *(Mallotus villosus),* a pelagic shoaling fish, play a significant role as a link between zooplankton production and the transfer of energy up the food web. Capelin are a key forage species for many marine organisms such as whales, seals, seabirds, and historically Atlantic cod (Lawson et al., 1998; Bundy et al., 2000; Montevecchi, 2001; Sherwood et al., 2007; Krumsick and Rose, 2012). DFO has recognized five biologically distinct capelin stocks in the NAFO subdivisions: 2J, 3K, and 3L (Eastern Newfoundland and Labrador; which exists as a stock complex that frequently mix); 3Ps (St. Pierre Bank); 4R, 4S and 4T (Gulf of St. Lawrence); 4W (Scotia Shelf); 3N and 3O (Southeast Shoal; a straddling stock that is internationally managed by NAFO).

Capelin spend the winter near the edge of the continental shelf where plastic is likely to be scarcer (Law et al., 2010; Eriksen et al., 2014). Each spring capelin typically move from offshore feeding areas to inshore bays, and then northward along the coast toward spawning areas where they are caught for human consumption (Nakashima, 1992). These are also, areas where plastic is known likely to be more plentiful (Barnes et al., 2009). During their migration to inshore spawning grounds, their feeding behaviours change presenting a higher occurrence of empty stomachs, suggesting feeding is less of a priority (Winters, 1970; Vesin et al., 1981; Davoren et al., 2003). Thus far, to my knowledge, no other capelin studies for plastic ingestion have been published in the literature.

Atlantic cod (*Gadus morhua*) is a benthopelagic demersal fish and an opportunistic feeder (Link and Garrison, 2002). Due to this foraging behaviour, plastic could be ingested directly by the cod (Foekema et al., 2013) or through secondary ingestion by the consumption of natural prey items that had ingested plastics (Rummel et al., 2016). Atlantic cod diet is primarily small or medium sized fish from benthopelagic waters (Cohen et al., 1990), below pelagic waters but above the ocean floor (approx. 150-200m below the surface) (Johansen et al., 2009). Atlantic cod continue feeding during early spring as they begin to spawn (Krumsick and Rose, 2012), increasing their intake of benthic organisms and plant material. By late summer, food intake is primarily benthopelagic fish (Cohen et al., 1990) in which plastic may be less likely to accumulate. However, studies have shown low-density plastics are commonly found near the surface or subsurface (Andrady, 2015). The sediment on the ocean floor has been found to collect a higher concentration of fouled or high-density plastics (Derraik, 2002; Van Cauwenberghe et al., 2013). However, the concentration of plastics on the ocean floor have not been examined in Newfoundland.

Historically, data has shown Atlantic cod prey on capelin (Krumsick and Rose, 2012), however recent data from DFO and MI has shown a shift in their preferred diet. Prey selection for cod in 3Ps is highly variable. Data from recent years (2013-2015) suggests that the dominated preferred diet by invertebrates includes sandlance (*Ammodytidae*) and snow crab (*Chionoecetes opilio*), where smaller individuals are consuming sandlance, and larger cod consume snow crab (Department of Fisheries and Oceans, 2016). Cod in the neighbouring NAFO Division 3O are not heavily consuming crab or sandlance. According to Marine Institute (MI) surveys conducted in NAFO division 3O from 2012, 2013, and 2014, have shown an even higher variability from year to year. These studies reported the most common prey recorded in the stomachs of Atlantic cod were as follows: toad crab (*Hyas araneus*) 38% (2012), polychaete 79% (2013), redfish 42% (2014).

Importance of Study

For over 500 years settlers of Newfoundlanders have fished waters abundant with Atlantic cod and capelin, creating cultural significance (Kurlansky, 1997; Lawson et al., 1998; Bundy et al., 2000; Hamilton and Butler, 2001; Rosenburg et al., 2005; Rose, 2007). Both are harvested annually through the recreational food fisheries (cod) or the annual capelin roll during June and July, and both species are also caught and sold commercially (Department of Fisheries and Oceans, 2015a, 2016). Newfoundlanders are often seen in the intertidal zone during June and July with buckets and cast nets collecting spawning capelin (CBC, 2015). Capelin are eaten smoked, flavoured or fried, and whole (Over, 2003). However, capelin harvested in Newfoundland are not limited to local consumers. DFO reported in 2009, 70.2% of the total capelin landings were commercial and exported to countries such as China, United States, Taiwan, and Japan, with a yearly export value of $26.6 million (Department of Fisheries and Oceans, 2011, 2014; Figure 2.1). The remaining 29.8% were sold (whole, and fresh or frozen) domestically as either bait or fishmeal (e.g., animal feed or fertiliser). Data from DFO did not specify if capelin sold domestically were also consumed (Department of Fisheries and Oceans, 2011, 2014).

2.4. Methods

2.4.1. Data Sharing

Every year DFO and MI conduct surveys of Atlantic cod and capelin for stock assessment, food web interactions, and environmental influences. These data are used to provide scientific advice on fishery management options and conservation goals. The DFO and MI surveys combine costly specialized vessels equipped with bottom trawl and acoustics. The surveys require specialized crew members, experienced in either vessel and trawl operations or gathering scientific data. In order to ensure that this collaboration met our respective goals and analysis requirements, agreements were formulated as to how we would collect and share the data. This took place through several meetings with both agencies.

2.4.2. Sampling Agreements

Atlantic cod were supplied from the DFO and MI spring surveys, in excess of those required for standard sampling. Sampling plastic protocols were identical for DFO and MI for each survey. During the surveys, DFO and MI technicians tagged and removed the entire GI tract from mouth to anus for each sample. Compromised samples (stomachs everted, incorrectly cut stomach, specimen dropped or contaminated with other samples), were discarded. Data collected for each Atlantic cod sample included: total weight, total length, sex, maturity, gonad weight, liver weight and gutted weight and stomach content.

Environmental factors were also provided for each survey, such as water temperature, location, and depth. Samples were placed into bags labelled by species and trip number and marked to indicate they were samples for microplastic analysis. Samples were immediately frozen after collection and stored for later dissection and analysis. All samples collected for plastic analysis were also used in prey analysis for both DFO and MI.

Once the samples were transferred to the laboratories for prey analysis a protocol was designed to ensure prevention of any loss of microplastic. During the prey analysis I worked side-by-side with both DFO and MI trained technicians. Sample bags were thawed two hours prior to GI tract analysis for Atlantic cod. I removed the stomach from the intestinal tract by detaching the stomach from the intestinal tract without removing the entire GI tract from the sample bag and placing it in a sterilised metal container. After prey analysis, including documentation and weighing of the stomach contents, I placed the contents in the original sample bag. I then carefully rinsed the metal container and the water was placed back in the sample bag along with the stomach and prey contents. The bag was resealed and frozen for plastic analysis at a later time. I sterilized the metal container between each sample dissection. The data collected during the prey analysis was then provided to me through email in the form of a data set.

A total of 460 Atlantic cod from two surveys was analysed. In collaboration with DFO, 142 Atlantic cod samples were collected during the 2016 Ecosystem Research Program Survey, May to July 2016, in two-week intervals. In collaboration with MI, 318 Atlantic cod samples were collected during their annual trawl survey from 26 April – 11 May 2016. Samples collected for plastic analysis were collected opportunistically from bottom trawl surveys. The randomly distributed set

locations were within depth-delimited strata. Figure 2.2 shows the locations for the MI and DFO surveys and sample collections. Total samples for each NAFO subdivision were: 3Ps (*n*=385), 3O (*n*=22), 3N (*n*=24) and 3L (*n*=28).

Capelin

DFO provided frozen whole capelin along with the data collected from the commercial harvesters during the surveys. This included location, date caught, gear used, and vessel identification number. In return, I agreed to provide DFO with plastic analysis as well as the length, sex and stomach contents.

DFO provided capelin samples caught in July 2015 from commercial fish harvesters in the NAFO subdivisions, 3Ps, 3L and 3K. Fifty capelin samples were collected from seven locations by commercial harvesters: Lawn, Burin Peninsula; Port de Grave, Conception Bay; Hickmans Harbour, Trinity Bay; Lumsden, Bonavista Bay; La Scie, White Bay; Comfort Cove and Leading Tickles in Notre Dame Bay (Figure 2.3).

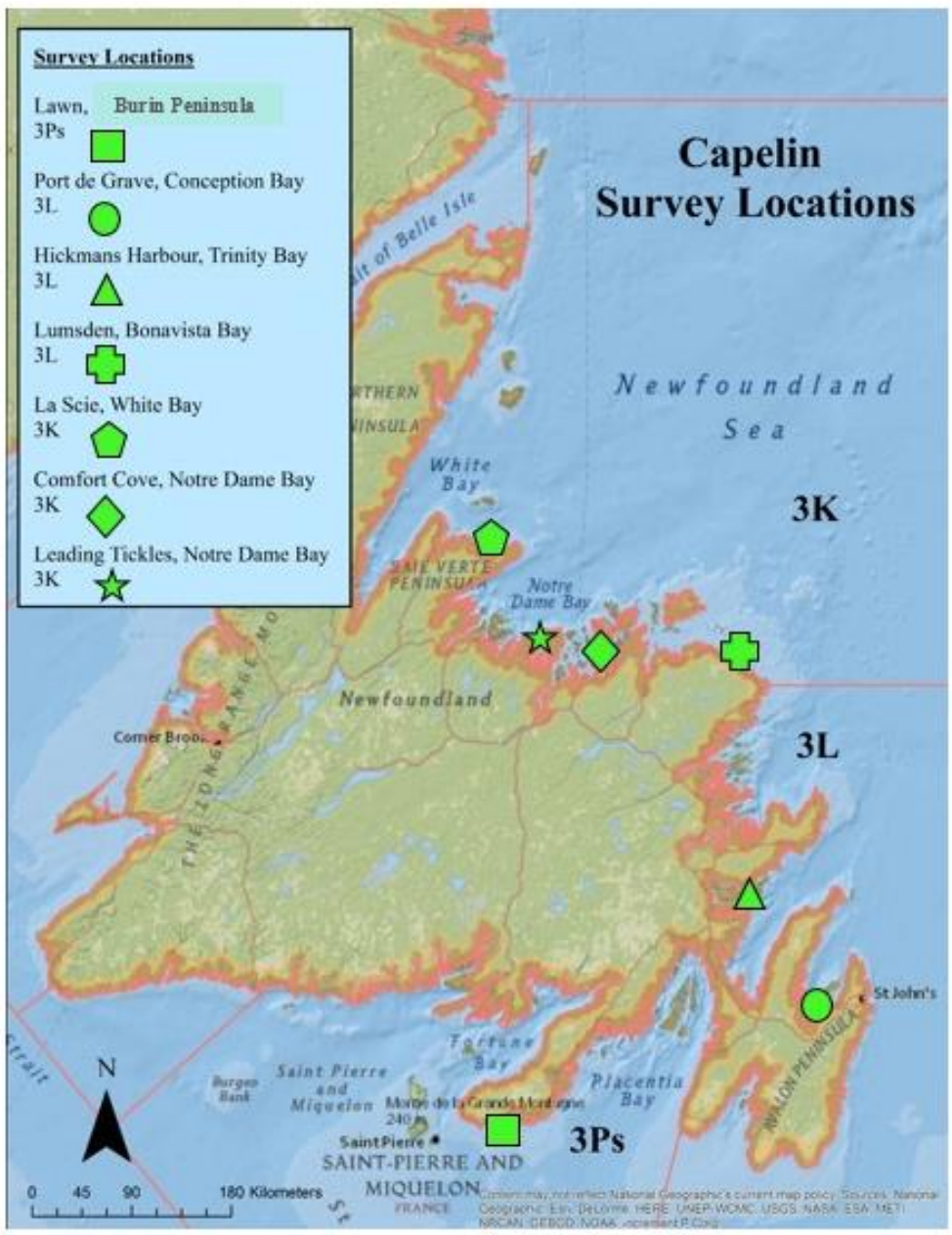

Figure 2.3. Map displaying capelin surveys conducted in Northwest Atlantic Fisheries Organization (NAFO) subdivisions 3Ps, 3L, and 3K in Eastern Newfoundland and Labrador from the following seven locations: Lawn, Burin Peninsula; Port de Grave, Conception Bay; Hickmans Harbour, Trinity Bay; Lumsden, Bonavista Bay; La Scie, White Bay; Comfort Cove and Leading Tickles in Notre Dame Bay of Newfoundland.

2.4.3. Laboratory Protocol

The protocol for dissecting Atlantic cod and capelin followed methods outlined in Liboiron et al. (2016). Before dissection, GI tracts were thawed in cold water for approximately 2 hours for Atlantic cod and 30 minutes for capelin. For Atlantic cod, a double sieve method was used to analyse stomach contents: a 4.75 mm (#4) stainless steel mesh sieve was placed over the top of a 1 mm (#18) stainless steel mesh sieve. For capelin, modifications were necessary due to their small size and the 4.75 mm (#4) stainless steel mesh sieve was not used. The 1 mm sieve was selected because this dimension is the lower cut-off point for microplastics (1.0-5.0 mm) (Wagner et al., 2014), and the recommended size for cod ingestion studies (European Commission, 2014). Microplastics in the range of 1.0-5.0 mm size can be reliably detected using the naked eye and can be further analysed using a microscope (Song et al., 2015).

The dissection process began by removing the entire GI tract from esophagus to the anus. After removing the stomach and intestinal content, it was gently cleansed and examined for embedded plastic. While continuously rinsing the sieve contents with cold water, the particles resembling non-organics were removed and placed in a petri dish for further analysis under a dissecting microscope with both reflected oblique and transmitted light (Olympus SZ61, model SZ2-ILST, with a magnification range of 0.5–12×). Visual sorting, probable microplastics (i.e., fragment, film, thread, fibres, and pellets) from organic food items and other anthropogenic debris followed. Colour, erosion and lack of cellular structure are the basis for visual sorting (Hidalgo-Ruiz et al., 2012). These samples were then examined for the absence or presence of cellular structure and erosion characteristics of plastics (Corcoran et al., 2009). As defined by Hidalgo-Ruiz et al. (2012) erosion found on microplastics is categorised as either- fresh, unweathered, incipient alteration, and level of crazing (conchoidal fractures), weathered, grooves, irregular surface, jagged fragments, linear fractures, subparallel ridges and very dedraged. In some instances, after the object was weighed and measured, it was broken open to inspect the internal structure. Unidentified debris under the dissecting microscope was examined using a compound microscope (magnification 10×, 40×). The probable plastics were air dried for two days in a folded paper filter, then stored in pre-labelled scintillation jars.

The following precautions were taken to ensure a safe working environment and the elimination of cross-contamination: all tools were rinsed or wiped down with water and paper cloths, including the microscope lens and plate, Petri dishes, and sieves. After each dissection, participants examined their hands and tools for any microplastics that may have adhered. All participants wore gloves, lab coat, and had their hair pulled back.

