

1 Low incidence of plastic ingestion among three fish species significant 2 for human consumption on the island of Newfoundland, Canada

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4 Max Liboiron^{1,2}, Jessica Melvin², Natalie Richárd^{1,2}, Jacquelyn Saturno^{2,3}, Justine Ammendolia²,
5 France Liboiron², Louis Charron², Charles Mather^{1,2}

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7 ¹Department of Geography, Memorial University of Newfoundland, St. John's, NL A1B 3X9, Canada

8 ²Civic Laboratory for Environmental Action Research (CLEAR), Memorial University of Newfoundland, St. John's,
9 Newfoundland A1B 3X9, Canada

10 ³School of Fisheries, Marine Institute of Memorial University of Newfoundland, St. John's, NL A1C 5R3, Canada
11

12 Abstract

13 This study reports the first baselines of plastic ingestion for three fish species that are common
14 food fish in Newfoundland, Canada. Species collections occurred between 2015-2016 for
15 Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*), and capelin (*Mallotus villosus*).
16 The frequency of occurrence (%FO) of plastic ingestion for both spawning Atlantic salmon
17 ($n=69$) and capelin ($n=350$) was 0%. Of the 1,010 Atlantic cod collected over two years, 17
18 individuals had ingested plastics, a %FO of 1.68%. This is the only multi-year investigation of
19 plastic ingestion in Atlantic cod for the Northwest Atlantic, and the first for capelin and salmon
20 in the region. Considering the ecological, economic, and cultural importance of these fish
21 species, this study is the beginning of a longitudinal study of plastic ingestion to detect future
22 changes in contamination levels.

23
24 **Keywords:** Atlantic cod, Atlantic salmon, Capelin, Plastic, Ingestion, Human consumption

25 **Highlights:**

- 26 • Ingestion rate (%FO) of plastics in Atlantic cod is 1.68%
- 27 • Ingestion rate (%FO) of plastics in Atlantic salmon and capelin is 0%
- 28 • First study of plastic ingestion rates in Atlantic salmon and capelin
- 29 • Multi-year baseline of plastic ingestion in Atlantic cod in the Northwest Atlantic
- 30 • Plastic ingestion rates for three food fish species in Newfoundland, Canada, are low

31 **Terms:**

- 32 • Frequency of occurrence (%FO): the number of individuals in a population or group that
33 have ingested plastics (not indicative of the number of particles ingested per individual)
34
35

36 Introduction

37 Since its rise as a consumer and commercial material in the 1940s (Meikle, 1997), plastic
38 production has increased exponentially, giving rise to an estimated production of 400 million
39 tonnes in 2017 (Hopewell et al., 2009; Plastics Europe, 2016). This growing rate of plastic
40 production has been reflected by increasing levels of plastic debris in the marine environment
41 (Barnes et al., 2009; Cózar et al., 2014; Thompson et al., 2004). Surveys from remote areas

1 report marine plastics in diverse ecosystems, including the Arctic, Antarctica, and isolated
2 islands of the Southern Ocean and South Pacific (Eriksson and Burton, 2003; Lavers and Bond,
3 2017; Mallory, 2008; Zarfl and Matthies, 2010). Yet, remote, northern regions can still constitute
4 a blind spot in marine plastics monitoring for a variety of reasons, including high costs, seasonal
5 restrictions, a lack of research infrastructure, and a lack of standardized monitoring procedures
6 adapted to harsh environments (Bergmann et al., 2017; McWilliams et al., 2017; Poon et al.,
7 2017).

8
9 The northeastern-most Canadian province of Newfoundland and Labrador (NL) comprises of a
10 section of coastal mainland (Labrador) and an island (Newfoundland). The province of NL is the
11 most sparsely populated province in Canada, with a population density of 1.4 persons/km²
12 (Statistics Canada, 2012). Much of this human population is concentrated along the coastline
13 with access to fisheries. To date, only a handful of studies have examined the marine plastic
14 landscape surrounding Newfoundland, despite the province's high degree of marine industrial
15 activity and its dependence on the ocean for food, culture, and livelihoods (Canadian Association
16 of Petroleum Producers, 2017, 2016; Fisheries and Oceans Statistical Services, 2016; The St.
17 Lawrence Seaway Management Corporation, 2016). Generally, remote islands are characterized
18 by a high representation of plastic debris from sea-based sources, most commonly through
19 fishing and shipping activities (UNEP, 2009). These activities are expected to be significant
20 contributors to the marine plastic landscape around the island of Newfoundland, considering the
21 waters surrounding the island contribute over 80% of the national fisheries landings (Fisheries
22 and Oceans Canada, 2017a) and facilitate the movement of over 160 million tonnes of shipping
23 cargo annually (The St. Lawrence Seaway Management Corporation, 2016).

24
25 Despite the low human population, land-based sources of marine plastics should also be
26 considered in Newfoundland. It is estimated that the province generates 493,595 metric tonnes of
27 solid municipal waste per year (Auditor General of Newfoundland and Labrador, 2014). In 2016,
28 a provincial highway litter audit reported that 42.7% of all collected litter from across the
29 province were of plastic origin (13,745 of 32,190 pieces) (Multi-Materials Stewardship Board,
30 2016). Terrestrial waste can be transferred to the marine environment through numerous
31 pathways typical of coastal regions such as wind and run-off, but some local waste management
32 practices may increase these rates in NL. Most rural municipal sewerage is not treated in the
33 province, and 760 sewer outfalls release 39,000 m³ of effluent daily (Government of
34 Newfoundland and Labrador, 2016). Small-scale incineration and open burning of waste, as well
35 as direct dumping remain an issue in rural, outport communities (Avery-Gomm et al., 2016;
36 Government of Newfoundland and Labrador, 2017; Henemen, 1988).

37 *The significance plastic ingestion by fish in Newfoundland*

38 Plastic ingestion by fish is a primary concern in Newfoundland. Since over 90% of surface water
39 marine plastics are less than 5 millimeters in size (Eriksen et al., 2014), they have a high degree
40 of bioavailability to a wide range of trophic levels in the marine food web. Marine plastics are
41 associated with contaminants that can take the form of ingredients and by-products of the plastic
42 material itself (such as UV stabilizers, softeners, flame retardants, non-stick compounds and
43 colourants), as well as contaminants adsorbed from the surrounding seawater (such as PCBs and
44 DDT) (Lithner et al., 2012; Mato et al., 2001; Rochman et al., 2015). The accumulation of toxic
45 chemicals in marine species can be transferred up the food chain via biomagnification, thus

1 potentially negatively affecting apex predators like humans (Ochi, 2009; Teuten et al., 2009;
2 Browne et al., 2013; Rochman et al., 2013; Koelmans et al., 2016). Industrial chemicals
3 associated with marine plastics have been correlated to negative health effects in humans,
4 including endocrine disruption, heart disease, and developmental disorders (Colborn et al., 1994;
5 Kim et al., 2002; Lang et al., 2008; Melzer et al., 2010). Despite these preliminary links further
6 research is required to better understand the full effects that plastics consumed at lower trophic
7 levels in the food web can have on top consumers (Cole et al., 2011; Engler, 2012). Nonetheless,
8 the burden of polluted wild food sources disproportionately affects rural and low-income
9 communities where country foods are relied on for sustenance (Shepard et al., 2002).

