A study of wrecked Dovekies (Alle alle) in the western North Atlantic highlights the importance of using standardized me....

Article *in* Marine Pollution Bulletin · September 2016

DOI: 10.1016/j.marpolbul.2016.08.062


Stephanie Avery-Gomm
The University of Queensland


Carley R. Schacter
Memorial University of Newfoundland

Max Liboiron
Memorial University of Newfoundland


Lorena M Rios
University of Wisconsin - Superior

Marine Pollution Bulletin 113 (2016) 75–80

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

A study of wrecked Dovekies (*Alle alle*) in the western North Atlantic highlights the importance of using standardized methods to quantify plastic ingestion

Stephanie Avery-Gomm [a,b,*], Michelle Valliant [c], Carley R. Schacter [c], Katherine F. Robbins [c], Max Liboiron [d], Pierre-Yves Daoust [e], Lorena M. Rios [f], Ian L. Jones [c]

[a] *Centre for Biodiversity and Conservation Science, University of Queensland, St. Lucia, Queensland 4072, Australia*
[b] *Wildlife Research Division, Environment and Climate Change Canada, 6 Bruce Street, Mount Pearl, Newfoundland, A1N 4T3, Canada*
[c] *Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3X9, Canada*
[d] *Department of Sociology and Program in Environmental Sciences, Memorial University of Newfoundland, St. John's, Newfoundland, A1C 5S7, Canada*
[e] *Canadian Wildlife Health Cooperative, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, C1A 4P3, Canada*
[f] *Program of Chemistry and Physics, Department of Natural Sciences, University of Wisconsin-Superior, Belknap and Catlin, P.O. Box 2000, Superior, WI 54880, USA*

A R T I C L E I N F O

Article history:
Received 20 May 2016
Received in revised form 23 August 2016
Accepted 24 August 2016
Available online 6 September 2016

Keywords:
Marine debris
Newfoundland
Plastic pollution
Seabird
Incinerated
Little auk

A B S T R A C T

Quantification of plastic ingestion across a range of seabirds is required to assess the prevalence of plastics in marine food webs. We quantified plastic ingestion in beached Dovekies (*Alle alle*), following a wreck in Newfoundland, Canada. Of 171 birds, 30.4% had ingested plastic (mean 0.81 ± 0.30 SE pieces per bird, mass 0.005 ± 0.002 SE g per bird). Most plastics were fragments of polyethylene and polypropylene. Surprisingly, 37% were burned or melted, indicating a previously unreported source of ingested plastics (incinerated waste). We found no relationship between plastic ingestion and age, sex or body condition. By comparing our results with a similar nearby study, we illustrate the need for researchers to adopt standardized methods for plastic ingestion studies. We underline the importance of using histological techniques to reliably identify gastric pathologies, and advise caution when inferring population level trends in plastic ingestion from studies of emaciated, wrecked birds.

1. Introduction

Marine plastic pollution is an emerging contaminant that has gained global attention in recent years (UNEP, 2014). A recent study estimates that the number of microplastics (i.e., 1–5 mm) in the world's oceans ranges from 15 trillion to 51 trillion pieces, and accounts for the vast majority of marine plastics globally (van Sebille et al., 2015). Plastics may be ingested by a diverse range of marine organisms, including zooplankton, fish, birds and marine mammals (STAP, 2012). Among seabirds, a reported 59% of all species have been documented to ingest plastic, and by 2050 it is predicted that 99% of seabird species will be subject to plastic ingestion (Wilcox et al., 2015). Ingested plastics are sometimes correlated with injuries including lacerations and ulcers but a direct cause and effect relationship between the ingestion of plastics and injury is unclear (Fry et al., 1987; Pierce et al., 2004). Of greater concern may be the emerging research which suggests that ingested plastics act as a vector for contaminant transfer into marine food webs (Mato et al., 2001; Rochman et al., 2013; Tanaka et al., 2013). At present, the ultimate consequences of plastic ingestion on seabird individuals and populations is not well understood (Rochman et al., 2015).