2.4.4. Analysis Protocol

The reported frequency of occurrence (%FO) was the proportion of sampled cod and capelin found to have ingested plastic, including mathematical means (±SE) for ingested plastic and mass. As per standardised practices (van Franeker, 2004), categories for plastics were fragments, sheets, threads, fibres, pellets, and foam. Documented dimensions were measured using a digital calliper (accurate to 0.01 mm), and the opacity (transparent or opaque), colour, and type of erosion. Air-dried plastics were measured regarding count and mass (in grams) using a Sartorius electronic weighing scale (accurate to 0.0001 g).

2.4.5. Raman Micro-Spectroscopy Protocol

Visual examination often is used as the only step to identify marine plastics. However, colour, size, and shape can be insufficient and often unreliable criteria to differentiate plastics from non-plastics, particularly at smaller scales (Hidalgo-Ruz et al., 2012; Song et al., 2015). Raman micro-spectroscopy is a technique used to identify specific polymers based on their vibrational energy states (Imhof et al., 2012; Lenz et al., 2015), and is more reliable for the identification of plastics than visual identification alone. All suspected plastic particles were washed with ethanol to remove any adhered organic particles. During the Raman micro-spectrometry stage, a trained research technician irradiated the samples with a monochromatic laser source. A silica wafer was used to calibrate the machine and as a background substance for analysis, because the wafer revealed a clear Raman spectrum with a single peak (at 520 cm-1. Samples were analysed using a Raman micro-spectrometer (Reinshaw InVia with 830 nm excitation) at a 20x Olympus objective, controlled by WiRE 3.4 software. Laser power did not exceed 5%, as high laser power can burn plastic samples. Many samples yielded high fluorescence upon initial analysis, and in these cases, laser power was reduced to 1% to reduce fluorescence. The WiRE 3.4 software indicated how the laser light interacted with the molecules of the plastic samples by observing the appearance of characteristic peaks at specific Raman shifts (Imhof et al., 2012; Lenz et al., 2015). The completed Raman spectrum of each particle was compared to reference spectra for common plastic polymers. These references included; polyethylene (PE), polyester (PET), polycarbonate (PC), polypropylene (PP), polyvinylchloride (PVC), polystyrene (PS), polyamide (PA), polyurethane (PU), poly (methyl methacrylate) (PMMA), acrylonitrile butadiene styrene (ABS), and cellulose acetate, all plastics that are common in marine pollution (Engler, 2012; Lenz et al., 2015; Bråte et al., 2016; Plastics Europe, 2016).

2.5. Results

2.5.1. Capelin

Out of the 350 capelin samples, none (0) had ingested plastic. Of these 350 samples, 151 were mature females, while the remaining samples (*n*=199) had trace gonads and sex was unknown. The mean length was 16.12 ± 1.39 cm. Out of the 350 samples 196 capelin had prey items in their GI tracts. Some samples had multiple items. The %FO of items found in the stomachs included fish eggs, 10% (*n*=36); krill, 6% (*n*=20); and undetermined material, 9% (*n*=31); or sand and/or granules, 56% (*n*=196), whereas the remaining 154 samples (44%) were found to be empty.

2.5.2. Atlantic cod

Five (5) of the 460 Atlantic cod GI tracts samples contained items identified as marine plastics, with an overall %FO of 1.1%. Each of the GI tracts contained only one plastic fragment. Cod samples found to have ingested plastics came from three NAFO subdivisions, 3Ps (*n*=2), 3O (*n*=2) and 3N (*n*=1) (Table 2.1; Figure 2.4). When isolated to the NAFO subdivision, the %FO are as follows: 3Ps (*n*=385), %FO of 0.5%; 3N (*n*=24), %FO of 4.2%; and 3O (*n*=22) at 9.1%. Division L had a rate of zero. Ingested plastics were in the stomachs of the GI tracts were clear. Plastics were of a variety of types: fragments (*n*=3), rope/thread (*n*=1), and a film (*n*=1). The mean length, width, and height of the five plastics were: 4.98 ± 2.79 mm, 1.36 ± 0.97 mm, and 1.26 ± 1.60 mm, respectively (range in the longest dimension was 1.5 mm – 9.0 mm and the shortest dimension 0.1

mm - 4 mm). The mean (+/- SE) mass of ingested plastic was 0.1417 ± 0.1388 g per fish (range 0.0001 g - 0.697 g). Four samples were not weathered, while one showed linear fractures, grooves, and irregular surfaces. All the fragments were opaque, and the pigmentations were white, beige, blue-green, red and white (Table 2.1.).

Table 2.1. Description of ingested marine plastics found in Atlantic cod *(Gadus morhua)* surveyed in Northwest Atlantic Fisheries Organization (NAFO) subdivisions 3Ps, 3N, and 3O*.

Sample ID	NAFO Sub-division	Type	Mass (g)	Size (longest side) mm	Polymer Type	Pigmentation	Erosion Patterns
F12333	3Ps	Fragment	0.0034	1.5	Undetermined	Blue-green	None
F32342	3Ps	Thread/ Rope	0.697	9	Polyethylene or Polyamide	Beige	None
J4475	3N	Fragment	0.007	5	Polycarbonate	Red and White	None
43896	3O	Fiber	0.0001	4	Polyethylene	White	None
43880	3O	Fragment	0.001	3	Polyvinylchloride	White	Linear fractures, irregular surface, and grooves

*see Figure 2.4 for the location of the subdivisions

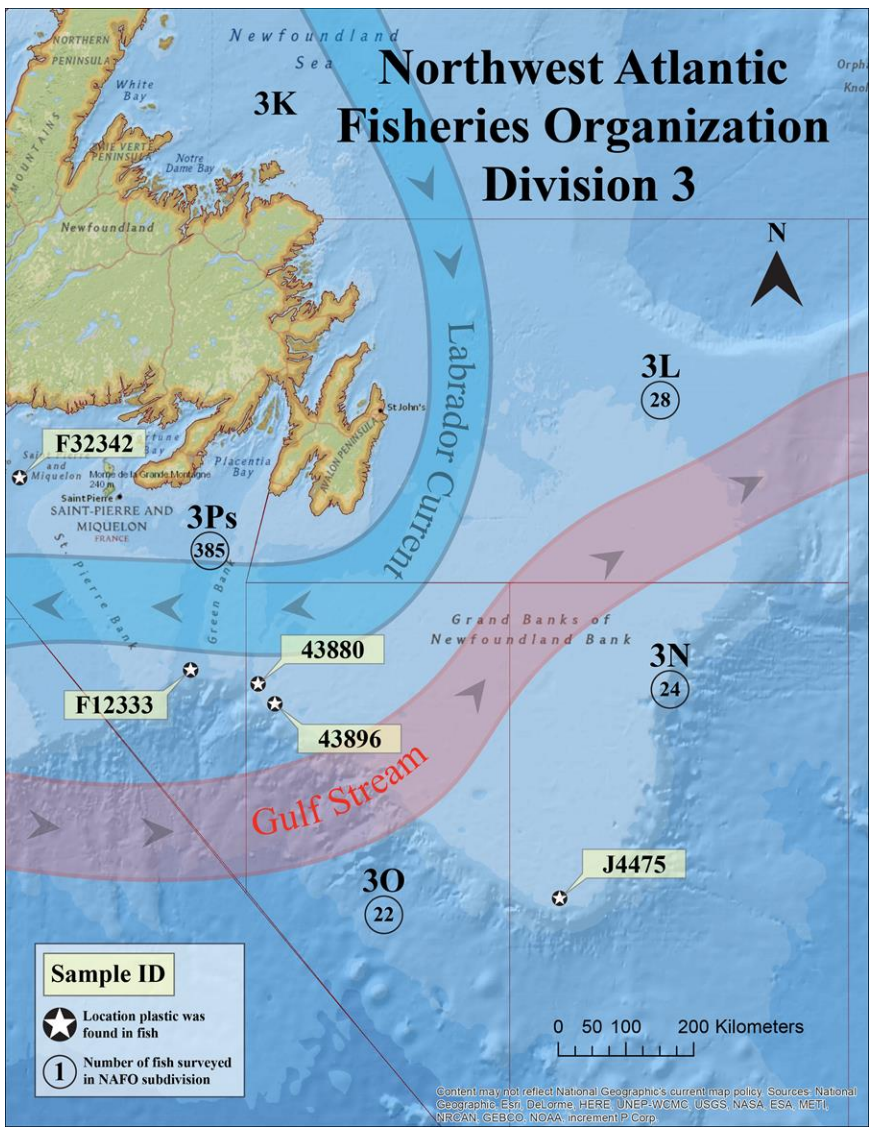

Figure 2.4. Map of survey locations in the Northwest Atlantic Fisheries Organization (NAFO) subdivisions. Total samples taken from each NAFO subdivision location are as follows: St. Pierre Bank 3Ps, (*n*=385), Southeast Shoal, 3N, (n=24), and 3O (*n*=22) and Eastern Newfoundland and Labrador 3L (*n*=28). Atlantic cod samples found to have ingested plastic come from three NAFO subdivisions, St. Pierre Bank 3Ps, (*n*=2), Southeast Shoal, 3N, (n=1), and 3O (*n*=2) and Eastern Newfoundland and Labrador 3L (*n*=0). When isolated to the NAFO subdivision, the %FO are as follows: 3Ps (*n*=385), %FO of 0.5%; 3N (*n*=24), %FO of 4.2%; and 3O (*n*=22) at 9.1%. Division L had a rate of zero. *Gulf Stream and Labrador Current are for illustration purposes only and do not represent seasonal variations

Out of the 460 Atlantic cod samples, 428 were found to have prey contents in the stomach. The stomach contents for the two highest %FO of items found in the NAFO subdivisions were: 3L, copepods 46% and polychaete 30%; 3N, Copepods 36% and amphipods 26%; 3O, mysids 19%, and amphipods 15%; and 3Ps, brittle star 21%, and amphipods 15%. Stomach contents of the five samples that ingested plastic included (some samples had multiple prey items): amphipod (*n*=4), plant material (*n*=3), brittle star *(Ophiuroidea sp.)* (*n*=1) and fish (*n*=2) (Redfish, cod, and capelin). Out of the total samples (*n*=460), 237 were female, 215 were male, and for 15 of the samples, sex was undetermined. All five fish that were found to have ingested plastics were female with maturity levels ranging from immature to recently releasing eggs (spent) and the mean length was 67.66 ± 28.02 cm (ranging from 45 to 99 cm). The mean water depth for collected samples was 111.6 ± 17.4 m (ranging 95 to 137 m). The mean temperature of sample locations was 2.16 ± 1.98 °C (only collected by MI).

2.5.3. Raman Micro-Spectroscopy

While Raman spectrometry is considered a way to reduce uncertainty in visual identification of marine microplastics, it did not always yield conclusive results. High fluorescence in sample F12333 (blue-green fragment) yielded no characteristic peaks; and therefore, the sample's polymer could not be identified, although visually it is identical to plastic nylon netting common in the area. Fluorescence manifests as an "umbrella peak" in the spectra, hiding characteristic peaks of the polymer when the light intensity emitted can be as strong as or stronger than that of the Raman scattering (Lenz et al., 2015). As a result, fluorescence is a common factor in poor Raman signal quality (Collard et al., 2015; Jochem and Lehnert, 2002). Sample ID F32342 (thread/rope) showed similarities to both polyethylene (PE) and polyamide (PA), whereas the red and white fragment (Sample ID J4475) showed strong similarities to polycarbonate (PC) but was missing the 1600 peak associated with PC (Figure 2.5). The white fibre (Sample ID 43896) found in NAFO subdivision 3O was confirmed to be PE (Figure 2.6), and the white fragment (Sample ID 43880) showed strong similarities to polyvinyl chloride (PVC). Altogether, the only particle confidently confirmed to a specific plastic polymer was sample 43896, the white fibre (polyethylene). Beyond this, the remaining particles only revealed strong similarities to common marine plastic polymers but not complete matches. This is not a surprising result when dealing with marine plastics. First, the marine environment can alter plastics from the manufactured state (from which Raman spectra reference libraries are drawn). This alteration can occur through photo-degradation, thermal degradation, and biodegradation as well as the integration of biological, synthetic or inorganic material in the marine environment (Andrady, 2011; Lenz et al., 2015). Also, the plastics themselves may not be composed of a single polymer but instead may include a mixture of additives (such as fillers, pigments, and dyes), which can modify or influence the Raman spectra (Van Lenz et al., 2015; Cauwenberghe and Janssen, 2014).

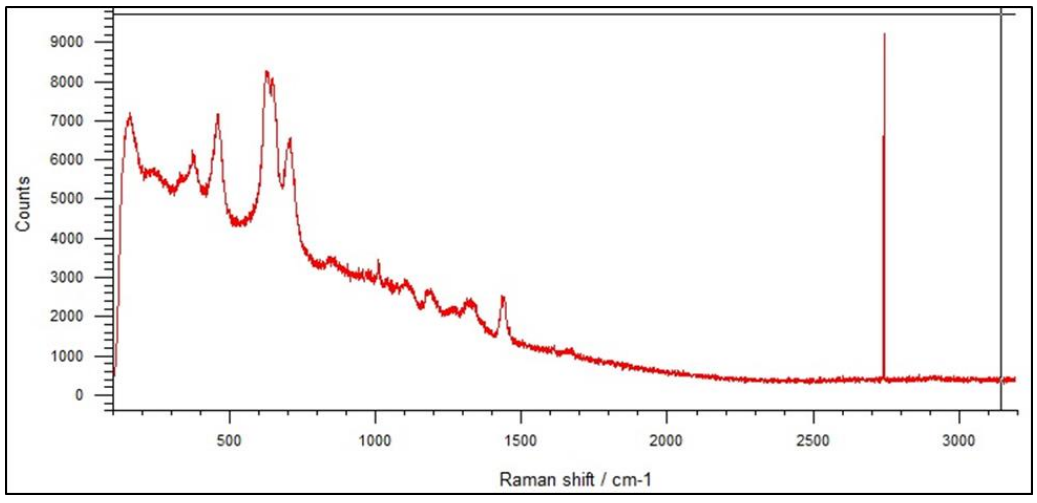

Figure 2.5. Raman spectrum for Sample ID J4475 (red and white) found in Northwest Atlantic Fisheries Organization (NAFO) subdivision 3N showed strong characteristics to polycarbonate (PC); however, is missing the 1600 peak. Peaks: 380, 450, 625-645, 700, 850, 1006, 1100, 1187, 1306, 1440, and possibly 1670.