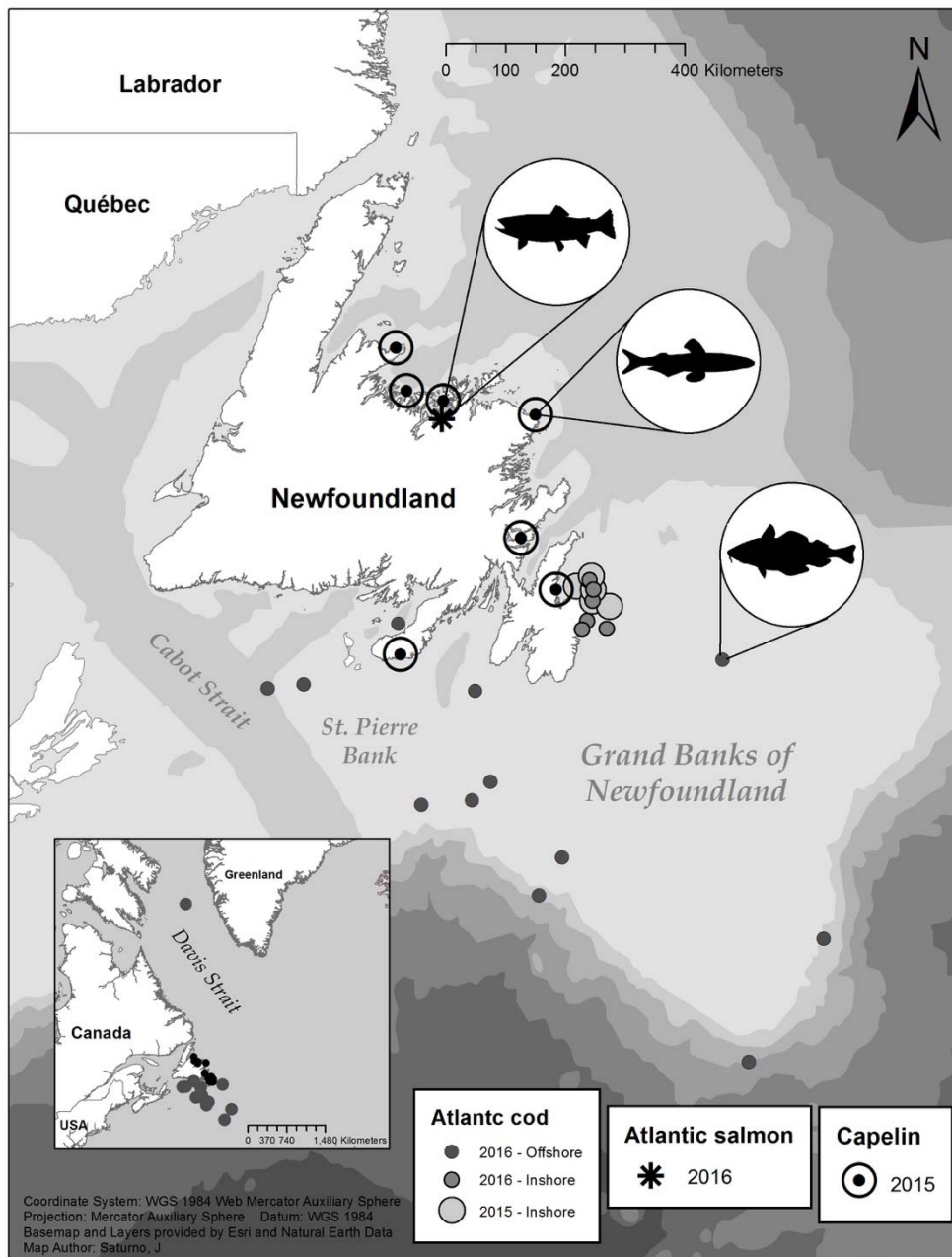
10
11 Despite the devastating collapse of the Atlantic cod (*Gadus morhua*) stock in Newfoundland and
12 the resulting moratorium on its fishery in 1992, fishing - both “recreational” (sustenance) and
13 commercial - remains financially and culturally important to the people of Newfoundland. In
14 2016, commercial fisheries employed over 17,000 individuals and landed over 220,000 tonnes of
15 catch (fish and shellfish) resulting in a production value of \$1.4 billion (Fisheries and Land
16 Resources, 2017). Within the context of the Newfoundland fishery, Atlantic cod yielded the
17 second highest landed value for all captured groundfish species in the country (~\$23 million),
18 while capelin (*Mallotus villosus*) yielded the highest landed value of all pelagic species (~\$13.5
19 million) (Fisheries and Oceans Canada, 2017b). The provincial recreational fisheries have one of
20 the highest participation rates in Canada with 71,382 participants in 2010 (Fisheries and Oceans
21 Canada, 2012a). Most of this participation comes from the recreational groundfish fishery for
22 Atlantic cod that does not allow the selling of catch (Fisheries and Oceans Canada, 2012a;
23 Government of Canada, 2017). Fishers retain their catch (as opposed to catch and release), and
24 fish are relied on for sustenance (Fisheries and Oceans Canada, 2012a). In a study of seafood
25 preferences in rural communities in Western Newfoundland, both Atlantic cod and Atlantic
26 salmon were the species most commonly consumed by respondents (81% and 42%,
27 respectively), while capelin was eaten less often, but remained a part of the diet of a majority of
28 respondents (Lowitt, 2013).

29
30 This research provides a baseline for plastic ingestion in these three common Newfoundland
31 food fish; Atlantic cod, Atlantic salmon, and capelin. This research: (1) builds upon and validates
32 the existing preliminary finding of a %FO of 2.45% in Atlantic cod collected in 2015 from the
33 eastern coast of Newfoundland (Liboiron et al., 2016) to generate a validated, multi-year baseline
34 for plastic ingestion in Atlantic cod of the Northwest Atlantic; and (2) establishes baselines of
35 plastic ingestion in Atlantic salmon and capelin on the island of Newfoundland. Based on a
36 review of English-language, published, scientific literature, no previous studies on the ingestion
37 of plastics by Atlantic salmon or capelin exist. Other studies of plastic ingestion in Atlantic cod
38 in the Northeast Atlantic have been conducted the North and Baltic seas (Bråte et al., 2016;
39 Foekema et al., 2013; Lenz et al., 2015a; Rummel et al., 2016). The production of a baseline of
40 plastic ingestion levels in these three species is the first step towards a reliable and useful long-
41 term monitoring program for the region, especially given their cultural, ecological, and economic
42 value on the island of Newfoundland.