One of the key seabird species used to monitor marine plastics is the Northern Fulmar (*Fulmarus glacialis*; OSPAR, 2008; MSFD-TSGML, 2013). Northern Fulmars are an ideal species for monitoring marine plastics at sea (van Franeker et al., 2011), but to assess the pervasiveness of plastic ingestion among seabirds generally it is important to quantify characteristics of plastic ingestion across a range of species (Moser and Lee, 1992; Avery-Gomm et al., 2013; Provencher et al., 2014) using standardized methods (van Franeker et al., 2011). For plastic ingestion studies that involve examination of the full stomach (proventriculus and gizzard), samples are obtained from three sources: direct collection of live individuals at sea, bycatch in fisheries, and beach cast birds which occasionally occur as large wrecking events involving hundreds to thousands of individuals (Stenhouse and Montevecchi, 1996). None of these approaches are without sampling biases, but taken together may be considered to be representative of plastic ingestion in the broader population.

Here we quantified the frequency and characteristics of plastics ingestion in a species of small pursuit-diving planktivorous seabird, the Dovekie (*Alle alle*), following a 'wrecking event' in eastern Newfoundland,

* Corresponding author at: Centre for Biodiversity and Conservation Science, University of Queensland, St. Lucia, Queensland 4072, Australia.
E-mail address: s.averygomm@uq.net.au (S. Avery-Gomm).

http://dx.doi.org/10.1016/j.marpolbul.2016.08.062
0025-326X/

Canada. Dovekies breed in large colonies in Russia, Norway and Greenland (Montevecchi and Stenhouse, 2002), and are abundant off eastern Newfoundland from coastal areas to far offshore areas such as the Grand Banks during the non-breeding season (Fifield et al., 2009; Fort et al., 2013; Montevecchi and Stenhouse, 2002). Using standardized laboratory methods, we quantified physical and polymer characteristics of ingested plastics and attempted to infer possible sources of this pollution. We tested for an association between Dovekie body condition and plastic ingestion, and whether or not there was evidence that ingested plastics may have caused gastric lacerations or ulcers - two commonly mentioned, but rarely quantified, impacts of plastic ingestion. Finally, we compared our results to other published and unpublished results for Dovekies from the region, highlighting the necessity of using standardized methods when reporting plastic ingestion.

2. Methods

On the 12–13th of January 2013, thousands of Dovekies were observed in Conception Bay, Newfoundland, following northerly gale force winds caused by a deep low-pressure system centered ~100 km to the east (Environment Canada, 2013). In inclement weather, with 28 km/h onshore winds, large numbers of Dovekies were observed washing up on Holyrood beach at the southernmost point of Conception Bay (47°23′9.95″N 53°7′59.44″W; Fig. 1). During this wrecking event a total of 230 freshly dead Dovekies were collected and frozen before being processed. Additional details on the wrecking event are available in the Supplementary materials.