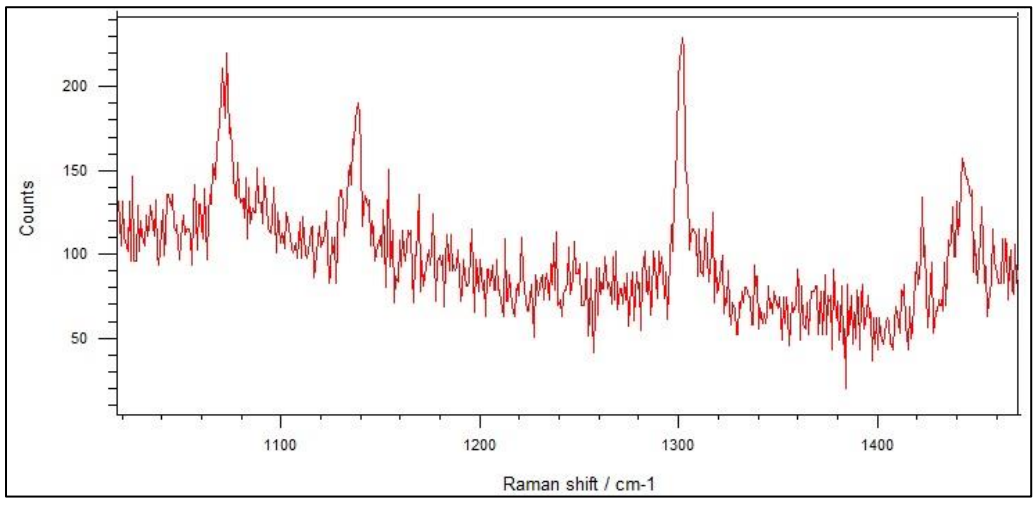

Figure 2.6. Raman micro-spectrometry for sample 43896 (white fibre) found in Northwest Atlantic Fisheries Organization (NAFO) subdivision 3O was confirmed to be polyethylene (PE). Peaks: 1080, 1130, 1300, and 1450.

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2.6. Discussion

The present study contributes baseline data on the ingestion of marine plastic debris by inshore capelin and offshore Atlantic cod in Newfoundland waters. Overall findings suggest these two species from NAFO division 3 are not ingesting substantial quantities of visible plastics (1.1%FO for Atlantic cod and zero for capelin). Other studies have similarly recorded low plastic recovery from species in the North Atlantic (Foekema et al., 2013; Bråte et al., 2016; Liboiron et al., 2016, Liboiron et al., 2018). It is therefore likely that the low rates of ingestion exhibited in the present study are related to geographical location, species behavior and biology.

Geographical Distribution of Plastics

Areas with higher populations densities have shown higher rates of plastic ingestion by fish (Foekema et al., 2013; Bråte et al., 2016). In the case of Foekema et al. (2013), a variety of species caught in the North Sea had %FO of 1.2% for fish caught in remote northern areas above 55°N, and 5.4% in the Southern North Sea. Their highest rate of >33% came from cod in the English Channel. In each case, fish caught in areas with higher human and industrial populations had higher frequencies of ingestion. Likewise, Bråte et al. (2016) found a frequency of occurrence of 3% in their total sample (*n*=302) of Atlantic cod from Norwegian waters. However, their most populated sample site, Bergen City Harbour, reported a %FO of 27%. In

Newfoundland, Liboiron et al. (2016) found a frequency of occurrence of 2.4% of Atlantic cod from samples collected nearshore during recreational fisheries.

Atlantic cod samples in this study taken from bodies of water adjacent to the southern shore of Newfoundland (NAFO subdivision 3Ps) had a %FO of 0.5% (*n*=385) respectively, and the sample site adjacent to St. John's (3L, *n*=28), the capital city and home to half the province's population, had zero ingested plastic. The two sample sites that were furthest offshore and not adjacent to Newfoundland shorelines (3N and 3O) had rates of 4.2% (*n*=24) and 9.1% (*n*=22), respectively. The overall mean of 1.1% is a low rate for Atlantic cod and aligns with other studies from northern waters in particular (Liboiron et al., 2016 for an overview). However, the geographic distribution of the plastics in this study where the waters adjacent to the highest populations had the lowest ingestion occurrence presents a counter-narrative where merely being close to populated shorelines is not the only reason for the occurrence of ingestion in cod. This finding is confirmed by the complete lack of plastics found in capelin, which were caught directly adjacent to shorelines where plastics are known to be concentrated (Barnes et al., 2009).

The findings presented in this study may have been influenced by the lack of Atlantic cod samples available in NAFO 3N, 3O and 3L. The three locations had considerably fewer samples than in 3P (Figure 2.4.). Previous studies that have examined Atlantic cod for ingested plastic have also shown inconsistent sampling between offshore and inshore. Foekema et al. (2013), sampled thirteen samples offshore (0%FO), and 69 samples inshore (14.9%FO). While Bråte et al. (2016) with a total of 302 samples, only sampled eight Atlantic cod from inshore waters where it was found to have the highest %FO of 27%. Due to the lack of inconsistent sampling sizes, results may have been skewed. Therefore, it is recommended using larger sample sizes for all locations to better understand the relationship between higher human population densities and plastic ingestion. Furthermore, there are not enough studies that examine ingestion rates in Atlantic cod in the North Atlantic to fully compare whether higher populations densities cause a higher occurrence of plastic ingestion in Atlantic cod.

Ingested plastics in offshore areas may have come from the Gulf Stream, which flows just south of Newfoundland, or from vessels travelling on the Gulf Stream. Although to pinpoint the origin of the ingested marine plastics is beyond the scope of this study, our samples displayed low levels of erosion and limited discolouring. Out of the five samples of ingested plastics collected from cod, four exhibited no indications of weathering and displayed limited amounts of biofilm that can accumulate after only a brief period in the water (Lobelle and Cunliffe, 2011; Muthukumar et al., 2011). This would suggest that the four plastics were not in the water for a considerable length of time or had sunk to the ocean floor shortly after reaching the ocean, and as such may have originated from vessel pollution (from litter, holding tanks, or ship wear) (Figure 2.7).

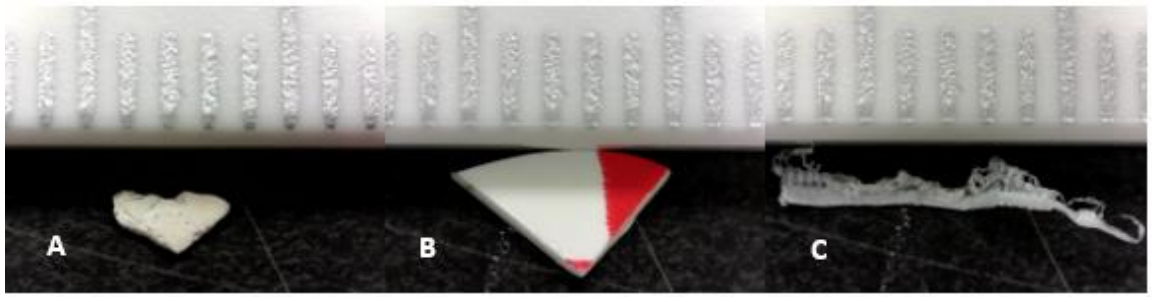

Figure 2.7. A picture is displaying three out of the five plastics found to be ingested by Atlantic cod *(Gadus morhua)* in the Northwest Atlantic Fisheries Organization (NAFO) Division 3. Sample ID 43880 (A), was a white fragment ingested in NAFO 3O and displayed linear fractures, irregular surface, and grooves, suggesting it was in the water for a considerable length of time before being ingested. Sample ID J4475 (B) is a red and white fragment ingested in NAFO 3N, and Sample ID 43896 (C) is a film ingested in NAFO 3O. Neither sample displayed discolouration or erosion. The ruler is in mm.

The Grand Banks (which underlies 3L, 3O, and 3N) is mixing of the cold waters of the Labrador Current with the warm waters of the Gulf Stream (Figure 2.4) ranging in depths from 25 to 100 meters. Most synthetic polymers, such as polyethylene and polypropylene, are buoyant and can be transported in seawater (Andrady, 2011; Plastics Europe, 2014), whereas polymers that are denser than saltwater (e.g., PVC) can be transported by underlying currents (Engler, 2012). These currents could explain the weathered plastic (Sample ID 43880 Figure 2.7) found in NAFO subdivision 3O (Table 2.1) that displayed strong characteristics of PVC.

Species Behaviours and Biology

Finding zero ingested plastic in capelin was unexpected. Since this is the first study to examine capelin for ingested plastic, there are no comparable studies. Understanding why the capelin sampled in this study had not ingested plastic is outside of the limits to the research questions presented here. However, it is possible to speculate why plastic was not found in capelin. As capelin mature and stage in shallow surface shoals, their behaviours change, suggesting feeding is less of a priority (Winters, 1970; Vesin et al., 1981; Davoren et al., 2003). Limiting feeding spawning during the time of sampling may have contributed to the findings. This would explain why almost half of the samples had empty stomachs. However, out of the 350 samples, prey items were found in the GI tracts of 196 samples. This is significant because even though these capelins were eating they were not ingesting plastic. This raises questions if the lack of plastic found in capelin was due to spawning behaviour or diet selection and should be investigated further. One way of determining the importance of spawning behaviour in relation to capelin ingestion of plastic would be to sample capelin during various times of the year, which would allow a comparison of capelin during spawning and non-spawning seasons.

Unlike capelin, Atlantic cod continue to feed during spawning (Krumsick and Rose, 2012). Moreover, all Atlantic cod samples found to have ingested plastic also had prey items throughout the GI tract. Because Atlantic cod are opportunistic feeders (Link and Garrison, 2002), plastic could be ingested directly by the cod (Foekema et al., 2013) or from secondary ingestion by the consumption of natural prey items that had ingested plastics (Rummel et al., 2016). Fish that had ingested plastic from 3Ps had ingested either invertebrates or plant material, and the fish that had ingested plastics from 3N had also eaten amphipods, while 3O samples contained fish and brittle stars. While these findings are in line with the type of things cod usually eat (Department of Fisheries and Oceans, 2016), they do not support a clear trend that would correlate secondary

plastic ingestion to prey. Brittle stars typically reside in benthic areas where plastics may be present, while amphipods and fish are distributed in the water column where plastics are less likely to be found (Derraik, 2002; Van Cauwenberghe et al., 2013; Andrady, 2015). This does not mean that there is no pattern to be found, but that we have too few samples to make a judgement in this case.

All five Atlantic cod samples that ingested marine plastics in this study were female with maturity levels ranging from immature to spent with a mean length of 67.66 ± 28.02 cm (ranging from 45 to 99 cm). Since most other studies surveyed found that sex is not a determining factor for plastic ingestion (Foekema et al., 2013; Bråte et al., 2016; Rummel et al., 2016) we can assume this is random, especially given the small sample of fish that ingested plastic.

Potential Effects of Ingested Plastics on Cod

All Atlantic cod that had ingested plastic in this study contained a single particle, suggesting marine plastics did not accumulate inside the digestive tract of these fish for extended periods, as found in other studies (Koelmans et al., 2014; Bråte et al., 2016). The gut retention time of natural food items for North Sea cod is reported to be 3.7 days (Daan, 1973), and ingested plastics would likely be retained in the GI tract the same period. GI blockage and false satiation are thus not likely in this case. However, chemicals associated with plastics may still transfer to tissue as plastics move through the GI tract. Koelmans et al. (2014) investigated the transfer of two plastic additives (nonylphenol (NP) and bisphenol A (BPA)) within the intestinal tracts of Atlantic cod and concluded that the field exposure to plastic additives was “negligible”. Koelmans et al.’s study involved modelling, and the toxicant transfer is still an emerging area of research.

2.7. Conclusion

The results of this study indicate that Atlantic cod and capelin from the Newfoundland region are not ingesting substantial quantities of plastics. While these results may indicate there is no environmental health concern, there has not been enough research to fully understand the health risks for humans ingesting fish, even with lower levels of ingested plastic. The transfer of chemicals from plastic to organisms could potentially be dangerous to humans who consume seafood. Therefore, regardless of plastic ingestion rates, caution should be observed when consuming marine organisms. This study was the first in-depth examination of microplastic ingestion by offshore cod and inshore capelin and serves as a baseline for monitoring the impacts of microplastic pollution on human food fisheries in this area.

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Chapter 3

Can citizen science methods be used to locate and identify microplastics ingested by Atlantic Cod *(Gadus morhua*)? A single-blind comparison across four methods.

Abstract

Marine plastics have become a global matter of concern given their potential impact on the marine environment. Assessing the impact of microplastics is difficult due to their widespread and patchy distribution in the ocean. One solution to this issue is through the use of citizen science methods and data. While citizen science data provides a possible solution to the problem of monitoring the spread of marine microplastics, we have little knowledge on the accuracy of citizen science methods, particularly as they relate to plastic ingested by marine organisms. To address this issue, I compared the success rates and identification rates for several methods to identify ingested plastic ranging from an expensive chemical laboratory method to a cost-effective citizen science kit. The four methods were: Method 1: 10% Potassium hydroxide (KOH) and visual analysis using a dissecting microscope with laboratory; Method 2: visual analysis with dissecting microscope with laboratory; Method 3: citizen science dissecting kit in the laboratory; and Method 4: citizen science dissecting kit in the field. KOH method as the most successful locating and identifying plastics. Significantly, methods involving lower levels of technology (e.g. Method 4), resulted in a relatively high success rate of locating and identifying plastics. Also, the results for citizen scientists using Method 4 were comparable to scientific Method 2. These results suggest that citizen science methods can be successfully used to monitor microplastics in fish.

3.2. Introduction

Marine plastics in the ocean have become a global issue, with a critical concern on the problem of microplastics ingested by fish and other marine species destined for human consumption (Rochman et al., 2015; Miranda and Carvalho-Souza, 2016; Schuyler et al., 2016). Microplastics are defined as particles <5-1 mm. They are distributed throughout trophic levels of the marine food web and are accessible for ingestion by a wide range of organisms (Farrell and Nelson, 2013; Setälä et al., 2014). There is growing evidence that microplastics can move toxic chemicals into food webs through ingestion, raising concerns about associated health risks for humans who eat fish (Mato et al., 2001; Rochman et al., 2013; Koelmans et al., 2016; Sussarellu et al., 2016). The potential impact of plastics on human health points to the need for ongoing and large-scale surveys of plastics in the marine environment. However, marine plastics are distributed widely and patchily, which makes it very difficult and costly to effectively survey the marine environment for this potentially serious pollutant.

Globally, there has been growing interest in the potential of citizen or community science to assist in monitoring pollution (Conrad and Hickey, 2011). Traditionally these programs have involved participants, who may not have science degrees, in the collection, processing, and analysis of pollution distribution collected from species and their habitats (Evans et al., 2005, Booney et al., 2014). In some cases, citizen science is the basis for community-based monitoring (CBM) that expands the role of citizens from “data collectors” to “citizens as scientists” through a process where concerned community

stakeholders collaborate to monitor, track and, perhaps most importantly, respond to issues of common environmental concern (Whitelaw et al., 2003, Lakshminarayanan, 2007). A CBM programme can help a community to determine when or whether a system has departed from the desired state, and it can measure the success of enacted management actions (Legg and Nagy, 2006). Scientists have begun to realize the value of citizen science partly due to the cost-effectiveness of data collection and as a way of accessing data over large spatial and temporal scales (Brossard et al., 2005; Holck, 2008; Levrel et al., 2010; Belt and Krausman, 2012).