43 Methods

1 Collection protocols

2 This study synthesises plastic ingestion data from different projects conducted around the island
3 of Newfoundland between 2015 and 2016. Accordingly, the geographical range of sampling
4 covered much of the island's inshore bays and harbours, as well as offshore regions in the Gulf
5 of St. Lawrence, the Grand Banks, as well as a collection site north of Labrador (Davis Strait)
6 (Fig. 1). "Offshore" was considered as any region >30 km from the shoreline whereas "inshore"
7 was an area <30 km from the shoreline (30 km was selected based on the distance indicated by
8 fishers whose activities are governed by these categories). Recreational fishers generally
9 captured fish within 10 km of the shoreline, while commercial and scientific collections almost
10 exclusively captured fish from at least 30 km offshore. Fish were collected using one of two
11 methods; (1) collaborations with existing scientific surveys (SS) offshore (Atlantic cod, Atlantic
12 salmon and capelin), and (2) citizen science surveys (CSS) of inshore recreational fisheries
13 (Atlantic cod). Gastrointestinal (GI) tracts were removed in the field for both Atlantic cod and
14 Atlantic salmon, while capelin were collected whole. Collection methods within the study are
15 outlined in Table 1. For further details regarding collection protocols refer to S1.
16



1
2 **Fig. 1.** Collection locations for the Atlantic cod, Atlantic salmon, and capelin from 2015-16
3 (map: J. Saturno).
4

5

1 **Table 1.** Summary of collection details for the Atlantic cod, Atlantic salmon and capelin
 2 examined for plastic ingestion rates.

Collection details	Atlantic cod (<i>Gadus morhua</i>)			Atlantic salmon (<i>Salmo salar</i>)	Capelin (<i>Mallotus villosus</i>)
Sample size (n)	204	348	458	69	350
Collection date (month, year)	October 2015	July - October 2016	April - June 2016	February - October 2016	July 2015
Collection method	Citizen Science Survey	Citizen Science Survey	Scientific Survey	Citizen Science Survey	Scientific Survey
Environment	Marine	Marine	Marine	Freshwater	Marine
Sample sites	(1) Quidi Vidi (2) Petty Harbour (3) Portugal Cove - St. Philip's	(1) Quidi Vidi (2) Petty Harbour (3) Portugal Cove - St. Philip's (4) Witless Bay (5) Bauline East (6) Brigus South	NAFO divisions: 3Ps, 3O, 3N, 3L, 0B (1) Placentia Bay (2) St. Pierre Bank (3) Fortune Bay (4) Burgeo Bank (5) Green Bank (6) Halibut Channel (7) Southern Grand Banks (8) Eastern Grand Banks (9) Davis Strait	Campbellton River	NAFO divisions: 3Ps, 3L, 3K (1) Bonavista Bay (2) Conception Bay (3) White Bay (4) Notre Dame Bay (5) Trinity Bay (6) Lawn
Variables recorded	Food presence ^a Body length (n=38) Sex (n=38) Sample site Inshore/ offshore ^c Date	Food presence ^a Body length (n=325) Sex (n=287) Sample site Inshore/ offshore ^c Date	Food presence ^a Sex (n=445) Depth of collection ^b Sample site NAFO division Inshore/ offshore ^c Date	Food Presence ^a Body length Sex Sample site Date	Food Presence ^a Body length Sample site NAFO division Date
Plastic identification methods	Visual	Visual Raman Spectrometry	Visual Raman Spectrometry	Visual Raman Spectrometry	Visual Raman Spectrometry

3 ^aFood was considered present if prey items were found in the GI tract (stomach and/or intestines)

4 ^bDepth of collection (range: 46-442m) data points were only obtained for Atlantic cod collected via Scientific Survey (SS) (n=458)

5 ^cOffshore collections occurred in any region >30 km from the shoreline whereas inshore was an area <30 km from the shoreline

6 Laboratory procedures

7 *Dissection of GI tracts*

8 We used standardized laboratory plastic ingestion protocols identical to Liboiron et al., (2016) to
 9 compare previous studies in the province, which in turn have been modified from those used in
 10 bird ingestion studies by van Franeker et al., (2011). GI tracts (or whole fish in the case of
 11 capelin) were thawed for at least 2 hours prior to dissection. Once thawed, an incision was made
 12 along the entire length of the tract, from the esophagus to anus. A double sieve method was used
 13 for analyzing Atlantic cod and salmon by rinsing and scraping GI tract contents onto stacked
 14 sieves with mesh sizes of 4.75 mm (#4) and 1 mm (#18) to aid in sorting items. Due to the small

1 size of capelin and their natural prey items (zooplankton), a single 1 mm sieve was used for
2 capelin GI tracts. Once digestive contents were transferred to sieves, the GI tracts were visually
3 and tactilely inspected for imbedded debris and thoroughly rinsed over the sieves. Sieve contents
4 were continuously and gently rinsed with cold water so any organic and inorganic debris were
5 removed from plastic particles to enable proper identification (Löder and Gerdts, 2015). Any
6 particles suspected to be non-food items (anthropogenic or not) were removed and transferred to
7 a petri dish for further analysis. The presence or absence of prey/digestive content in the stomach
8 and/or intestines was noted. Although rare, there were some instances of relatively intact fish in
9 the GI tract contents that were viable for secondary plastic ingestion analysis ($n=5$) but were too
10 degraded for a positive species identification. These prey fish were analysed following the same
11 methods described above in an attempt to identify the secondary ingestion of plastics.

12 *Contamination*

13 Plastic contaminants were occasionally found on the external surface of the stomach and/or
14 intestines for Atlantic cod ($n=15$). These particles were identified as wood splinters and paint
15 chips that visually matched the inventory of field tools and were assumed to have been
16 transferred during sample collection. To control for microplastic contamination in the
17 laboratory, we used a cotton laboratory coat, wore gloves, tied hair back, rinsed all tools under
18 tap water before and after each sample, and used daily control sticky dishes to capture particles
19 settling from the air. In the event that a fibre was found in a GI tract, it was compared to fibres
20 present in the control dish.

21 *Quantification of plastics*

22 Visual identification of microplastics through use of a microscope has been confirmed as a
23 reliable method for identifying particles greater than 1 mm in size (Song et al., 2015). As such, in
24 deference to a protocol that could be used by citizen scientists, the lower limit of plastics
25 identifiable by this research is restricted to 1 mm in the largest dimension.

26
27 Each particle that was identified as a probable plastic was individually wrapped in filter paper
28 and dried for at least five days. Once dried, particles were visually examined under a
29 stereomicroscope (Olympus SZ61 stereomicroscope, maximum magnification 45X), and a
30 compound microscope (Ecoline, Eco Series, maximum magnification 100X). Particles identified
31 as plastic at this stage were set aside to be verified by Raman micro-spectrometry (see section
32 below).

33
34 Plastic ingestion is most commonly quantified in the literature as a frequency of occurrence
35 (%FO), a metric that is indicative of the number of individuals in a sample population that
36 ingested plastic(s) (Cannon et al., 2016; Peters et al., 2017; Provencher et al., 2016; Rochman et
37 al., 2015; Romeo et al., 2015; Rummel et al., 2016). Following this metric, we recorded and
38 quantified the presence or absence of plastic(s) for each individual fish and calculated the
39 population average (%FO). The colour, weight, length, type and weathering details of plastics
40 were recorded. Plastics were weighed on a scale sensitive to 0.0001 g and length measurements
41 were taken from the longest axis of the plastic using digital calipers sensitive to 0.01 mm. There
42 remains a debate in the literature over the designation of plastic size classes in terms of micro,
43 meso, and macro designations (GESAMP, 2015). Following the designations used by Bråte et al.