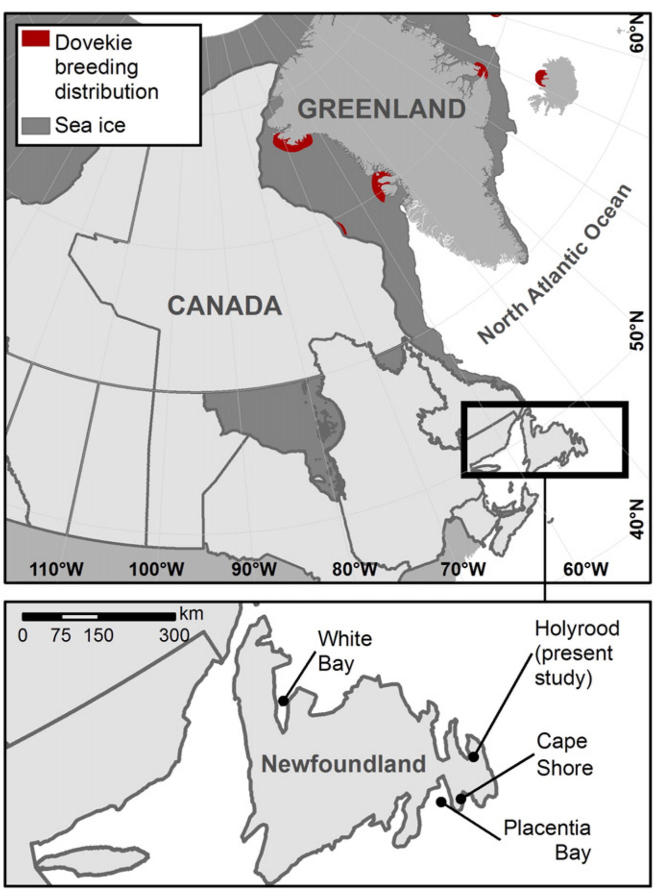

Fig. 1. Locations of key sites in Newfoundland, Canada where Dovekies were sampled for the present study and other studies referred to in Table 1. The dark grey area indicates Multisensor Analyzed Sea Ice Extent (MASIE) for January 12, 2013, obtained from the National Snow and Ice Data Centre (NSIDC). Map projection: Canada Albers equal area conic.

Of the 230 birds collected during the wrecking event, 181 were used in this study. A subset of 10 randomly selected Dovekies was sent to a veterinary pathologist for an independent professional assessment of cause of death. For the remaining 171 Dovekies, we assessed body condition following protocols described by van Franeker (2004). In brief, subcutaneous fat, intestinal fat and the condition of pectoral muscles were scored from 0 to 3 and the body condition index was the sum of these scores. Sex was determined genetically by sending ~1 g of pectoral muscle tissue from each bird to the Genomics and Proteomics Facility of the CREAIT Network, Memorial University of Newfoundland (Supplementary materials; Fridolfsson and Ellegren, 1999). Age was recorded as juvenile or subadult/adult (>1 year) based on the development of the suborbital ridge (Rosing-Asvid et al., 2013).

For each bird, the proventriculus and gizzard (the ‘stomach’) were removed for content analysis following the standardized protocol for assessing plastic ingestion (van Franeker, 2004). Both the proventriculus and gizzard were opened along their length and their contents were flushed out over a 1.0 mm mesh sieve, and then transferred to a petri dish for sorting under a dissecting microscope. Plastic items were separated from other ingested matter visually under a compound microscope (Hidalgo-Ruz et al., 2012), and air dried for 30 days.

After being rinsed, the interior surfaces of the proventriculus and gizzard were examined under a dissecting microscope for evidence of lesions (abnormalities) such as lacerations, erosions, or ulcers. Lacerations were recognized as cuts or punctures, erosions as decreased thickness, and ulcers as complete loss of a portion of the mucosa (mucous membrane). Stomachs suspected to have lesions were analyzed by a veterinary pathologist to confirm if pathologies were present using standard histological methods. First, stomachs were fixed in 10% buffered formalin. Next, samples of putative lesions were dehydrated in graded ethanol and CLEAR-RITE 3® (xylene substitute; Richard-Allan Scientific, Mississauga, Ontario, Canada), and embedded in paraffin blocks. Sections 5-μ-thick were cut with a microtome and stained with hematoxylin and eosin (Bancroft et al., 1994). These were then examined by compound microscopy to better characterize the lesions.

For each bird, the total mass of ingested plastic was measured using an analytical scale (accurate to 0.0001 g), and the number of ingested plastic items was recorded. Prevalence rate is reported as the proportion of sampled Dovekies found to have ingested plastic, and arithmetic means (±SD) for number of ingested plastic and mass are reported for the full sample size ($N = 171$). Individual plastic items were categorized as industrial plastic (small virgin plastic pellets, or ‘nurdles’) or user plastic (e.g., fragments, sheets, threads, foam) according to standardized practices (van Franeker, 2004). Additionally, the dimensions of each piece were measured using digital calipers (accurate to 0.01 mm) and colour was recorded. Plastics were classified as macro- (20–100 mm), meso- (5–20 mm) and micro-plastics (1–5 mm; Barnes et al., 2009). To determine the type of synthetic polymers being ingested by Dovekies, samples were analyzed using Fourier transform infrared spectroscopic (FT-IR), a Nicolet IR 2000 FT-IR spectrometer equipped with ZnSe crystal (Rios and Jones, 2015). The positive identification of each polymer was done using virgin plastic resin pellets as standards. The polymer matching reference spectrum was accepted at 60–85%.