Citizen scientists can play a key role in monitoring marine plastics (Zettler et al., 2017). Despite the potential, some scientists remain concerned about the accuracy of citizen science data (Crall et al., 2011; Law et al., 2017). However, Zettler et al. (2017) reviewed several citizen science marine plastic programs that have successfully collected data that were subsequently used in peer-reviewed scientific research. These programs included clearing marine debris from coastal waters, data collection via mobile applications (uploading pictures of plastic), and sampling for laboratory-based analyses. In some cases, citizen scientists collected and directly analysed samples to produce data. Zettler et al.'s (2017) analysis of citizen science methods for marine plastic research indicates that citizen science methods may be used to accurately identify and locate ingested plastic in marine food sources. In this paper, I build on Zettler et al.'s (2017) work to determine the accuracy of citizen science methods for locating and identifying plastic ingested by fish destined for human consumption.

This research is significant to the local context, the island of Newfoundland. Fish remains a vital component of the local diet: a survey from the west coast of the island reported that the weekly diet consisted of up to 82% seafood, with Atlantic cod being the preferred choice (Lowitt, 2013). Local consumption of Atlantic cod also occurs through the recreational food fishery, with the Department of Fisheries and Ocean (2012) reporting a very high level of participation by residents. The importance of cod to the local population raises the importance of understanding the ingestion rates of marine plastics by Atlantic cod.

The purpose of this study was to compare the success and identification rates to locate and identify marine plastics in the gastrointestinal (GI) tracts of Atlantic cod (*Gadus morhua*) for several methods ranging from an expensive chemical laboratory method to a cost-effective citizen science kit. A key goal of the research was to determine whether monitoring marine plastics without the means of a laboratory, microscopes, or professional scientific tools compared favourably to more formal laboratory methods and techniques. My findings indicated methods involving lower levels of technology (e.g. citizen science method), resulted in a relatively high success rate. The results for the citizen science method were also comparable to scientific methods conducted in a laboratory. These results suggest that citizen science methods can be successfully organised to monitor microplastics in fish.

3.3. Methods

3.3.1. Microplastic Methods and Protocol

The research presented involved a single-blind study across four methods for identifying marine plastics in the GI tracts of Atlantic cod. Participants were not aware of whether GI tracts contained plastic or not. The four methods and the protocols were as follows:

Method 1: 10% Potassium hydroxide (KOH) and visual analysis using a dissecting microscope in a laboratory.

The controlled chemical laboratory was located at the Marine Institute (MI) and was equipped with a fume hood, a deionised (DI) water system, incubator, dissecting microscope, mesh stainless steel sieves, sinks, and scientific tools such tweezers, wash bottles and Petri dishes. The glass jars were rinsed three times with Ultra-purified water (milliQ) to remove any fibres or other contaminants before placing samples into KOH. Following the protocol in Rochman et al. (2015) (Appendix C), specimens were submerged in 10% Potassium hydroxide (KOH) solution in an incubator at 45-50 degrees C for one to two weeks. The 10% KOH solution dissolves organic material leaving only plastics and undissolved lipids. Participants poured the dissolved samples into a 1 mm (#18) stainless steel mesh sieve, and the jar was thoroughly rinsed into the sieve to remove all the contents. Any material identified as a possible plastic was extracted with tweezers, placed in a Petri dish, and examined under a dissecting microscope with both reflected oblique and transmitted light (Olympus SZ61, model SZ2-ILST, with a magnification range of 0.5–12×) or using an unaided eye. Classification of the samples was either, plastic, non-plastic or undetermined. Participants wore lab coats and had their hair pulled back to eliminate contamination.

Method 2: visual analysis with dissecting microscope in a laboratory

Method 2 used visual analysis with a dissecting microscope in the Civic Laboratory Environmental Action Research (CLEAR), a laboratory located at Memorial University, equipped with dissecting microscope, sink, stainless steel mesh sieves, and scientific tools such as tweezers, wash bottles and Petri dishes. Following the protocol in Liboiron (2016), specimens were visually analysed using a dissecting microscope in the laboratory. The participants emptied each sample bag using a double sieve method, which involves stacking a 4.75 mm (#4) stainless steel mesh sieve above a 1 mm (#18) stainless steel mesh sieve. To remove all contents from the bag it was thoroughly rinsed, and the excess water poured back into the sieve. The dissecting process began by detaching the stomach from the intestines using scissors and setting the intestines aside. The stomach was cut open from the one end to the other removing mucus and remaining food with a gentle cleanse using a wash bottle. If mucus was present in the stomach, it was gently separated from stomach contents using a wash bottle or fingers. The participant used the same protocol to dissect the intestines. Any material identified as a possible plastic was extracted with tweezers into a Petri dish and examined under a dissecting microscope with both reflected oblique and transmitted light (Olympus SZ61, model SZ2-ILST, with a magnification range of 0.5–12×) or using an unaided eye and classified as plastic, non-plastic or undetermined. Participants wore lab coats and had their hair pulled back to eliminate contamination.

Method 3: citizen science dissecting kit in a laboratory

Method 3 used a citizen science dissecting kit in a laboratory in the CLEAR laboratory. The citizen science kit included: instructions (Appendix A), a spotter's guide for plastics (Figure 3.1) and non-plastics (Figure 3.2), a 9x13 inch cake pan, a pair of scissors, stainless steel mesh strainer (7 7/8 cm), gloves, tweezers, a sport water bottle, a glass dish (7.6 cm diameter), a handheld magnifying (5x) glass and a dissecting diagram (Appendix B). Using the dissecting diagram was not necessary during this method as the GI tracts had already been removed from the fish. The costs of this equipment, which is an important consideration in citizen science methods, was less than CAD$20 at a local dollar store. Participants used the CLEAR laboratory and the instructional document that gave step-by-step instructions on how to dissect the GI tract, and how to look for and identify microplastics in the provided samples. Once familiarised with the materials and the protocol, participants set up the dissecting station by placing the cake pan in the sink and the stainless-steel mesh strainer inside the cake pan. All the tools were set aside and were within an easy reach. The participants emptied each sample bag into the mesh strainer, and the bag was rinsed thoroughly so that all the contents were deposited into the mesh strainer or cake pan. The dissecting process began by detaching the stomach from the intestines using scissors and setting the intestines aside. The stomach was cut open from one end to the other, and mucus and remaining food was removed with a gentle cleanse using a wash bottle. Participants used the same protocol to dissect the intestines. Any material identified as a possible plastic was extracted with tweezers into a glass dish. The spotter's guide and handheld magnifying glass to distinguish the sample as plastics, non-plastics or undetermined. Participants wore lab coats and had their hair pulled back to eliminate contamination.

Method 4: citizen science dissecting kit in the field

Method 4 used the same citizen science dissecting kit as Method 3. Rather than testing for plastics in a laboratory setting, the participants did the testing in the field. Participants tested for plastics twice at the facilities of a non-profit organization with emphases on traditional fishing knowledge and skills of their ancestors called *Fishing for Success*, and twice at a private home. Participants read over the instructional document and familiarized themselves with the dissecting process. Participants were also provided with a dissecting demonstration. Participants set up the dissecting station by placing the cake pan in the sink or on top of a counter and the mesh strainer inside the cake pan. The remaining steps followed the protocol described above for Method 3. At no time were participants aided in locating microplastics or identifying the material. Because this was an uncontrolled environment, participants were not asked to wear lab coats or have their hair pulled back.

Figure 3.1. Plastic Spotter's Guide is showing the identification guide for marine plastics commonly found in fish.

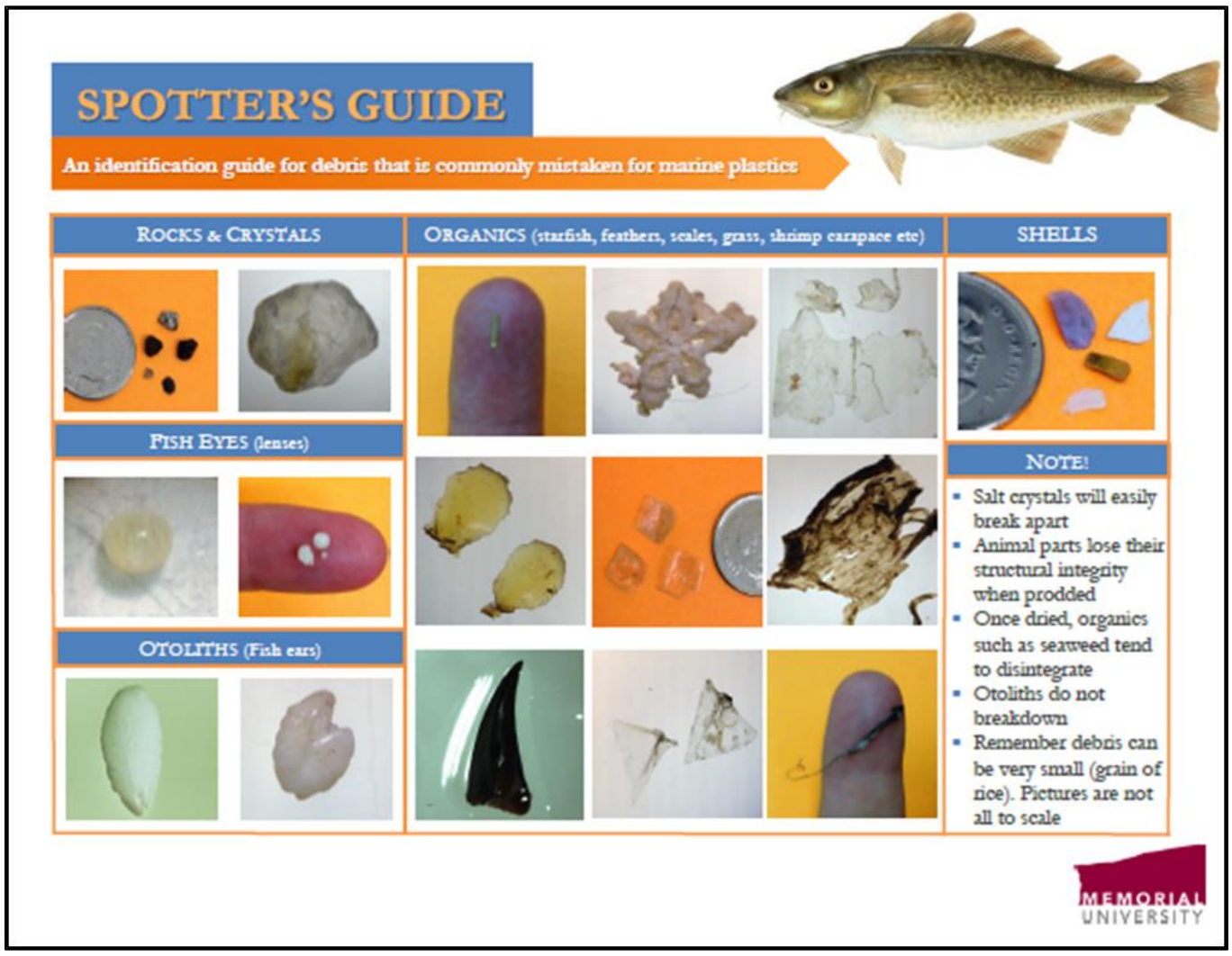

Figure 3.2. Non-plastic Spotter's Guide is showing the identification guide for debris most commonly mistaken for marine plastics.

3.3.2. Atlantic cod samples

A total of 276 Atlantic cod GI tracts were used for this study. Of this total, 100 samples came from DFO's 2016 offshore Ecosystem Research Program survey, and 176 samples came from the 2016 Newfoundland recreational food fishery. Zero samples were inspected for plastic ingestion prior to the trials. Samples from DFO were juvenile Atlantic cod with GI tracts averaging 3.75g and were delivered whole and frozen. The recreational fishery samples were collected from fish harvesters at wharves at Petty Harbour, Newfoundland and averaged 122.37g. The GI tracts from the recreational food fishery were immediately removed, placed in sample bags and frozen for later use. DFO samples were primarily used for the 10% KOH method as their smaller mass expedited the dissolving process. The remaining DFO samples and the recreational fishery samples were randomly distributed throughout the other methods. Each method was used to analyse 69 samples. Three trials were conducted for each Method with 23 samples for each trial (Figure 3.3).

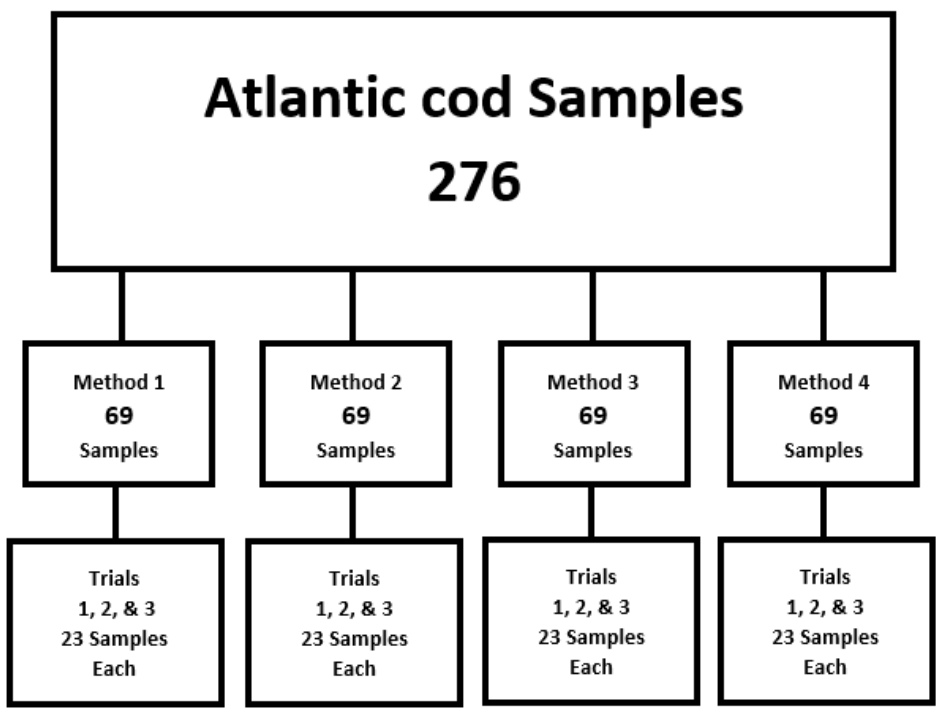

Figure 3.3. The figure displays how the total number of fish (*n*=176) were distributed across the four methods and trials.