1 (2016) and Cannon et al. (2016), and the recommendation of Provencher et al. (2016) for
2 standardization, plastics smaller than 5 mm in maximum length were classified as microplastics,
3 while plastics from 5-20 mm were classified as mesoplastics (and those over 20 mm as
4 macroplastics, though such plastics are rare in ingestion studies). When plastics were measured
5 they were not stretched or manipulated to facilitate a longer axis, but were measured according to
6 their size at time of recovery (Avery-Gomm et al., 2016; Liboiron et al., 2016). The size of
7 ingested plastics is important to record because it is necessary to evaluate how much surface area
8 plastics occupy in the GI tract and the surface area available for the transfer of contaminants
9 (Jabeen et al., 2017; Mato et al., 2001; Provencher et al., 2016; Rummel et al., 2016; Silva-
10 Cavalcanti et al., 2017).

11
12 Plastic types were categorized as either: industrial resin pellets, sheet/film (i.e. plastic bags),
13 threads (i.e. fishing line, rope), foamed plastics (i.e. polystyrene packaging), fragments (i.e. hard
14 plastics), fibres (i.e. from clothing), microbeads (i.e. from personal care products) or other. In
15 order to evaluate the state of plastic erosion each particle was assessed for: discolouration,
16 fraying, fracturing, pits and grooves, and adhered particles (following Corcoran et al., 2009), as
17 well as burning or melting (following Avery-Gomm et al., 2016).

18 *Raman micro-spectrometry*

19 Raman micro-spectrometry was conducted on particles obtained from: Atlantic cod collected in
20 2016, Atlantic salmon and capelin. This analysis was not available for the Atlantic cod collected
21 in 2015. However, no plastics from 2015 were visually ambiguous. Particles were washed in
22 ethanol and allowed to dry in preparation for Raman micro-spectrometry. Samples were then
23 placed on a silica wafer of known Raman spectrum (520 cm⁻¹ peak) and analysed using a Raman
24 micro-spectrometer (Reinshaw InVia with 830 nm excitation) set at a 20x Olympus objective. To
25 ensure samples were not burnt during the processing, laser power did not exceed 5%, and in
26 cases of high fluorescence (in 4/5 samples), laser power was reduced to 1%. Results were
27 analysed by using WiRE 3.4 software. A reference spectra was used to compare the Raman
28 spectrum of each experimental sample to the following common marine plastic polymers:
29 acrylonitrile butadiene styrene (ABS), cellulose acetate, polyamide (PA), polycarbonate (PC),
30 polyethylene (PE), polyethylene terephthalate (PET), poly (methyl methacrylate) (PMMA),
31 polypropylene (PP), polystyrene (PS), polyurethane (PU) and polyvinylchloride (PVC) (Bråte et
32 al., 2016; Engler, 2012; Lenz et al., 2015b; Plastics Europe, 2016).

33 **Data Analysis**

34 When frequency of occurrence for a species was >0% (Atlantic cod), the relationship between
35 %FO and the following variables were evaluated: 1) food presence, 2) sex, 3) depth of collection,
36 4) sample site, 5) NAFO management area, 6) distance offshore, 7) day of year (DOY), 8)
37 month, and 9) year. Each variable was modelled using a generalized linear model with a
38 binomial error structure. Assumptions of residuals homogeneity, independence and normality
39 were visually tested and met for all models using lag plots, residual versus fit plots, and quantile-
40 quantile (QQ) plots. Models were then tested with a ANOVA for hypothesis testing; the null
41 hypothesis was rejected when $p < 0.05$ (95% confidence interval). All analyses were carried out
42 using R Studio version 1.0.143. All graphs were generated using Prism Graphpad version 7.0

1 (Prism and Graphpad, 2016) and maps were generated using ArcGIS version 10.5.1 (ESRI,
2 2014).

3
4 Plastic characteristics (type, colour, length, mass, weathering and polymer) were quantified as a
5 percentage of all particles. The Joint Research Centre of the European Commission recommends
6 that for the standardization of plastic ingestion research, in addition to the %FO, the following
7 metrics be reported: “1) abundance by number (average number of items per individual), and 2)
8 abundance by mass (weight in grams, accurate to 4th decimal)” (Galgani et al., 2013, p. 78). Both
9 of these metrics were calculated for the entire sample population of Atlantic cod ($n=1,010$), as
10 well as for the sub-population of individuals that had ingested plastic ($n=17$). Particles that could
11 not be weighed (because they were lost prior to weighing or were too small to register) were
12 assigned a mass of 0 g for calculations involving the entire sample population, and removed for
13 calculations for the sub-population of individuals with ingested plastics.

14 Results

15 A total of 1,429 individuals from 3 species were collected and plastics were found to have been
16 ingested by 17 individuals (all Atlantic cod), resulting in a multi-species %FO of 1.19%
17 ($n=1,429$, $SD=0.110$). Although %FO of plastics is commonly reported for multi-species
18 populations (Anastasopoulou et al., 2013; Davison and Asch, 2011; Neves et al., 2015; Phillips
19 and Bonner, 2015), this study will focus on %FO for separate species so that trends can be
20 clearly examined (Table 2). This is particularly important in species with 0%FO, to avoid false
21 connotations of ubiquitous plastic consumption by all fish species (Liboiron et al., 2018).

22
23 **Table 2.** Summary of the results for plastic ingestion rates (%FO) among three fish species
24 collected from Newfoundland.

25

Species	Collection year(s)	Sample size (n)	Mean plastic ingestion rate (%FO \pm SD)
Atlantic cod (<i>Gadus morhua</i>)	2015-2016	1,010	1.68 \pm 0.129
Atlantic salmon (<i>Salmo salar</i>)	2016	69	0
Capelin (<i>Mallotus villosus</i>)	2015	350	0

26

27 Atlantic cod

28 A total of 1,010 Atlantic cod were collected and 17 fish were found to have ingested plastic, a
29 %FO of 1.68% ($n=1,010$, $SD=0.130$). None of the GI tracts found to contain plastic had any
30 holes or perforations. One GI tract that contained plastic had green paint from a green splitting

1 table on the exterior wall prior to dissection; this paint was discounted as contamination. All but
2 two of the 17 fish with ingested plastic ($n=15$) had ingested only one plastic, while two of the
3 fish collected in 2015 had ingested two plastics each. The mean number of plastics ingested per
4 individual for the entire sample was 0.019 particles/individual ($n=1,010$, $SD=0.150$). Of the sub-
5 population of Atlantic cod that had ingested plastic, the mean number of plastic particles
6 ingested per individual is 1.12 particles/individual ($n=17$, $SD=0.330$).
7