We used Generalized Linear Models to test for the effect of age, sex, and condition index on prevalence (binomial distribution with logit link; McCullagh and Nelder, 1989), and number (negative binomial distribution with log link, to account for high occurrence of zero counts; O'Hara and Kotze, 2010) of ingested plastics. Post-hoc comparisons of fixed effects within these models were tested using Wald's Chi-squared test and are presented with χ^2-values, degrees of freedom, and p-values (Ver Hoef and Boveng, 2007). We ensured our models were not overdispersed by ensuring that the ratio of the residual deviance over residual degrees of freedom was ~1 (Venables and Ripley, 2002). A General Linear Model was used to test for an effect of age, sex, and condition index on the mass of plastic ingested. A Pearson's Chi-squared test was used to test for an association between plastic ingestion and the presence of gastric ulcers and other lesions. Statistical analyses were conducted using packages 'car' (Fox and Weisberg, 2011) and 'MASS' (Venables and Ripley, 2002) in R version 3.1.1 (R Core Team, 2014).

3. Results

All of the 10 birds independently necropsied at the Animal Health Division, NL Forestry and Agrifoods Agency had atrophied pectoral muscles, no visible subcutaneous or visceral fat and no evidence of trauma, indicating that these birds died of starvation (L. Rogers 2013, *pers. comm*, December 2). This was consistent with our own assessment of body condition, as all of the Dovekies examined scored as 'mortally emaciated' (scores ranged from 0 to 1, on a scale of 0 to 9) based on significant reduction in pectoral muscle mass and fat reserves (N = 171). The age ratio of Dovekies was 24 juvenile: 147 subadult/adult, and the sex ratio was 51 female: 62 male: 58 unknowns (slightly male-biased, Binomial p = 0.044).

Plastic debris was found in the stomachs of 30.4% of Dovekies sampled (52/171; Table 1). The mean number of plastic items ingested was 0.81 ± 0.30 SE pieces per bird (range 0–50). The mean mass of ingested plastic was 0.0050 ± 0.0020 SE g per bird (range 0 g–0.2700 g). For all ingested plastic items (n = 142) the mean (±SD) mass was 0.0060 ± 0.0300 g, while mean length, width, and height were 5.19 ± 4.18 mm, 2.1 ± 1.46 mm, and 0.68 ± 0.88 mm, respectively. Only one piece of plastic was an industrial pre-production pellet (a nurdle, 0.7%). The remaining 141 items were fragments (42.3%), sheet or film plastic (30.1%), or threads (26.1%; Fig. 2A). The majority of ingested plastics (64%) were microplastics (1–5 mm), 35% were mesoplastics (5–20 mm), and only one piece was macroplastic (20–100 mm). The colours of ingested plastics were predominantly black (25.4%) and brown (19.7%), followed by white (14.8%), green (14.1%), clear (11.3%) and various other colours such as grey, red, tan and yellow. 37.3% of the plastics showed evidence of being recently burned or melted. The FT-IR analysis showed that the primary synthetic polymer in the samples was polyethylene (77.6%), although polypropylene was also prevalent (20.9%). Polyvinyl chloride (PVC) and nylon 6.6 were also found (0.7% for both polymers).