3.3.3. Microplastic samples

A total of seventeen marine plastics were implanted in the 23 samples for each experiment (Table 3.1). Multiple types, colour, and sizes of marine plastics were implanted to assess the impact of different plastic types on identification rates. Marine plastics selected for this study, apart from the industrial pellets and the clear film, were collected from birds from a plastic ingestion study in Newfoundland, Canada (Avery-Gomm et al., 2016) and shoreline plastics from Bermuda. Plastics sizes chosen for this study ranged from 1.5 - 6.0 mm, which is in the range for the cut-off point that can detect microplastics with the naked eye and can be reliably detected using a microscope (Song et al., 2015). The types of marine plastics chosen were industrial pellets, fragments, threads, and films (Table 3.1). The colours selected were as follows: beige, blue, black, clear, green, light blue, opaque, white, white and blue, and yellow. Sizes ranged from 1.5 to 6.0 mm in length, 0.1 mm to 4 mm

in height and 0.5 to 4 mm in width. Table 3.1 provides a summary of the types of marine plastics, their characteristics, the fish sample, and where they were implanted in the GI tract.

Table 3.1. Implanted marine plastics in GI tracts of Atlantic cod *(Gadus morhua)* based on a random number generator. Length x Height x Width in mm [LXHXW] defined size.

Number	Type	Colour	Size	Fish Sample Identification #	Location
1.	Pellet	Yellow	3x3x3	3	Stomach
2.	Fragment	Opaque	2x1x1	3	Intestine
3.	Fragment	Beige	3x1x2	9	Stomach
4.	Pellet	Opaque	4x4x4	9	Stomach
5.	Fragment	White	2x1x2	10	Stomach
6.	Fragment	White and Blue	3x1x1	10	Intestine
7.	Thread	Yellow	4x2x1	11	Intestine
8.	Thread	Light Blue	3x0.5x0.5	11	Stomach
9.	Fragment	Opaque	6x1x3.5	16	Stomach
10.	Fragment	Green	3.5x1x2	16	Intestine
11.	Pellet	Black	4x4x3	17	Stomach
12.	Fragment	White	1.5x1x1	19	Intestine
13.	Fragment	Blue	5x1x4	23	Intestine
14.	Film	Clear	4x0.1x2	23	Stomach
15.	Film	Black	4x0.2x2	24	Stomach
16.	Thread	Light Blue	6x0.5x0.5	24	Intestine
17.	Fragment	Opaque	4x1x4	25	Intestine

3.3.4. Implanting microplastics

For each method, 69 fish were selected. Prepared GI tracts samples were weighed and placed in numbered sample bags. The sample bags were divided into three trials, with 23 samples for each trial. A random number generator was used to determine which of the 23 GI tract samples would have implanted plastics. The use of the random number generator meant that several GI tract samples had multiple marine plastics implanted. The random number generator was also used to determine whether the microplastics were implanted in either the stomach or the intestines. Marine plastic samples were inserted into the GI tract through the opening of the stomach by detaching the oesophagus from the stomach during filleting or by making a small incision in the intestines. In Methods 2, 3 and 4, samples were refrozen once prepared with implanted marine plastics and thawed before the experiment began. Freezing the samples allowed the GI tracts to stay intact, as they tend to break down at room temperature.

3.4.5. Method commonalities

Detailed notes were taken throughout the process to ensure that each method was carried out using the correct protocol and to identify processing errors, unforeseen environmental factors, and to record comments made by the participants. Participants were asked to identify microplastics in the GI tracts of fish using the tools at hand for each method. The participants were timed in seconds (s) using a stopwatch from the beginning to end of the dissection process to determine the total duration of each GI tract dissection. Participants were instructed to advise the note-taker when they discovered

microplastic fragments. The material extracted from the GI tract was classified as plastic or non-plastic. The process to classify plastics used either a dissecting microscope with both reflected oblique and transmitted light (Olympus SZ61, model SZ2-ILST, with a magnification range of 0.5–12×), a handheld magnifying glass (5x), or an unaided eye (depending on method). The plastics were air-dried in a Petri dish for 24 hours and then re-evaluated to confirm correct classification. Participants were asked to classify the type of plastics found as either fragment, film, pellet, or thread (by previous training or using the spotter's guide), and they were asked to identify the colour of the found plastic. The unidentified material that participants could not identify was categorised it as 'undetermined'. Undetermined material was placed in a paper filter, air dried it, re-examined it under a dissecting microscope and reassessed. Any implanted plastic not found by participants was recovered, cleaned, air dried and stored before the next phase of the experiment. Participants were also asked to estimate the size and describe the content in both the stomach and intestines for each sample. Assessment of stomach and intestine content was as follows: (1) empty, (2) a little content, (3) half full, (4) three-quarters full and (5) totally full (stomach is completely stretched out). After each dissection, participants examined their hands and tools for any plastics that may have adhered. In Methods 2-4 the presence of mucus was noted as either 'yes' or 'no'.

Trials for Methods 1 and 4 did not exceed two hours, and in the case of Method 2 and 3 that went beyond two hours, the trials were conducted over a two-day period to avoid fatigue. All participants, in all methods, were given breaks as needed. For Methods 1-3, the following precautions were made to ensure there was no cross-contamination: all tools were rinsed or wiped down with water and paper cloths, including the microscope lens and plate, Petri dishes, and sieves. Method 4 did not follow these precautions.

3.3.6. Participants

Participants (*n*=21) were not randomized across the four methods (Table 3.2.). There are regulatory and logistical reasons why it was not possible to randomly select participants for this experiment. First, in accordance with Memorial University's protocol, technicians working in a chemical laboratory require special training. Participants (*n*=3) selected for Method 1 had completed the Workplace Hazardous Materials Information System through Memorial University. Participants (*n*=6) selected for Methods 2 and 3, had experience working in a biological laboratory (working with microscopes and other laboratory equipment), and previous experience identifying microplastics. Participants selected for Method 3 were not familiar with using the citizen science kits. Participants (*n*=12) for Method 4 had no experience working with marine plastics. While the selection of participants was not randomized, this approach accurately reflects how the four methods would be used in real life scenarios. Participants using KOH will always be trained technicians, while those using citizen science kits in the field will likely be citizen scientists with limited experience of marine plastic pollution identification. In this sense, the methods and the practices are closely linked and reflect how they would be deployed in practice. This study was cleared by the Interdisciplinary Committee on Ethics in Human Research (ICEHR) at Memorial University of Newfoundland for research involving human subjects. All participants signed an informed consent form (Appendix D).

Table 3.2. The table is displaying the different methods used, description of the participant's experience, mode of dissection and plastic identification.

Method #	Method Name	Type of Experience	Mode of Dissection	Mode of Plastic Identification
1	10% KOH and visual analysis using a dissecting microscope with full laboratory	Microplastic and Biochemical Laboratory Experience	Implemented in a biochemical laboratory using 10% KOH and scientific tools	Dissecting microscope with both reflected oblique and transmitted light (Olympus SZ61, model SZ2-ILST, with a magnification range of 0.5–12×) or unaided eye
2	Visual analysis with dissecting microscope with full laboratory	Microplastic and Laboratory Experience	Implemented in a biological laboratory using Scientific Tools	Dissecting microscope with both reflected oblique and transmitted light (Olympus SZ61, model SZ2-ILST, with a magnification range of 0.5–12×) or unaided eye
3	Citizen science dissecting kits in laboratory	Microplastic and Laboratory Experience	Implemented in a biological laboratory using tools from a citizen science kit	Unaided eye or handheld magnifying (5x) glass and spotter's guide
4	Citizen science dissecting kits in the field	No Microplastic Experience	Implemented in a private/public facility using tools from a citizen science kit	Unaided eye or handheld magnifying (5x) glass and spotter's guide

3.3.7. Statistical analyses

To draw conclusions from the data, inferential statistical analyses were performed, as well as qualitative observations.

Method Comparison: A Fisher's exact test (Fisher, 1935) was used to assess the success rates for locating planted plastics for each method and to identify the global differences across all Methods. Testing for pairwise differences in the ability of each method to successfully find planted plastic I used two-proportion z-tests. P values (p=0.05) from z-tests were adjusted using the Holm adjustment to account for familywise error (Holm, 1979).

If the participant was able to successfully find the planted plastic using their assigned method, they were then asked to identify the plastic. The ability of participants using each method to successfully identify the implanted plastic was assessed in the same manner as described above using only the data from participants who did successfully find the plastic.

Finally, to investigate the relationship between the total processing time and the Method used, I performed an ANOVA to test for global differences. Pairwise differences between methods were tested using pairwise t-tests using the Holm adjustment to correct for familywise error.

Regression model: Logistic regression (Hosmer and Lemeshow, 1989; Trexler and Travis, 1993) was used to determine if sampling method, plastic height, length, width, or colour, and the presence of mucus had a significant influence on detecting implanted microplastics in the GI tract of fish. Sampling Method 1 (i.e. KOH) was excluded from the logistic regression analysis as fish stomachs were not examined before the process. The dichotomous dependent variable was, YES plastic was found (1), or NO plastic was not found (0). Before the analysis began, a multicollinearity test between the plastic measurements (length, width, and height) was conducted with a correlation analysis (Pearson correlation coefficient (r) = ±

0.70). A full model tested all the independent variables. Backward stepwise regression and Akaike's Information Criterion (AIC) to determine which variables in the full model should be kept in the reduced, final model. A likelihood ratio test and McFadden's R-squared index were used to assess the model fit of the final selected model (McFadden, 1974). The significance of all variables was evaluated at $\alpha = 0.05$.

3.3.8. Qualitative Observations

In order to provide qualitative reflection on the participants and the different methods, descriptive notes were taken throughout each trial to provide additional insights into the central research question: 'Can citizen science methods be used to locate and identify marine plastics in the GI tracts of Atlantic cod?' Participant comments on issues associated with locating or identifying the implanted plastics were recorded to provide additional data on the research question. I noted the differences in the protocol approach from each participant. For example, participants in Method 1 – 3 had been previously trained to dissect and look for microplastics in GI tracts. These techniques included how the intestines were cut during the dissection process and the use of a faucet. Observing the participants for the duration of each method allowed for improvements and suggestions to better develop not only the citizen science kits but also protocols for all methods. Detailed observations on the types of non-plastic items extracted from the GI tracts. These detailed notes will assist in improving the spotter's guide and will benefit changes for future instructional demonstrations.

3.4. Results

3.4.1. Method comparison

Locating plastic: There were significant differences between Method 1 and all other methods ability to successfully find plastics ($p = 0.00017$, Fisher's Exact Test, Figure 3.3, Table 3.3). Method 1 found plastics 94.0 ± 3.4% of the time which was significantly higher than Methods 2, 3, and 4 (Table 3.4). There were no significant differences found for success rates for finding plastic between Methods 2, 3, and 4 (66.7 ± 6.6%, 58.8 ± 6.9% and 70.6 ± 6.4%) respectively (Table 3.4).

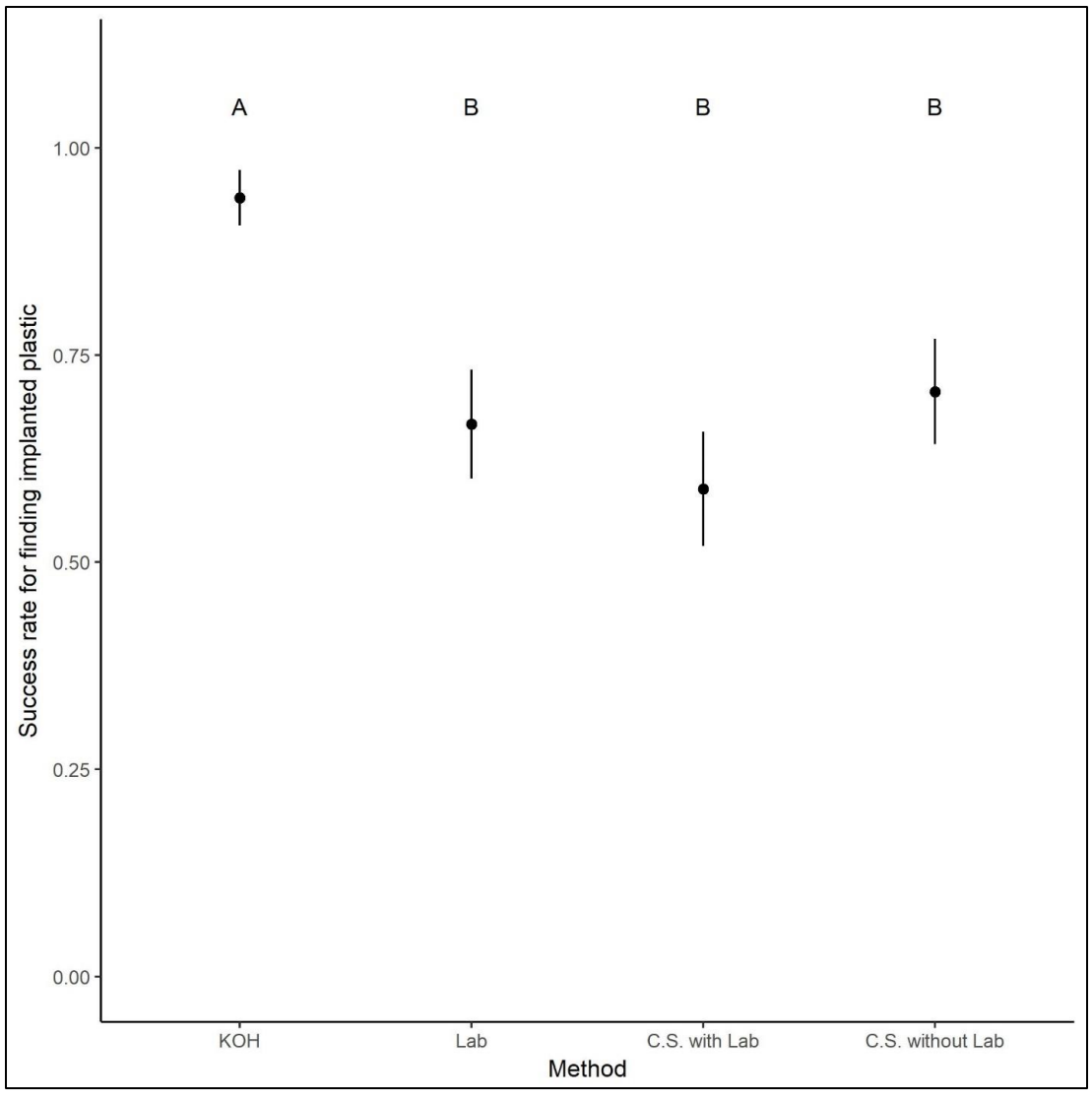

Figure 3.4. Success rates for finding implanted plastic for each method. Points represent the mean success rate with bars showing the standard deviation and significant differences in the success rate of each method is indicated by letters.

Table 3.3. Mean and the standard deviation for the success rate at finding implanted plastic for each method, Potassium hydroxide (KOH), laboratory, citizen science with laboratory and citizen science without a laboratory.