8 The %FO of the 2015 citizen science survey collection did not significantly differ from
9 individuals collected in 2016 ($p=0.730$, $df=1$, $n=552$). Therefore, the subsequent analyses of
10 these datasets were combined (with the exception of the analysis of year as an independent
11 variable) and are hereafter referred to as the citizen science survey (CSS). A total of 12 of 552
12 fish from the CSS of Atlantic cod had ingested plastic, yielding a %FO of 2.17% ($n=552$,
13 $SD=0.150$). The scientific survey (SS) of Atlantic cod found plastic in 5 out of 458 fish collected
14 for a %FO of 1.09% ($n=458$, $SD=0.100$). The comparison of the CSS and SS collections yielded
15 no significant difference in plastic ingestion ($p=0.170$, $df=1$, $n=1,010$), and are therefore
16 presented together as a total %FO (1.68%) for the species in Table 2.
17

18 The results of binomial generalized linear model fits of plastic ingestion are presented in Table 3.
19 Notably, both food presence and sex were each significantly related to %FO ($p<0.05$), while
20 there was no evidence of a significant relationship between %FO and temporal or regional
21 variables ($p>0.05$). All 17 individual cod that had ingested plastic had also ingested other food
22 items. Although most individuals had consumed food (71%), the presence of food in the stomach
23 was a significant factor in positively predicting the presence of ingested plastic ($p<0.01$),
24 whereby individuals that ingested prey were more likely to contain plastic. Sex was identified for
25 10 out of the 17 individuals with ingested plastic. Of these 10 individuals, 9 were females.
26 Females were determined to have a significantly higher %FO than males ($p<0.05$).
27
28

1 **Table 3.** Independent variables found that had a statistically significant effect (indicated by
 2 bolding) on the %FO of plastic ingestion in Atlantic cod (Atlantic salmon and capelin were not
 3 tested, due to an absence of ingested plastics). Dashes represent the scenario in which there was
 4 not enough data present to conduct analysis. [IV= explanatory variable; CSS=2015-2016 citizen
 5 science surveys; SS=scientific surveys]

IV	Metric	CSS	SS	Combined
Food presence	n	552	456	1,008
	df	1	1	1
	Pr(>X ²)	0.002	0.110	0.001
Body length	n	363	-	-
	df	1	-	-
	Pr(>X ²)	0.799	-	-
Sex	n	324	441	765
	df	1	1	1
	Pr(>X ²)	0.360	0.012	0.020
Depth	n	-	458	-
	df	-	1	-
	Pr(>X ²)	-	0.160	-
Sample site	n	540	458	999
	df	5	7	14
	Pr(>X ²)	0.220	0.064	0.050
NAFO	n	-	458	998
	df	-	4	4
	Pr(>X ²)	-	0.077	0.056
Inshore/Offshore	n	552	458	1,010
	df	1	1	1
	Pr(>X ²)	0.440	0.410	0.590
DOY	n	348	458	997
	df	1	1	1
	Pr(>X ²)	0.360	0.380	0.086
Month	n	348	458	997
	df	1	1	1
	Pr(>X ²)	0.830	0.710	0.120
Year	n	-	-	1,010
	df	-	-	1
	Pr(>X ²)	-	-	0.360

1 Atlantic salmon

2 None of the 69 Atlantic salmon that were caught in 2016 had ingested any plastics, yielding a
3 %FO of 0% for the species. Notably, only 20% of individuals had ingested food. This proportion
4 was considerably less than what was found in Atlantic cod (see above), though salmon are
5 caught while spawning, while cod are not.

6 Capelin

7 Of the 350 capelin collected, no individuals were found to have ingested plastics (%FO=0%).
8 Similar to the Atlantic salmon sample, the proportion of fed individuals was low at just 37%.
9 Also like Atlantic salmon, capelin are caught while spawning.

10 Plastic forensics

11 The most common plastic type was fragments ($n=6$), followed by threads ($n=5$), then fibres and
12 sheet/film plastics ($n=4$ per category) (Fig. 2A). The particle lengths (longest dimension) ranged
13 from 1.6 mm to 12.1 mm, with a mean length of 5.6 mm ($n=16$, $SD=3.140$). The majority of
14 particles were microplastics ($n=11$) (Fig. 2B). Size could not be recorded for three plastic
15 particles that were visually identifiable as microplastics, either because they were lost prior to
16 analysis ($n=2$), or were too small to register by the calipers ($n=1$).

17
18

1 **Table 4.** Plastic characteristics and measurements for particles recovered from Atlantic cod
 2 captured in Newfoundland from 2015 to 2016. Offshore (OS) and inshore (IS) collection sites
 3 are noted per location, where offshore was considered >30 km from the shoreline and inshore
 4 was <30 km from the shoreline.

Location	Polymer	Type	Mass (g)	Length (mm)	Colour	Erosion
Quidi Vidi (IS)	Δ	Thread	0.0028	9.0	Green	Irregular surface
Quidi Vidi (IS)	PVC ^a	Thread	*	3.5	White	Fraying
St.Philip's (IS)	Δ	Sheet	0.0017	6.5	White	None
St.Philip's (IS)	Δ	Sheet	0.0011	9.7	White	None
St.Philip's (IS)	Δ	Thread	∅	8.2	Green	None
St.Philip's (IS)	∅	Fibre	∅	∅	Blue	Fraying
Petty Harbour (IS)	Δ	Fragment	0.0010	4.2	White	Pitting, adhered particles, grooves, linear fractures
Petty Harbour (IS)	Δ	Fragment	0.0002	2.0	White	Pitting, adhered particles, grooves, linear fractures
Petty Harbour (IS)	Δ	Fragment	0.0018	2.8	White	Grooves, linear fractures
Witless Bay (IS)	ABS ^a	Fragment	0.0003	1.6	Grey	None
Witless Bay (IS)	PET	Fibre	0.0015	12.1	Pink	None
Bauline East (IS)	PE	Thread	0.0005	6.0	Green	Small pits, grooves
Brigus South (OS)	Δ	Film	0.0002	3.4	Green	Discoloured, melted, frayed
Brigus South (OS)	∅	Fibre	∅	∅	Blue	Fraying, discolouration
Halibut Channel (OS)	Δ	Fibre	*	*	Blue	None
Burgeo Bank (OS)	PE ^a	Thread	0.6970	9.0	White	None
Grand Banks (OS)	PC ^a	Fragment	0.0070	5.0	Red/white	None
Grand Banks (OS)	PE	Thread	0.0001	4.0	White	None
Grand Banks (OS)	PVC ^a	Fragment	0.0010	3.0	White	Linear fractures, irregular surface, grooves

^a suspected

* too small to register

∅ particle lost, data could not be gathered

Δ no result

5
 6 The masses of the plastic for which weight could be determined ($n=14$), ranged from 0.0001 g to
 7 0.6970 g, with a mean mass of 0.0510 g ($n=14$, $SD=0.190$; Table 4). The masses were not
 8 recorded for 5 particles, either because they were lost prior to weighing ($n=3$) or were too
 9 lightweight to register ($n=2$). The high standard deviation is attributed to the mass of one of the
 10 particles (a large piece of rope); when this particle was removed, the mean mass of the remaining
 11 13 particles was 0.0015 g ($n=13$, $SD=0.002$). The mean weight of plastic for the entire sample
 12 population of Atlantic cod (including individuals that had not ingested plastic) is 0.0007 g per
 13 individual ($n=1,010$, $SD=0.022$). Although this metric is recommended to be reported in
 14 ingestion studies for the sake of standardization (Galgani et al., 2013), considering that only 17
 15 cod had ingested any plastic, it is more useful here to report the mean weight of plastics among
 16 individuals for which plastic ingestion had occurred. Of the sub-population of Atlantic cod that
 17 had ingested plastic(s), the mean weight of plastic is 0.0420 g per individual ($n=17$, $SD=0.170$).