When considering the prevalence of plastic ingestion among Dovekies (N = 171) we found a significant interaction between sex and condition index $\chi^2_1 = 4.38$, p = 0.036, n = 104). To explore this, we analyzed males and females separately, but found no effect of age ($\chi^2_1 = 0.38$, p = 0.54, n = 48) or condition index ($\chi^2_1 = 0.90$, p = 0.34, n = 48) on plastic prevalence in females. Similarly, for males, neither age ($\chi^2_1 = 0.11$, p = 0.74, n = 55) nor condition index ($\chi^2_1 = 3.07$, p = 0.08, n = 55) were statistically significant at $\alpha = 0.05$. Regarding variation in the number of plastics ingested by Dovekies, there was a significant effect of condition index on the number of ingested plastics per Dovekie ($\chi^2_1 = 7.52$, p = 0.006, n = 103), with higher numbers present in birds with lower condition index. However, this relationship was not robust to the removal of one outlier, a bird that had ingested 50 pieces of plastic ($\chi^2_1 = 1.82$, p = 0.18, n = 102). When considering the mass of plastics ingested by dovekies (N = 171), we detected no effect of sex ($F_{1,95} = 0.58$, p = 0.45, n = 103), age ($F_{1,95} = 0.36$, p = 0.55, n = 103), or condition index ($F_{1,95} = 2.04$, p = 0.16, n = 103).

Areas of dark discolouration of the inner surface of the stomach (proventriculus and gizzard) were observed in 25 of 171 of Dovekie stomachs (14.6%; Fig. 2B), and lesions on these 25 stomachs were examined using standard histological methods (Bancroft et al., 1994). Although samples were adequately preserved, when observed microscopically none of the areas of discolouration qualified as an ulcer (defined as complete loss of a portion of the mucosa, including koilin and glandular portion). The only changes associated with the areas of discolouration included focal loss of the koilin and possible decreased thickness (erosion) of the glandular portion of the mucosa, with no associated hemorrhage, inflammation or fibrosis of repair. The exact cause of these changes could not be determined, but none were considered to be clinically significant. The absence of ulcers negated the need for analysis of the relationship between ulcers and plastics, and we did not detect a significant relationship between ingestion of plastic and presence of other lesions ($\chi^2_1 = 0.05$, p = 0.81).

4. Discussion

In the present study of wrecked Dovekies, we found that 30.4% of our sample had ingested plastics. Although overall the number and mass of plastics ingested by Dovekies was small (Table 1), the prevalence of plastic ingestion in this study is much higher than in other Dovekies from the region (Fife et al., 2015) or in other alcid species in Newfoundland, including Common Murre (*Uria aalge*, 7%), Thick-billed Murre (*U. lomvia*, 9%), Razorbills (*Alca torda*, 0%), or Atlantic Puffins (*Fratercula arctica*, 7%; Bond et al., 2013; Muzaffar, 2009; Provencher et al., 2014) This difference may be related to the difference in average size of prey items between small-bodied Dovekies and these larger-bodied alcids, which feed primarily on small fish. Dovekies, in contrast,

Table 1
Characteristics of plastic ingestion by Dovekies in Newfoundland, Canada from the present study, in comparison with other studies from the region employing different methodologies.

Study	Year	Location	Collection method	Analysis methods	N	Prevalence	Mass ± SD (range)	Number ± SD (range)
Present Study	January 2013	Holyrood, Newfoundland 47°23′9.95″N 53°7′59.44″W	Beached birds	Proventriculus & gizzard, 1.0 mm sieve w/ microscope[a]	171	30.4%	0.0049 ± 0.0280 g (0.000–0.2670)	0.8070 ± 3.910 (0–50 pieces)
Present Study, excluding burned & melted plastics	January 2013	Holyrood, Newfoundland 47°23′9.95″N 53°7′59.44″W	Beached birds	Proventriculus & gizzard, 1.0 mm sieve w/ microscope[a]	171	27.5%	0.0044 ± 0.0272 (0.000–0.2670)	0.4970 ± 1.2385 (0–12 pieces)
Fife et al., 2015	January 2013	White Bay, Newfoundland 50°9′22.88″N 56°26′30.24″W	Beached birds	Gizzard only, visual inspection w/ microscope	65	13.8%	0.0183 ± 0.0205 g	0.1538 ± 0.4043 (0–2 pieces)
Rosing-Asvid et al., 2013 *unpub data*	March 2011	Placentia Bay, Newfoundland 47°12′6.92″N 54°25′43.81″W	Shot at sea	Proventriculus, visual inspection	50	0.00%	Not applicable	Not applicable
Robertson et al., 2006 *unpub data*	December 2003	Cape shore, Newfoundland 46°57′33.29″N 54°2′58.08″W	Oiled beached birds	Proventriculus & gizzard, visual inspection	73	1.4%	Not reported	Not reported