Method	***Mean***	***Std. Deviation***
KOH	0.94	0.033
LAB	0.666	0.066
C.S. with lab	0.588	0.068
C.S. without lab	0.706	0.064

Table 3.4. Pairwise two-proportion z-tests comparisons of the difference in success rate at finding implanted plastic for each method, Potassium hydroxide (KOH), laboratory, citizen science with laboratory and citizen science without a laboratory.

Comparison	*Difference*	*z value*	*p value*
KOH – LAB	0.273	3.446	0.002**
KOH – C.S. with lab	0.351	4.152	0.0001***
KOH – C.S. without lab	0.234	3.073	0.008**
LAB – C.S. with lab	0.078	0.819	0.825
LAB – C.S without lab	-0.039	-0.426	0.825
C.S. with lab – C.S. without lab	-0.117	-1.243	0.641

* Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Identifying plastic: Method 1 was significantly different than all other Methods, except for Method 3 (p = 0.00212, Fisher's Exact Test, Figure 3.5, Table 3.5). When plastic was found, participants using Method 1 (100.0 ± 0.0%) identification rates were significantly higher than participants using Methods 2 and 4 (76.4 ± 7.3%, 86.1 ± 5.8%) respectively. No significant differences were found among Method 2, 3, and 4. However, Method 3 was able to identify plastic 90.0 ± 5.4% of the time, and was comparable to Method 1 (z=2.21, p=0.108) (Table 3.6).

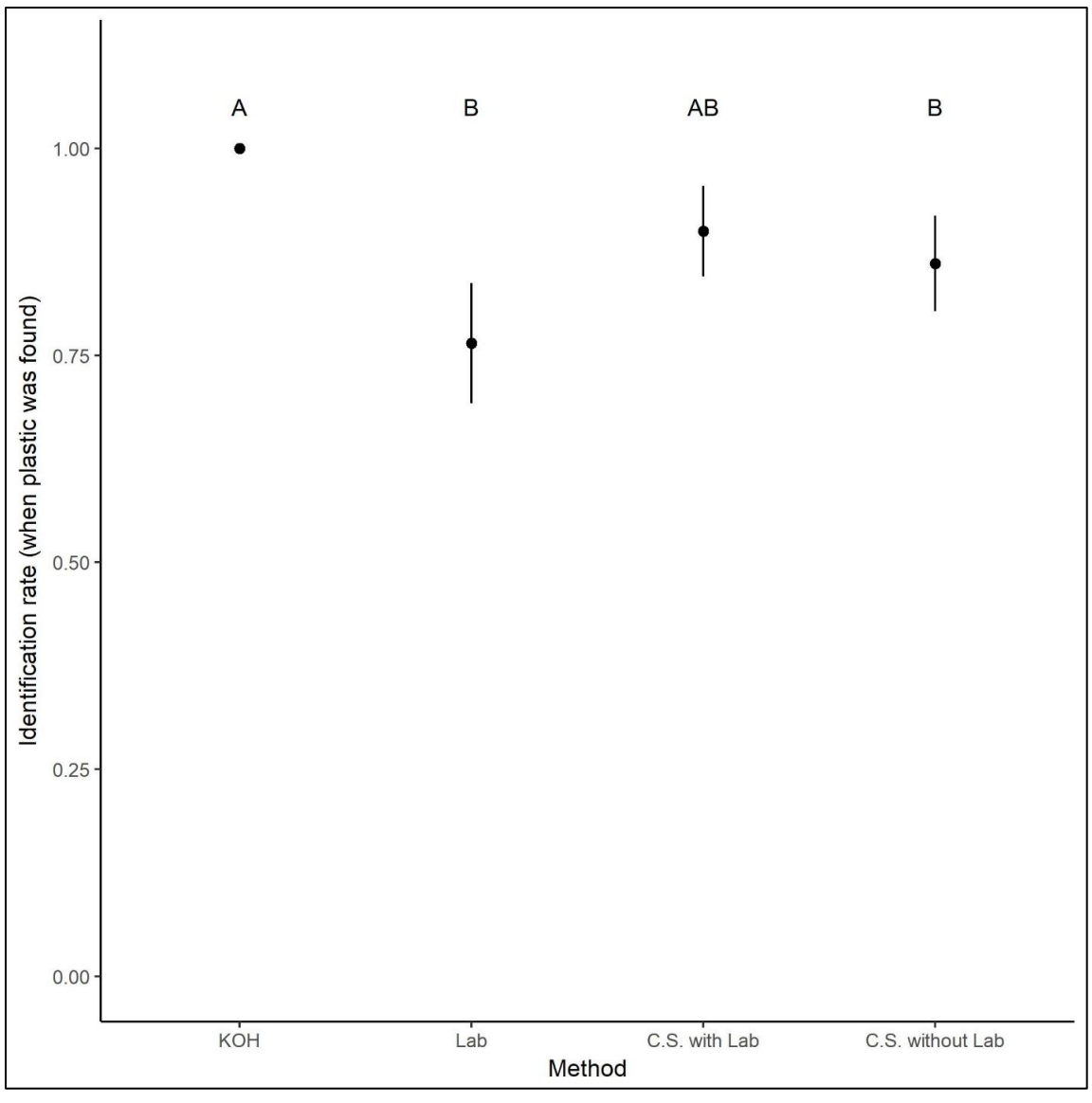

Figure 3.5. Observed identification rate of planted plastic when the plastic was successfully found. Points show the mean identification rate with bars showing standard deviation. Significant differences in the success rate of each method are indicated by letters. for each method; Potassium hydroxide (KOH), laboratory, citizen science with laboratory and citizen science without a laboratory. Letters indicate significance.

Table 1.5. Mean and the standard deviation for the identification rate for found implanted plastic for each method, Potassium hydroxide (KOH), laboratory, citizen science with laboratory and citizen science without a laboratory.

Method	***Mean***	***Std. Deviation***
KOH	1	0
LAB	0.764	0.072
C.S. with lab	0.9	0.054
C.S. without lab	0.861	0.057

Table 3.6. Pairwise two-proportion z-tests comparisons of the difference in identification rate for implanted plastic for each method, Potassium hydroxide (KOH), laboratory, citizen science with laboratory and citizen science without a laboratory.

Comparison	***Difference***	***z value***	***p value***
KOH – LAB	0.235	3.502	0.002**
***KOH* – C.S. with lab**	0.1	2.211	0.108
KOH – C.S. without lab	0.138	2.635	0.041*
***LAB* – C.S. with lab**	-0.135	-1.431	0.456
LAB – C.S. without lab	-0.096	-1.036	0.599
C.S. with lab – *C.S. without lab*	0.038	0.482	0.629

* Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Timing: There were significant differences between Method 1 and all other Methods for the total processing time (minutes) ($F(3,271)=41.53$, $p < 0.00001$, Figure 3.6, Table 3.7). Method 1 was significantly faster (3.02 ± 1.5 m) than Method 2, 3, and 4 (9.77 ± 3.4 m, 9.73 ± 4.7 m and 10.59 ± 6.7 m) respectively. No significant differences were found between Methods 2, 3 and 4 (Table 3.8).

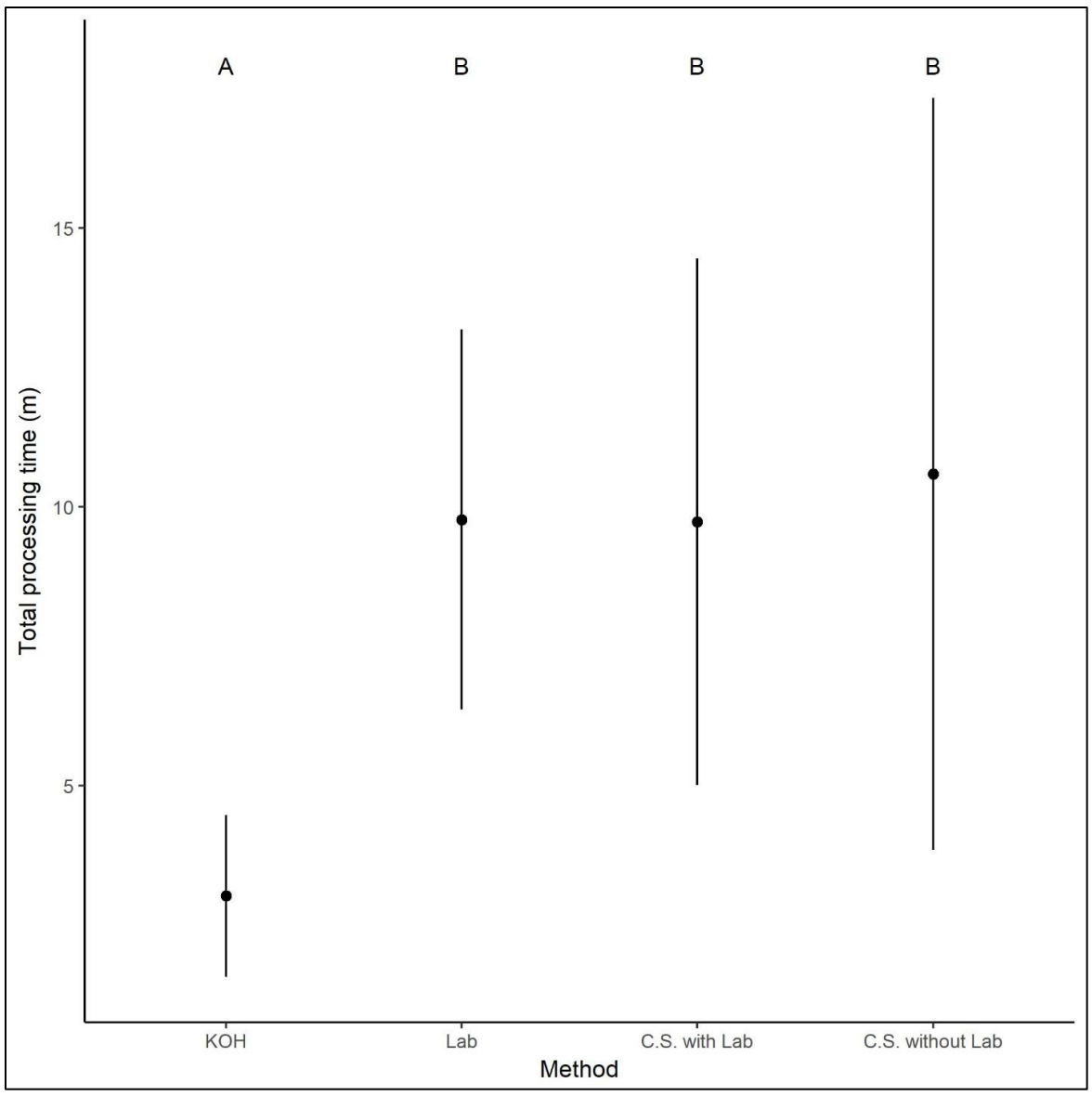

Figure 3.6. Total processing time for each Method in minutes (m). Points indicate the mean time spent for each method and bars show the standard deviation. Significant differences in the time spent on each method is indicated by letters. Potassium hydroxide (KOH), laboratory, citizen science with laboratory and citizen science without a laboratory. Letters indicate significance.

Table 3.7. Mean and the standard deviation for the total processing time in minutes for each method, Potassium hydroxide (KOH), laboratory, citizen science with laboratory and citizen science without a laboratory.

Method	***Mean***	***Std. Deviation***
KOH	3.018	1.451
LAB	9.769	3.409
C.S. with lab	9.728	4.722
C.S. without lab	10.590	6.746

Table 3.8. Pairwise comparisons for differences for total processing time and pairwise t-tests for each method, Potassium hydroxide (KOH), laboratory, citizen science with laboratory and citizen science without a laboratory.

Comparison	*Difference*	*T value*	*P value*
KOH – LAB	-6.750	-8.735	<0.00001 ***
KOH – C.S. with lab	-6.709	-8.682	<0.00001 ***
KOH – C.S. without lab	-7.571	-9.797	<0.00001 ***
LAB – C.S. with lab	0.0408	0.053	0.957
LAB – C.S. without lab	-0.821	-1.066	0.791
C.S. with lab – C.S. without lab	-0.862	-1.119	0.791

* Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.4.2. Factors influencing plastic detection using a regression model

Correlation analysis was used to determine if independent variables were autocorrelated. The analysis found no variables were correlated at >0.7 (Hazra and Gogtay, 2016); therefore, all plastic measurements were included in the full model. The independent variables in the full model included mucus presence, colour, length (mm), width (mm), height (mm) and sampling methods (Methods 2, 3 and 4). Using backward stepwise regression and AIC criterion to find the most parsimonious model, I retained mucus presence, colour and height (mm) (Table 3.8.). The presence of mucus significantly reduced the probability of finding implanted plastics (P=<0.001). A unit increase in plastic height increased the probability of the plastic being found; however, this variable was less significant than mucus presence and colour (P=0.071) (Tables 3.8 and 3.9). Beige served as the reference variable for the colour analysis. There was no significant difference between beige and any other colour in the probability of finding the plastic, except for light blue. There was a significant decrease in the probability of finding the plastic if the plastic was light blue, (P=0.014). Additionally, there were large standard errors for the blue, green, and clear. This was likely due to the fact that for these colour levels, all of the observations within each level were either found or not found. Mucus presence and colour significantly reduced the model deviance, as did height, but to a much lesser extent (Table 3.9).

Table 3.9. Mucus presence, colour and height (mm) in the reduced model after conducting backward stepwise regression and AIC criterion to find the most parsimonious model.

Variables	*Estimate*	*Std. Error*	*z Value*	*p Value*
Intercept	1.561	1.185	1.317	0.187
Mucus Presence	-3.919	0.769	-5.096	3.47e-07 ***
Colour Black	1.090	1.401	0.778	0.436
Colour Blue	19.329	2990.050	0.006	0.994
Colour Clear	-20.066	3002.369	-0.007	0.994
Colour Green	19.698	2977.255	0.007	0.994
Colour Light Blue	-3.791	1.551	-2.444	0.014 *
Colour Opaque	1.253	1.2184	1.029	0.303
Colour White	0.151	1.302	0.116	0.907
Colour White and Blue	0.816	1.734	0.471	0.637
Colour Yellow	-0.540	1.422	-0.380	0.703
Height mm	0.794	0.440	1.805	0.071

* Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3.10. Analysis of deviance table showing that mucus presence and colour significantly reduced the model deviance, as did height. Df = Degrees of Freedom, Deviance Resid. = Deviance Residual, Pr(Chi) = Chi-Square Test.