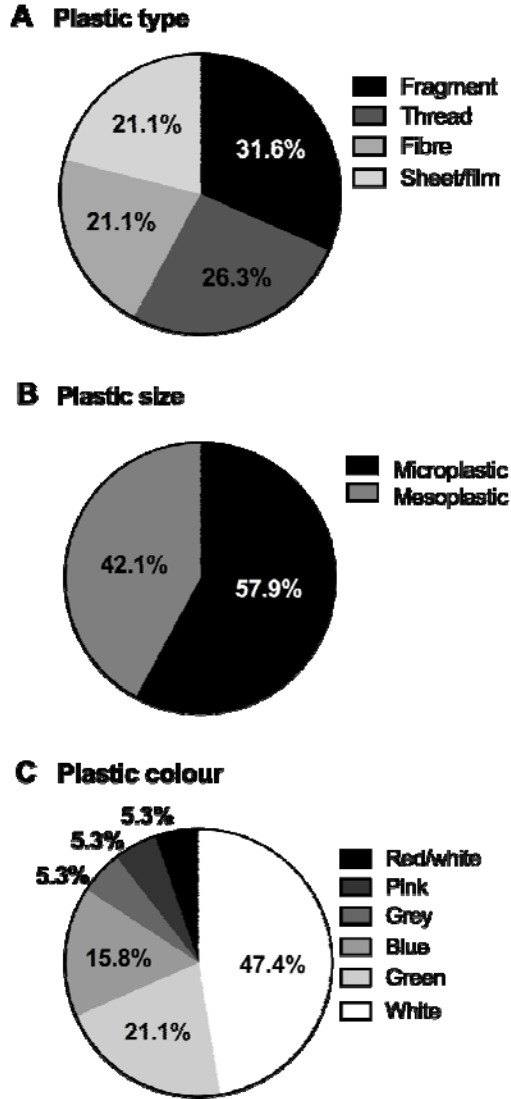
18
 19 Nine of the particles (47%) had no discernable weathering or erosion patterns, while the rest of
 20 particles (53%, $n=10$) had various forms of weathering, including pits and grooves ($n=5$),
 21 fracturing ($n=4$), adhered particles ($n=2$), fraying ($n=4$), discolouration ($n=2$), melting ($n=1$) and
 22 irregular surface textures ($n=2$) (Table 4). There was a higher proportion of particles recovered
 23 from offshore cod that displayed considerable weathering (29%, $n=2$) relative to particles from

1 inshore fish (17%, $n=2$), but the frequency of occurrence of the weathered particles did not vary
2 between offshore and inshore fish ($p=0.510$, $df=1$, $n=19$).

3
4 It is worth noting that three plastic particles were microfibres and the possibility of
5 contamination needs to be addressed. Two particles were enmeshed in a conglomerate of
6 partially digested material (Fig. 3C), eliminating the possibility of contamination via atmospheric
7 deposition, as is common with microfibres (Fries et al., 2013; Hidalgo-Ruz et al., 2012; Nuelle et
8 al., 2014; Woodall et al., 2015). Although the third microfibre was small enough to be
9 atmospherically deposited, it did not match any fibres present in the control dishes and instead
10 was embedded in a bundle of red algae. As such, we do not consider any of these microfibres to
11 be a product of contamination.

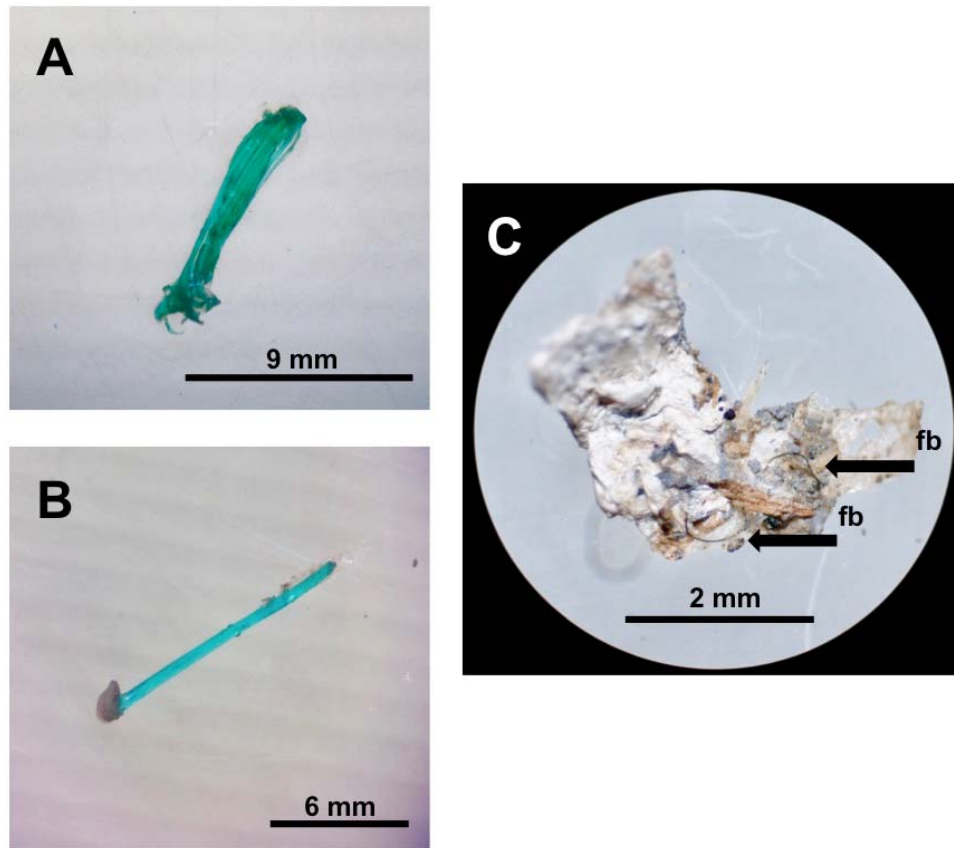
12
13 The following plastic polymer types were identified for particles recovered from Atlantic cod
14 using Raman micro-spectrometry: polyethylene (PE), polyvinylchloride (PVC), polyethylene
15 terephthalate (PET), acrylonitrile butadiene styrene (ABS), and polycarbonate (PC). For more
16 details on spectrometry results, see S2.

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Fig. 2 The characteristics for plastic particles (n= 19) recovered from Atlantic cod captured in Newfoundland from 2015 to 2016 for the following: (A) plastic type, (B) plastic size, as microplastics (<5 mm) or mesoplastics (5-20 mm) and, (C) plastic colour.