[a] (van Franeker et al., 2011).

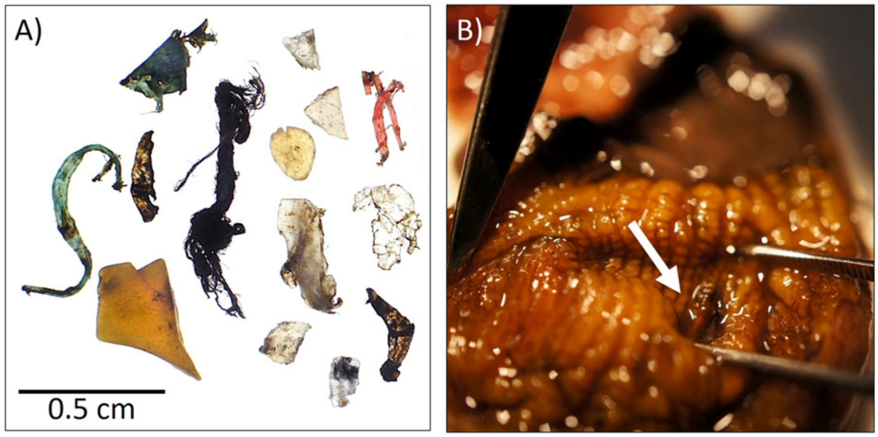

Fig. 2. (A) The majority of user plastics ingested by Dovekies in Newfoundland included fragments, sheet plastic, and threads plastics, 37.3% of which were burned or melted. (B) An area of discolouration on the inner surface of a Dovekie stomach that could easily be misidentified as an ulcer.

forage by pursuit diving for zooplankton, mostly for copepods (*Calanus* spp.), amphipods (*Themisto* spp.), krill (*Thysanoessa raschii*) and larval Arctic cod (*Arctogadus glacialis*; Gaston and Jones, 1998; Montevecchi and Stenhouse, 2002). Dovekies may have directly ingested plastics, or these birds may have indirectly ingested plastic via prey (e.g., small fish) that had ingested plastic beforehand. This kind of 'secondary ingestion' has been suggested previously for Pacific alcids (Robards et al., 1995). All Dovekies in this study were in poor body condition, therefore it is difficult to conclude whether the observed rate of plastic ingestion is representative of normal behavior.

Though there is a sex-related difference in foraging behavior during chick rearing at sea, when the males accompany and provision fledglings after the females depart (Harding et al., 2004), the distributions of male and female Dovekies overlap during winter (December/January; Fort et al., 2013). In our study, we found no sex-related differences in plastic ingestion. This is not surprising, as sex-related differences in plastic ingestion have not been documented in other species either (Spear et al., 1995; Acampora et al., 2014). We also found no evidence that juvenile Dovekies ingested more plastic than older birds, which is contrary to the limited number of studies which have investigated this relationship (Spear et al., 1995; van Franeker et al., 2011; Avery-Gomm et al., 2012; Acampora et al., 2014). It is possible our lack of a significant difference between age groups is due to a small sample of juveniles, or that this trend is not present in Dovekies (Fife et al., 2015). We also found no statistically significant correlation between Dovekie body condition and plastic ingestion. This may be due to the relatively small plastic loads observed, or the lack of variation in body condition. For the subset of Dovekies that ingested plastics, plastic loads were equivalent to (average) 0.013% $\pm$ 0.04% of the individual Dovekies' body mass, and all birds were mortally emaciated. Of course, the usefulness of correlative relationships for understanding the impacts of plastic ingestion on seabirds is limited because of the difficulty separating cause from effect (Ryan, 1987), and so our results and those of other studies (e.g., Connors and Smith, 1982; Day, 1980; Furness, 1985a, 1985b; Lavers et al., 2014) should be interpreted cautiously. Ultimately, controlled feeding experiments will be required to determine the impacts of plastic ingestion on seabird health (subject to approval from the appropriate Animal Care and Ethics Committee; e.g., Ryan, 1988).