	Df	*Deviance Resid.*	*Df*	*Resid. Dev*	*Pr(>Chi)*
NULL			152	197.429	0.187
Mucus Presence	1	47.729	151	149.700	4.895e-12 ***
Colour	9	72.962	142	76.738	3.987e-12 ***
Height (mm)	1	4.505	141	72.233	0.03379 *

* Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.4.3. Qualitative Observations

Throughout the Method 4 trials, two issues were observed and recorded across the four groups: the non-plastic Spotter guide (Figure 3.2) and the magnifying glass (5x). Several participants stated there was not enough non-plastic material represented in the guide. Three participants suggested adding pictures of worms, and more animal carcasses to eliminate organic material mistaken as plastic. However, the three participants who removed worms and animal carcasses (isopods and

brittle stars), could identify the content through the process of eliminating characteristics of plastic (located on the Spotter's guide) and found the material to be organic. All participants in Method 4 found the magnifying glass (5x) to be inadequate to identify material extracted from GI tracts, plastic or non-plastic.

The light blue thread (3x0.5x0.5 mm and 6x0.5x0.5 mm) was most commonly not found among all the methods. During Method 4, observational notes recorded when the sieve filled with water the 3x0.5x0.5 mm light blue thread detached from mucus and stomach content. Swirling the stomach contents and mucus with a gloved hand separated the thread from the mucus and stomach content. The thread floated to the surface, allowing the participant to locate and identify the plastic quickly.

The implanted plastics not located by participants were recovered by myself, either in the sieve, outside of the sieve, or on the gloved hands of the participants. Observations recorded during Method 2, noted that one participant was using a faucet to cleanse samples. The lost plastics may have been due to the unnecessary splashing during the dissecting process. Also, during Method 4 while recovering a lost plastic, it was found to adhere to a participant's glove with mucus.

3.5. Marine debris in specimens not implanted

Participants found three microplastic fragments not deliberately implanted in three different GI tract samples. These were microplastic samples that fish had ingested while in the marine environment. With a total sample of 276 fish, this is a 1.1% incidence of ingestion. The inshore Atlantic cod sample containing plastic was collected from a recreational fisherman from the wharf at Petty Harbour. The fish was caught within 5 km of the harbour. The plastic appeared to be a white fragment and measured 1x0.1x0.5 mm. The total of 176 Atlantic cod samples from this area indicated a incident rate of plastic 0.56%. The remaining two plastic fragments came from two offshore samples (*n = 100*) provided by DFO, which were caught in the Northwest Atlantic Fisheries Organization (NAFO) 3Ps. Unfortunately, exact locations for these samples were not provided by DFO. The first plastic was a piece of black film that during analysis broke into two pieces. The larger piece of film was 6x2x6 mm, and the smaller piece was 3x1x3 mm. The second plastic from the offshore sample was a white fragment measuring 2x0.1x1 mm. The incident rate for plastics found in offshore Atlantic cod samples is 2%.

3.6. Discussion

Comparison of methods

While Method 1 using trained scientists was the most successful method of locating plastics, this research found that Method 4 involving citizen science methods is an effective way of identifying ingested plastics in marine organisms. The results for citizen scientists using Method 4 were comparable to scientists using Methods 2 or 3. A controlled environment did not influence the outcome of the citizen science approaches, as Method 3 and 4 had comparable results for identifying marine plastics to Method 4. The small difference in success rate between Methods 2 and 3 indicate that using the citizen science kits is an effective alternative for dissecting GI tracts for locating marine plastics not only for citizen scientists but for laboratories without funds for dissolving chemicals or scientific tools. Given the lack of differences in successfully finding plastics between

Methods 2-4, citizen scientists can practice monitoring programs that use visual identification to find plastics in fish with minimal training and a kit. However, citizen scientists were unable to locate all implanted plastics, nor did they accurately identify all plastics in the GI tracts of Atlantic cod. Incorporating a training video into the training protocol for new scientists, lab technicians, or students learning how to locate and identify marine plastics in GI tracts of fish is recommended.

Method 1 was significantly faster than Methods 2-4. The lack of time needed to pick through the undigested stomach contents and mucus contributed to the significant difference in method times. However, the timed results for Method 1 did not include the two weeks it took for the contents to dissolve in KOH, before the sieving process. The timed results for citizen scientists using Method 4 were not distinguishable from trained scientists using Method 3. This study did not test the repeated use of the citizen science kits among scientists and citizen scientists. Therefore, future studies should examine if repeating the dissecting procedure with the same individual decreases the amount time it takes to dissect fish while looking for ingested plastics.

Factors influencing plastic detection

The presence of mucus significantly reduced the probability of finding implanted plastics. Based on this result, it ise recommended that when dissecting a GI tract with mucus, more time should be spent examining the contents, including separating the mucus as much as possible with the use gentle washing, and gloved hands. During Method 4, one participant allowed the sieve to fill with water and used their hand to separate the mucus from the stomach contents. The thread was observed floating to the surface, allowing the participant to locate and identify it quickly. When dissecting fish, it is also recommended to observe hands after sifting through mucus closely.

There was a significant decrease in the probability of finding the plastic if the plastic was light blue, as compared to beige. When participants did not find the light blue thread, I noted the that the thread intertwined in either mucus or stomach contents. Threads were a challenge to locate, with added difficulty in the presence of mucus. It is recommended to investigate ways to safely break down the mucus for scientists or citizen scientists to use, such as bleach diluted with water.

This study found that when the height of the plastic decreased so did the probability of locating the plastics. These findings are in line with previously reported error rates using visual sorting by experienced technicians, (Eriksen et al., 2013; Hidalgo-Ruz et al., 2012) with the error rate increases with the decreasing size of particles. Using a technique such as a float test may eliminate the relevance of plastic size. However, this study did not examine the size of plastics using the float test. Recommendations for future studies are to examine if the size of plastic is affected by the float test.

Participants

The participants suggested several improvements to the dissection process, which could potentially improve the chances of locating and identifying plastics in both citizen science and laboratory methods. For example, a participant requested a glass of water to see if the items they extracted from the GI tracts would float. Participants then used the float test to distinguish plastics from small rocks when they either floated or sunk in the glass of water, respectively. Another suggested improvement to the dissection process was to let the pan fill up with water, filling the bottom of the sieve and allowing plastics

to rise to the surface. Participants would swirl the contents of the sieve attempting to dislodge any attached plastics to either GI tract contents or mucus. This study did not test if all plastics would float using the float test it cannot be determined if this technique would be reliable for all plastic types. Recommendations are for future studies that examine the types of plastics that float using the float test.

Participants did not identify any non-plastic items as plastics. Non-plastic items included prey content found in the GI tract. When in question, prey content was extracted and examined closer using either a microscope or the spotter's guide. In all trials, participants were able to eliminate prey content from plastics.

Improving method protocols

These results can be used to improve monitoring protocols for both citizen science and accredited studies to increase both the ability to locate and accurately identify plastics successfully. The following recommendations for improving protocols are suggested:

Citizen science methods:

1. Adding more non-plastic items commonly found in the GI tracts of Atlantic cod, such as worms and animal carcasses (shrimp, crab pieces and brittle stars) to the Spotter's Guide.
2. Include protocol for citizen scientist to examine hands and workstation after each dissection thoroughly.
3. Use a stronger magnifying glass (i.e. 10x magnification) to improve the visible difference between characteristics of plastic and non-plastic material.

Laboratory science methods:

1. Eliminate or control the use of faucets during dissection and identification.

For both citizen and accredited scientists:

1. When dissecting a GI tract with mucus and prey contents, researchers need to spend more time examining the contents, including separating the mucus as much as possible with the use of water and gloved hands.
2. Use a float test to differentiate between plastic and non-plastic.
3. Include vials with examples of marine plastics and commonly mistaken items associated with plastics in either the lab or the citizen science kits.

Marine debris in specimens not implanted

Finding plastics not implanted is not a surprising result. Participants found three non-implanted plastics out of 276 fish samples collected (176 from Petty Harbour fishing wharf and 100 offshore provided from DFO), with an incident rate of ingested marine plastic at 1.1%. A recent study that examined ingestion rates of plastic from inshore Atlantic cod in Newfoundland, Canada, found a prevalence rate of 2.4% (Liboiron et al., 2016). These findings are within range of the marine plastics found during this study. Therefore a monitoring program for ingested plastic is warranted for persons consuming fish caught for human consumption within the waters of Newfoundland, Canada.

3.7. Conclusion

This study compared the success rates to locate and identify marine plastics in the GI tracts of Atlantic cod across four methods. The purpose was to examine if citizen science methods could locate and identify plastic using lower levels of technology. It was found that citizen science methods can successfully locate and accurately identify implanted plastics without the means of a laboratory, microscopes or professional scientific tools. The ability to locate and identify implanted plastics had no significant difference between laboratory and citizen science methods using visual identification as the primary methodology. Most importantly, methods conducted in the field were statistically comparable to methods conducted in a laboratory.

During this study, three variables reduced the probability of finding implanted plastics, height, colour, and mucus. Results found the smaller the height of the plastic, the more difficult it was to locate. This would indicate why the light-blue thread (height 0.1 mm) was most likely not found across all methods. The presence of mucus had a considerable influence in detecting implanted microplastics in the GI tract of fish. It is recommended future studies investigate techniques that dilute mucus when attempting to locate plastic in the GI tracts of fish.

Marine plastics have become a global matter of concern given their wide distribution and their impact on the marine environment. Assessing the impact of microplastics is, however, difficult due to the widespread and patchy distribution of marine plastics in the ocean. This makes it very difficult to monitor the global distribution of marine plastic pollution in the environment. The study presented here demonstrates one solution to this issue. This study suggests that citizen science methods can be utilized as a resourceful approach to monitoring marine plastics in fish and used as a baseline for future studies.

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Chapter 4

4.1. Introduction

The context of this study is the global problem of plastics in the marine environment. Marine plastics are a problem because they are widespread and patchily distributed throughout the globe (Derraik, 2002; Edyvane et al., 2004; Barnes et al., 2009; Ogata et al., 2009; Obbard, 2014), consequently making it difficult to assess the local and regional impacts of plastic pollution. Marine plastic is also a problem because of the potential impact on consumers of fish (Rochman et al., 2015), due to the transfer of toxic chemicals associated with plastics from marine organisms to humans (Mato et al., 2001; Rochman et al., 2013; Koelmans et al., 2016; Sussarellu et al., 2016).

The aim of this thesis was to contribute to our knowledge of plastics in the marine environment through two discreet studies. The first study examined and reported the frequency of occurrence of plastic ingested in capelin *(Mallotus villosus)* and Atlantic cod *(Gadus morhua)* in the Northwest Atlantic Fisheries Organization Division (NAFO) Division 3. The second study compared the success and identification rates for several methods ranging from an expensive chemical laboratory method to a cost-effective citizen science kit. The remainder of this conclusion is structured into three sections. First, I discuss the results of the ingestion studies, followed by the results of the citizen science methods analysis. Finally, I draw conclusions on the significance of this research and outline future research priorities.

4.2. Ingestion Analysis

I sampled 350 inshore capelin, and 460 offshore Atlantic cod for ingested microplastics in the NAFO Division 3. This study quantified the frequency of occurrence in these two-species using visual analysis and Raman Micro-Spectroscopy. This study found that capelin had a frequency of occurrence of ingested plastic of zero, and Atlantic cod had a frequency of occurrence of 1.1%.

Capelin

This was the first study of plastic ingestion in capelin in Newfoundland and represents the first-time capelin have been examined for plastic in published literature. The research reported the frequency of occurrence of plastic in capelin as none. The absence of plastic in capelin is most likely related to spawning behaviour. Capelin tend not to feed during spawning (Winters, 1970; Vesin et al., 1981; Davoren et al., 2003), thus lowering the risk of ingesting plastic. However, the data presented in this research showed half the capelin samples consumed prey, and/or granules. This indicates spawning behaviour is not the only reason for finding zero plastic in capelin. Future research is necessary to better understand if seasonal variations and feeding behaviours influence plastic ingestion rates in capelin. The contribution to the field of marine plastics would enable comparing spawning behaviour and plastic ingestion for future capelin research and pelagic foraging fish within the North Atlantic region.

Atlantic Cod

The research found the frequency of occurrence was 1.1% in offshore Atlantic cod. These results are consistent with studies that similarly recorded low plastic recovery from species in the North Atlantic (Foekema et al., 2013; Bråte et al., 2016; Liboiron et al., 2016, Liboiron et al., 2018). It was speculated geographical location, species behaviour, and biology contributed to the low plastic ingestion rates. The research also found that the highest occurrence of ingested plastic was found offshore, possibly contributed by vessel pollution. Whereas, samples closest to human populated locations showed little to no ingested plastic. However, a problem that emerged in the sampling methodology may have influenced the findings.

Sampling conditions did not allow an equal amount of fish from each subdivision. The samples collected from each NAFO subdivision were 3Ps (*n*=385), 3N (*n*=24), 3O (*n*=22) and 3L (*n*=28). Guidelines suggest sampling 25–50 individuals of a given species for plastic ingestion (Hanke et al., 2013; Lusher et al., 2013), with larger samples required for species with variable or low ingestion rates (Lavers and Bond, 2016) or for studies aimed at quantifying trends in plastic over time (Hanke et al., 2013). In order to gain a better sense of the role of geographical location in the incidence of marine plastics, future studies should be designed along these lines.

The samples numbers presented in this study from NAFO 3N, 3O, and 3L fall within the suggested sampling guidelines, however, they are on the lower end of the scale. It is recommended future Atlantic cod studies collect an equal number of samples and a greater number of samples in these NAFO subdivisions, particularly for a long-term monitoring program. Although the sampling methodology was not ideal, it was the first study for plastic ingestion in offshore Atlantic cod in NAFO subdivisions 3Ps, 3L, 3N, and 3O and will contribute as a baseline for future studies.

4.3. Citizen Science Methods for Marine Plastic Pollution

A possible solution to monitoring the widespread and patchy distribution of marine plastics is to utilize citizen science methods. In order to understand if citizen science can provide a possible solution, it was necessary to investigate data accuracy from citizen science methods. To address this issue, I compared the success and identification rates for several methods ranging from an expensive chemical laboratory method to a cost-effective citizen science kit. The four methods were: Method 1: 10% Potassium hydroxide (KOH) and visual analysis using a dissecting microscope with laboratory; Method 2: visual analysis with dissecting microscope with laboratory; Method 3: citizen science dissecting kit in the laboratory; and Method 4: citizen science dissecting kit in the field. To draw conclusions from the data, statistical analyses were performed, as well as qualitative observations. The descriptive analyses included a Fisher's exact test and logistic regression, while the qualitative observations were analysed through descriptive notes.

The results found citizen science methods can successfully locate and accurately identify implanted plastics. There was no significant difference in the ability to locate and identify implanted plastics between the lab (Method 2) and citizen science

methods (Method 3 and 4) using visual identification as the primary methodology. These results suggest that citizen scientists could monitor marine plastics in fish.

This study also brought attention to problems associated with detecting plastic in the GI tract with the presence of mucus. While other factors such as colour and height hindered detectability, mucus presence was found to be the most significant reason for not locating plastic. Therefore, it is highly recommended taking extra precautions while examining any fish for plastic in the presence of mucus.