1
2
3 **Fig. 3** Selection of plastics found in Atlantic cod (*Gadus morhua*) collected by CSS in
4 2015/2016: (A) green fishing line from 2015, (B) a green polyethylene fishing line thread from
5 2016 and, (C) a fibre enmeshed in a conglomerate of partially digested material; the black arrows
6 labeled “fb” indicate the exact location of the fibre.

7 Discussion

8 A total of 1.19% of studied Newfoundland food fish individuals (Atlantic cod, Atlantic salmon
9 and capelin) were found to have ingested plastic, with an ingestion rate of 0% for capelin, 0% for
10 Atlantic salmon, and 1.68% for Atlantic cod. The geographical range of collections was broad,
11 and spanned both the southern and eastern coasts of the island, and northern Labrador (Davis
12 Strait). Atlantic cod is a culturally and economically important species on the island of
13 Newfoundland. In total, 17 individual cod ingested at least one plastic particle.

14
15 The majority (58%) of ingested plastics were microplastics (1-5 mm) and the remainder were
16 mesoplastics between 5 mm and 10 mm in size. This is at first a surprising result considering that
17 microplastics are expected to be the most common neuston marine plastic by number (Eriksen et
18 al., 2014), and plastics ingested by fish are often dominated by the microplastic size class
19 (Foekema et al., 2013; Lusher et al., 2016, 2013; Murphy et al., 2017; Neves et al., 2015; Peters

1 et al., 2017). Yet in previous studies, both Bråte et al. (2016) and Rummel et al. (2016) used
2 identical methods to ours to identify plastics in Atlantic cod and in both cases, plastics greater
3 than 5 mm in size were present in the highest proportion.

4
5 Half of all ingested particles showed no weathering, and in several particles weathering was
6 minimal. There seemed to be no difference of weathering condition in particles recovered from
7 individuals collected offshore when compared to inshore fish. Particle colours were often fresh
8 and bright, fragments had sharp edges, and most fibres were minimally frayed. This lack of
9 weathering is indicative of short residence times in the ocean, which may point toward a local
10 source (Endo et al., 2005; Liboiron et al., 2016).

11
12 Green threads were recovered in Atlantic cod from both sampling years (Fig. 3). This particular
13 shade of green is common in the fishing industry's most common fishing gear: polyethylene,
14 polypropylene, and polysteel ropes and threads (Knowlton et al., 2016; Murray and Cowie, 2011;
15 Nedostup and Orlov, 2010; Turner, 2016). The green thread recovered from an Atlantic cod
16 individual in 2016 was confirmed by Raman micro-spectrometry to be polyethylene and green
17 was the second most common colour of particles recovered from cod in this research.

18
19 All polymers identified in Raman spectrometry ($n=8$) were of a higher density than seawater,
20 except polyethylene (Table 4). A density greater than that of seawater likely leads to the
21 movement of these high density particles from the surface of water to the benthos (Browne et al.,
22 2010; Claessens et al., 2011; Nuelle et al., 2014), placing them in the feeding zone of
23 benthopelagic feeders such as Atlantic cod. Polyethylene was the most commonly identified
24 polymer and is ubiquitously used in the production of fishing gear. Polyethylene in its pristine
25 form is buoyant in seawater (Turner, 2016), though biofouling or ingestion by vertically
26 migrating species can transport this material to greater depths (Lusher et al., 2013; Ye and
27 Andrady, 1991).

28
29 Plastics ingestion by Atlantic cod has been examined over a wide geographic region beyond this
30 study, with a particular focus on the Northeast Atlantic. These published results have been highly
31 variable. Rummel et al. (2016) and Bråte et al. (2016) report %FO values of $\leq 3\%$ for Atlantic
32 cod in the North, Baltic and Norwegian seas. By contrast, earlier studies reported higher %FO
33 values for Atlantic cod in the same region; ranging from 13% (Foekema et al., 2013) to 39%
34 (Lenz et al., 2015a) in the North Sea, and 21% in the Baltic Sea (Lenz et al., 2015a). Low plastic
35 ingestion levels reported for Atlantic cod ($<3\%$ FO) that are comparable to the results reported
36 here stem from studies that similarly rely on the visual identification of plastics (Bråte et al.,
37 2016; Rummel et al., 2016). Meanwhile, reports of relatively high plastic ingestion levels in
38 Atlantic cod ($>10\%$ FO) have stemmed from studies that employ acid digestion of digestive
39 contents and can identify plastics < 1 mm in size (Foekema et al., 2013; Lenz et al., 2015a), thus
40 increasing the size range of identifiable plastics and the resultant %FO for the sample population.
41 Acid digestion protocols are not suitable to the long-term monitoring developed by CLEAR for
42 its inaccessibility to citizen scientists, and so are not used in this study (see Liboiron et al., 2016).

43
44 The wide range of sample sizes may also explain the wide range of %FO values reported for
45 Atlantic cod. Outside of this research, sample sizes for Atlantic cod plastic ingestion research
46 range from 7 (Rummel et al., 2016) to 302 (Bråte et al., 2016) individuals. The ideal minimum

1 sample size for plastic ingestion studies is species specific and depends on the %FO for that
2 species (larger sample size required for species exhibiting a lower %FO), as well as any
3 interannual variability in that average and whether the purpose of the research is to detect
4 changes over time (Provencher et al., 2016; van Franeker and Meijboom, 2002). There is not yet
5 enough records of plastic ingestion in Atlantic cod across various sample sizes to conduct an
6 analysis of the species dataset and determine at which sample size the variance in reported %FO
7 decreases, as described by van Franeker and Meijboom (2002).

8
9 To our knowledge, this is the first published study that examines plastic ingestion by Atlantic
10 salmon. The majority of salmon examined in our study (84%) were collected within the
11 Newfoundland summer recreational fishing season (Fisheries and Oceans Canada, 2015). During
12 this period, Atlantic salmon return from the ocean to spawn in freshwater rivers in
13 Newfoundland (Porter, 1975). It is possible that individuals were not actively feeding during this
14 time - indeed, only a small proportion (20%) of individuals collected in this study had food in
15 their stomach. A previous study found 1 plastic in 4 individual Chinook salmon destined for
16 human consumption in California, USA, for a %FO of 25%, though the sample size is too low to
17 generalize for the species (Rochman et al., 2015). Ingestion studies on the zooplankton that are
18 food sources for salmon speculated that secondary ingestion of microfibers would be widespread
19 in salmon (Desforges et al., 2015). It is not clear whether these studies are comparable to this
20 one, as the species, methods, and locations are different.

21
22 Similar to Atlantic salmon, this is the first study of plastic ingestion by capelin. Sampling of
23 capelin for this study was also restricted to the summer when capelin are subject to human
24 consumption during their spawning period (Lilly, 1987). Similar to Atlantic salmon, the
25 proportion of fed individuals collected in this study was relatively low (37%). The human
26 consumption of capelin may be of particular interest considering that the species is often
27 consumed whole (MacDonald, 2016; Over, 2003).

28
29 The 0% ingestion rate in capelin and Atlantic salmon mirror a null rate in Silver hake in the
30 region of southern Newfoundland, as well as 41% of all fish species reported in English-
31 language scientific literature (Liboiron et al., 2018). While the null rate may be due to the species
32 spawning behavior, it may be part of a broader trend.

33
34 Geographically, sampling was relatively broad, however, the west and north coasts of
35 Newfoundland as well as coast of Labrador were not examined in this study. Future monitoring
36 of plastics in important food fish species should address these regions.

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33 Supplementary

34 S1: Collection methods

35 The following text outlines collection methods in detail.

36 Scientific surveys

37 *Atlantic cod*

38 Atlantic cod were collected in 2016 during the following trawl surveys: 1) Ecosystem Research
39 Program surveys conducted by the Department of Fisheries and Oceans (DFO) aboard the vessel
40 RV *Teleost*, and 2) Transatlantic Added Value survey conducted by the Marine Institute (MI) of

1 Memorial University of Newfoundland aboard the vessel RV *Celtic Explorer*. These surveys are
2 primarily aimed at assessing stock sizes of various species of fish and invertebrates. DFO trawl
3 research evaluates the status of Atlantic cod stocks, while MI uses acoustic and trawl methods to
4 assess different species (Fisheries and Oceans Canada, 2012b; Marine Institute (Foras na Mara),
5 n.d.). Surveys occurred over the southern and eastern Newfoundland shelf, as well as in the
6 Davis Strait.

7
8 Both surveys examined individual Atlantic cod for prey analysis prior to our microplastic
9 analyses for an unrelated project. Trained technicians from DFO and MI dissected each stomach
10 from the GI tracts and transferred them to separate, sterile metal trays, where digestive contents
11 were analyzed. Following this analysis, each stomach and all of its contents were placed into a
12 plastic bag with the corresponding GI tract. To ensure all particles were transferred, the metal
13 tray was thoroughly rinsed with a wash bottle and this water was also transferred to the plastic
14 bag, which was then sealed and frozen for later plastic analysis.

15 *Atlantic salmon*

16 Atlantic salmon were collected at a fish-counting fence in the Campbellton River. Located 220 m
17 from the mouth of the river, this fence is constructed annually by DFO and used by researchers
18 to study salmon returning to the river (Downton et al., 2001). Atlantic salmon were collected and
19 sacrificed so that physiological samples like blood, isotopes, and fatty acids (not related to the GI
20 tract) could be used for three other research projects. Physiology studies were conducted under
21 Fisheries and Oceans Canada Experimental Licenses provided to Memorial University, Carleton
22 University, and DFO. Donations of intact GI tracts were made for this study after the
23 physiological sampling.

24 *Capelin*

25 Capelin were collected by commercial fishers around the island of Newfoundland and donated to
26 this study by DFO. Capelin were caught using several fishing methods (cast net, purse seine, and
27 tuc seine). Whole individuals were frozen immediately after collection and stored for later
28 dissection and analysis.

29 Citizen science surveys

30 Atlantic cod were collected from the 2015 and 2016 Newfoundland commercial and recreational
31 fisheries. All individuals examined in this survey were collected directly by fishers from six
32 coastal communities located along the Eastern Avalon Peninsula, and the GI tracts were donated
33 to researchers at the wharf when fish were landed. Three of these communities were visited in
34 2015, and revisited in 2016 (Petty Harbour, Quidi Vidi, and Portugal Cove-St. Philip's) with the
35 addition of three new communities further south in 2016 (Witless Bay, Bauline East, and Brigus
36 South). Atlantic cod were caught by one of the following methods: hand-line, jigger, or gill net.
37 Such methods are advantageous over scientific trawling as no anthropogenic debris accumulates
38 in the net for possible consumption by captured fish (Davison and Asch, 2011).

39
40 In the cod surveys from 2015, around 30 fishers participated per collection site whereas in 2016,
41 between one and seven fishers participated per site. This sampling method is based on fishers'
42 knowledge of fish aggregations ("good fishing spots"), which increases sampling efficiency

1 (Liboiron et al., 2016). This sampling method has proved efficient by providing a means of
 2 sampling the human food web directly; all fish analysed were consumed by humans (see also
 3 Neves et al., 2015; Rochman et al., 2015). The removal of GI tracts (from esophagus to anus)
 4 occurred at the wharf and was done either by the researcher or the fisher, depending on the
 5 fisher's preference. Fish were always identified as Atlantic cod by the researcher(s) and other
 6 data about individual fish (length, sex) was recorded when possible. All GI tracts were
 7 individually bagged and frozen for further analysis.

8 S2: Raman Spectrometry

9 Two of the three microfibrils identified in 2016 were lost prior to Raman micro-spectrometry and
 10 therefore excluded from the analysis. A total of 10 particles from Atlantic cod caught in 2016,
 11 one particle from Atlantic salmon, and two particles from capelin were subjected to Raman
 12 micro-spectrometry. Of the 13 particles analysed with Raman micro-spectrometry, three yielded
 13 conclusive polymer identifications, five yielded suspected polymer identifications, two particles'
 14 polymers could not be identified (but were visually identifiable as plastics), two were confirmed
 15 to be non-plastic and one microfibre was too small to produce a result (Table 4).

16
 17 **Table S2.** Summary of results for particles subjected to Raman micro-spectrometry analysis.
 18 Results indicated whether particles were confirmed as plastic (Y, Yes or N, No), whether a
 19 polymer could be identified (Y, Yes or N, No), and, within particles for which a polymer was
 20 identified, the confidence of the polymer result (C, conclusive or S, suspect). Dashes indicate
 21 information could not be determined in the analysis.
 22

Species	Particle type	Particle colour	Plastic (Y/N)	Polymer (Y/N)	Confidence (C/S)	Polymer	Density (g/cm ³)*
Atlantic cod (<i>Gadus morhua</i>)	Fibre	White	Y	Y	S	PVC	1.15-1.96
	Fragment	Grey	Y	Y	S	ABS	0.90-1.53
	Fibre	Pink	Y	Y	C	PET	1.38
	Film	Green	Y	N	-	-	-
	Fibre	Green	Y	Y	C	PE	0.92-0.96
	Fibre	Blue	Y	N	-	-	-
	Fibre	White	Y	Y	S	PE	0.92-0.96
	Fragment	White+Red	Y	Y	S	PC	1.36
	Fibre	White	Y	Y	C	PE	0.92-0.96
	Unknown		N	-	-	-	-
	Unknown		N	-	-	-	-
	Fragment	White	Y	Y	S	PVC	1.15-1.96
Capelin (<i>Mallotus villosus</i>)	-	-	N	-	-	-	-
	-	-	N	-	-	-	-
Atlantic salmon (<i>Salmo salar</i>)	-	-	N	-	-	-	-

* Densities listed here correspond to the pure polymer, according to Wolfram Alpha LLC (2017). Actual particle densities will vary from that of their pure form. Seawater density ~ 1.02-1.04 g/cm³ (Wolfram Alpha LLC, 2017)

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 24
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