These findings were essential to the study of marine plastic for a several reasons. First, the data presented here are preliminary results that can be used to further investigate the accuracy of citizen science methods monitoring plastic ingested by fish. Second, these findings contribute to the field of citizen science and marine plastics research. Although, 10% KOH was found to be the most successful detecting and identifying plastics, there was no significant difference between Method 2, 3 or, 4. Thus, building on Zettler et al. (2017) work that citizen science can be a reliable solution for marine plastic pollution.

4.4. Thesis Contributions

Both studies contributed to existing research on marine plastic pollution. The frequency of occurrence for ingested plastic in caplin and cod expanded the limited data available on North Atlantic fish. Analysis of citizen science methods to locate and identify marine plastics in Atlantic cod indicated the reliability of citizen science data.

The frequency of occurrence for ingested plastic in offshore Atlantic cod can be used in comparison with other North Atlantic cod studies. The frequency of occurrence for plastic ingestion in North Atlantic cod between offshore and inshore range from 1.2 to 27% (Foekema et al., 2013; Bråte et al., 2016; Rummel et al., 2016; Liboiron et al., 2016). While cod have been examined for plastic ingestion in several North Atlantic locations, including Newfoundland, this was the first time that capelin have been studied. However, it is not the first time zero ingested plastic findings have been published for a North Atlantic species. Liboiron et al. (2018) found silver hake (*Merluccius bilinearis*) also ingested zero plastic. As more plastic ingestion studies are conducted in North Atlantic species, possible ingestion rates trends may begin to emerge allowing for comparisons between studies. The study presented here will contribute to those comparisons.

Zettler et al. (2017) argued that citizen scientists can play a key role in helping us monitor marine plastics. In this paper, I built on Zettler et al.'s (2017) work to determine the accuracy of citizen science methods for locating and identifying plastic ingested by fish destined for human consumption. This work also contributes to the idea that global datasets for plastic ingestion in fish can be utilized using citizen science methods, as there was no significant difference between scientific and citizen science methods, expanding on scientific data regarding marine plastic pollution.

While both studies contributed to existing research for marine plastic pollution, they also represent a possible synergy for research in marine plastics, utilizing both scientific and citizen science methods.

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Appendix A

Citizen Scientists and Marine Plastics Dissecting Materials and Instructions

Materials

Spotter's Guide

Dissecting Diagram

9x13 inch cake pan,

Scissors,

Stainless steel mesh strainer (7 7/8 cm),

Gloves,

Tweezers

A sport water bottle

Glass dish (7.6 cm diameter)

Handheld magnifying (5x) glass

Instructions

1. Dissecting station should be on a clean and even surface. Prepare the station by placing the cake pan on the table or counter. Set aside the strainer, scissors, magnifying glass, water bottle and garbage bag.

2. Place the fish in the cake pan. Following the dissecting diagram, use the scissors to remove the entire GI tract (outlined in red) from the mouth to the anus. Gently place the guts in the strainer. Try to get it all in one piece with no nicks!

3. Using scissors, carefully cut the stomach from the intestines. Move intestines to one side of colander; you will dissect them after the stomach.

4. Cut the stomach from one end to the other allowing the contents to gently fall onto strainer. Using the water bottle carefully and slowly pour water over the contents to separate and remove all debris from the stomach. Use your fingers to separate the stomach contents and mucus.

5. Using the magnifying glass carefully inspect the empty stomach lining for any remaining contents.

6. Using the Spotters Guide, look through the stomach contents closely. Remember microplastics are small—the size of a grain of rice or smaller! It can take up to half an hour to look at one fish if it had a full stomach.

7. You can remove items you know are not plastic and place them in a garbage bag. Once you have completed looking through all the stomach contents you will be ready to cut open the intestines.

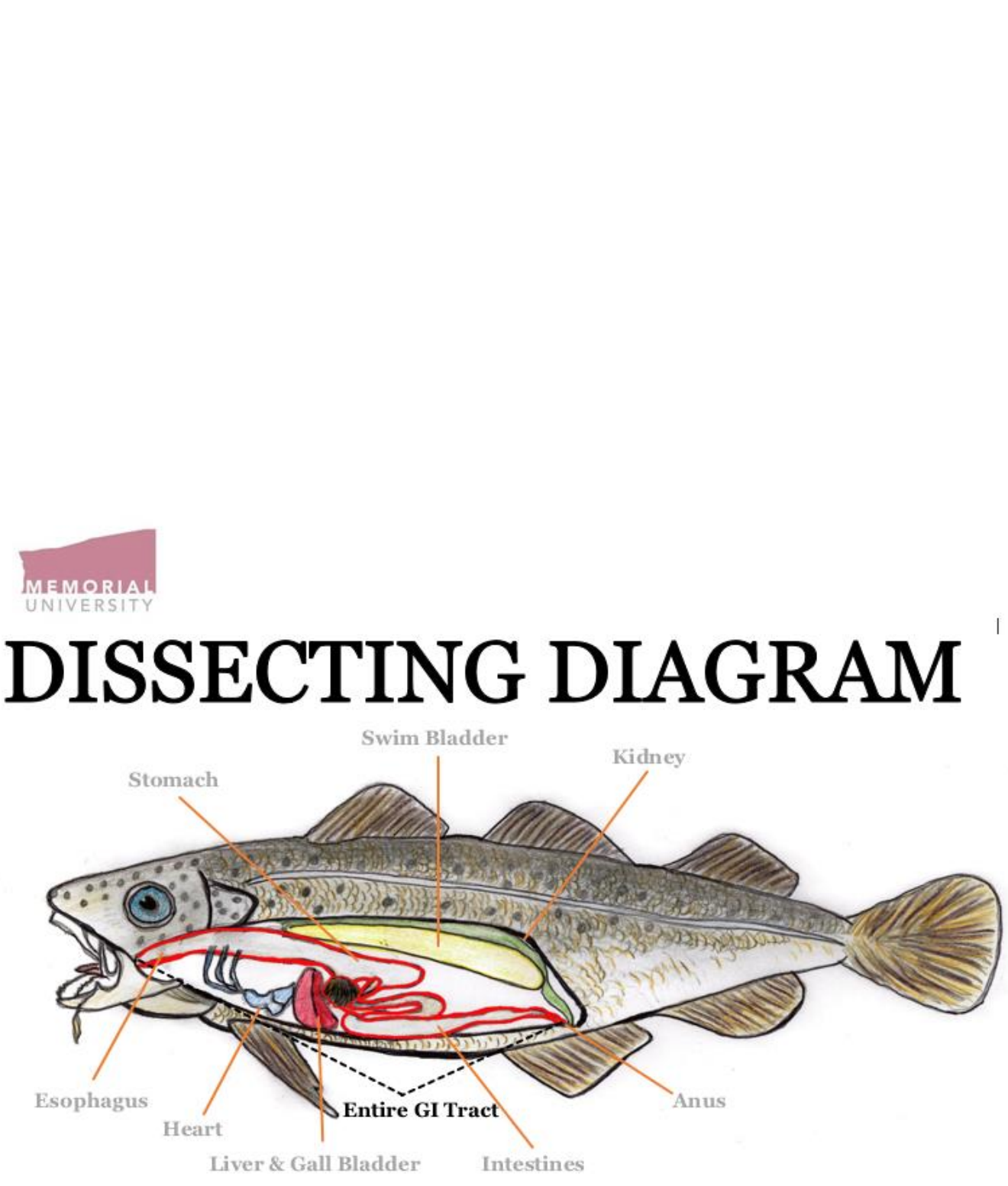
8. Using sharp scissors, cut from one end of the intestines to the other and gently rinse with water. Make sure you inspect the empty intestines using the magnifying glass for any plastics or debris that may still be attached.

9. Repeat steps 6 and 7.

10. Place any possible plastics in a paper filter and let dry for 48 hours. It is easier to identify plastics when they are dry. Use your spotters guide to reassess your findings.

Appendix B

DISSECTING DIAGRAM



When removing the **Entire GI Tract** cut from the **Esophagus** to the **Anus**
Discard the Heart, Liver, Gall Bladder, Swim Bladder and Kidney

Created by Natalie Richárd M.Sc. Student Department of Geography and CLEAR

Appendix C

10% Potassium Hydroxide (KOH) Protocol

Materials

1000ml Beaker

Purified Water (Nano)

4 Liter Glass Container Properly Labeled with NFPA Label

400g KOH Pellets

Hood (very important not to conduct without the using, fumes will burn eyes and throat)

Protective Gloves

Protective Eyewear

Scale

Scoopula

Measuring plate for KOH

Protocol

1. Before you begin make sure you are wearing gloves and protective eyewear.
2. Using the 1000ml beaker fill the 4L glass container with purified water.
3. Tare the scale with the plate you plan to use to measure out the KOH pellets.
4. To be more accurate measure out 200g of KOH pellets twice to total 400g. You should have two plates of KOH 200g each.
5. One you have measured out 400g of pellets it is important to act quickly but safely. The pellets will begin to soften and melt.
6. Bring your KOH pellets and purified water filled glass container to the hood and turn on.
7. Using the scoopula begin to place the pellets into your 4L glass container. Do this as quickly as possible but try not to spill over. If you do quickly pick up pellets and place into glass jar.
8. Once you have place 400g of KOH pellets into the 4L glass container, gently swirl. You will notice the reaction will cause the glass container to become hot.
9. Your KOH will be ready to use once the reaction is completed. The glass container will cool.
10. Keep stored in the hood at all times.
11. When using KOH always use a hood. The reaction can cause severe throat and eye irritation.
12. If you have any spillage while using KOH clean up with paper towels and soak in water. Dispose paper towels in a proper container.
13. IMPORTANT Never use Prepared KOH with metal for any extended time. It will cause a reaction and begin to melt. Use only plastic or glass.

Appendix D

Informed Consent Form

Title:	Can citizen scientists identify microplastics ingested by Atlantic Cod (*Gadus morhua*)? A single-blind comparison across four methods
Researcher:	Natalie Richard, Memorial University Department of Geography St John's, Canada NL A1B 3X9, nr8136@mun.ca
Supervisor(s):	Max Liboiron (mliboiron@mun.ca) and Charlie Mather (cmather@mun.ca), Memorial University Department of Geography St John's, Canada NL A1B 3X9

You were invited to take part in a research project entitled "Can citizen scientists identify microplastics ingested by Atlantic Cod (*Gadus morhua*)? A single-blind comparison across four methods."

Although the data has already been collected, I am required to go through a process of informed consent. This process will remind you what the research was about and it will describe your participation. It also provides you with the option to withdraw from the study, which will mean that the data I collected will be destroyed. If you decide to continue to allow me to use the data I collected, this form provides you with information on the risks and benefits of participating. Please read this carefully. You may contact the researcher if you have any questions, or you would like more information (Natalie Richard nr8136@mun.ca).

Purpose of Study:
The objective of this study was to create and test a protocol that may help citizen scientists locate and identify marine plastic in the gastrointestinal (GI) tract of food fish. This study hopes to determine if the dissection kits I have created will enable citizens to locate and identify marine plastics in fish caught for food without a scientific laboratory. This study is significant for several reasons. First, if citizens can locate and identify plastics caught recreational fish, then a marine plastic citizen science monitoring program could be feasible. Second, this would empower citizens to know what their fish have ingested.

Your participation in this study:
Participants were asked to dissect both the stomach and intestines of Atlantic cod. You were given written and oral directions and in some cases, if needed, a demonstration. Participants were supplied with all the necessary tools for the dissections and a plastic spotter's guide to help identify plastics if any are found.

Withdrawal from the Study:

- The data for this study has already been collected. If you would like to withdraw from the study, you may indicate this in the form below. If you decide to withdraw, any data collected form you for this study will be destroyed and will not be part of the analysis.
- If you agree to continue to allow me to use the data collected during your participation in this study, there will be a time where you will no longer be able to withdraw your consent. The time frame in which you can no longer withdraw consent is four weeks before the thesis is going to review, 31st of December 2017.

Possible Benefits:

- Participants learned about marine plastics and the anatomy of Atlantic cod.
- The scientific/scholarly community may benefit from the participant's contribution to science by allowing the data to be used in understanding if a citizen science marine plastic monitoring program is feasible.

Possible Risks:
Potential risks included: cutting self with scissors, upset stomach due to smell or the site of dissecting fish guts.

Confidentiality:
The final data will be presented in aggregated forms or without personal identifiers such as age, gender, etc. The average reader will not be able to identify individuals from this data, though note that informed readers such as other participants or those familiar with the project may be able to identify you. To protect confidentiality as much as possible, the consent forms will be stored separately from the collected data, so that it will not be possible to associate a name with any given set of responses. You were requested not to put your name or other identifying information on the materials used.

Recording of Data:
Using a piece of paper, I recorded whether the participants found plastic or not, the time that it took to complete the dissection and any verbal comments made by the participants.

Storage of Data:

- Data have been stored on a hard drive. All files are password protected and stored on password-protected and encrypted devices. Consent forms will be stored in a separate location that will be only obtained by myself or advisors.
- Data will be kept for a minimum of five years, as required by Memorial University's policy on Integrity in Scholarly Research. After such time, the data will be deleted.

Reporting of Results:

- The data is intended for publication in the journal, *Citizen Science: Theory and Practice.*
- Upon completion, my thesis will be available at Memorial University's Queen Elizabeth II library and can be accessed online at http://collections.mun.ca/cdm/search/collection/theses.

The data collected from participants (the ability to find plastics) will be reported statistically, and any comments made that would improve the quality of this study, will be summarized in the thesis and publication.

Questions:
You are welcome to ask questions before, during, or after your participation in this research. If you would like more information about this study, please contact Natalie Richard (nr8136@mun.ca), Max Liboiron (mliboiron@mun.ca) and Charlie Mather (cmather@mun.ca),

Consent:
Your signature on this form means that:

- You have read the information about the research.
- You have been able to ask questions about this study.
- You are satisfied with the answers to all your questions.
- You understand what the study is about and what you will be doing.
- You understand that you are free to withdraw participation in the study without having to give a reason and that doing so will not affect you now or in the future.
- You understand that if you choose to end participation **during** data collection, any data collected from you up to that point **will be retained by the researcher unless you indicate otherwise**.
- You understand that if you choose to withdraw **after** data collection has ended, your data can be removed from the study up to 1st of December 2017.

I allow data collected from me to be archived in a password-protected hard drive.	☐ Yes ☐ No

By signing this form, you do not give up your legal rights and do not release the researchers from their professional responsibilities.
Your Signature Confirms:

☐ I understand that I am being asked to give my retroactive consent to use the data collected through my participation in this research. I have had adequate time to think about this and had the opportunity to ask questions, and my questions have been answered.

☐ I agree to participate in the research project understanding the risks and contributions of my participation, that my participation is voluntary, and that I may withdraw my participation.

_____ _____
Signature of Participant Date

Researcher's Signature:
I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in the study, any potential risks of the study and that he or she has freely chosen to be in the study.

_____ _____
Signature of Principal Investigator Date