Gastric ulcers and lacerations are two commonly cited physical impacts linked to plastic ingestion, however they are rarely characterized or quantified. Of the few seabird plastic ingestion studies that report ulcers (Pettit et al., 1981; Ryan and Jackson, 1987; Sievert and Sileo, 1993; Pierce et al., 2004), only one study used histological methods to identify them (Fry et al., 1987). In this study we observed areas of dark discolouration and potential laceration of the inner surface of the stomach (which we suspected to be ulcers) in 14.6% of the birds (Fig. 2B). However, none could be confirmed as ulcers when examined using histological methods, and they were determined not to be of clinical significance to the birds. Further, we found no relationship between Dovekie plastic ingestion and these areas of dark discolouration. For seabirds that carry larger plastic loads plastic-related ulcers may be more prevalent, but evidence of this in the literature is scarce. Further, lesions such as erosions and ulcers are nonspecific and can result from a variety of causes, particularly parasitism. It is therefore risky to attribute such lesions to plastic ingestion before ruling out other causes. Future studies should use histological techniques to characterize gastric pathologies and statistical analyses to evaluate whether observed lesions are likely plastic related.

The vast majority of ingested plastics were post-consumer user plastic (99.3%), which is consistent with the globally reported decrease in the proportion of industrial plastics ingested by seabirds (Ryan, 2008; van Franeker and Law, 2015). The majority of user plastics were fragments, sheet plastic and threads, and nearly half were black or brown in colour. A comparison of our findings with at-sea sampling of plastic in the North Atlantic (at 40° latitude off the coast of Cape Cod, Massachusetts) shows that the dominant type (fragment) of ingested plastics are similar, although the relative proportions of thread, sheet and foam plastic differ (Morét-Ferguson et al., 2010). High counts of threads in our Newfoundland sample were likely due to the nearby commercial fishery that uses nylon and polypropylene line and net. Fishing nets are subject to wear during fishing activity and are sometimes lost at sea. Many of our film plastics appeared to be fragments of garbage bags, and we found the vast majority of plastics to be polyethylene and polypropylene - two polymers which make up the majority of single use plastics (Plastics Europe, 2015). There are currently no studies that describe the colour of plastic pollution in the western North Atlantic, at latitudes comparable to where our sample of Dovekies would have fed, therefore we could not assess whether Dovekies exhibit colour selectivity when they are ingesting plastic items (Lavers and Bond, 2016).

Curiously, 37.3% of the ingested pieces of plastic appeared partially burnt or melted (Fig. 2A). The presence of burned or melted plastics in the coastal waters of Newfoundland likely originated from coastal waste disposal sites, with open burning or incineration (Newfoundland and Labrador, 2014), shoreline garbage burn piles (which are still a common practice in remote outport communities of Newfoundland; H. Nowak 2016, *pers. comm* Jan. 27), or from waste incinerated on fishing vessels (Chen and Liu, 2013). Although tens of millions of Dovekies spend the winter non-breeding season spread widely in Newfoundland offshore water (Fifield et al., 2009; Fort et al., 2013), storms and strong winds, presumably combined with poor food availability, occasionally drive birds to land. This is what occurred just prior to the wrecking event that supplied our sample of emaciated beach-cast Dovekies. These birds were likely in poor condition due to a combination of factors, including abnormal ocean conditions (sea surface temperature anomaly of +2 °C above average; Fisheries and Oceans Canada, 2013, 2014), and would have died at sea, but were retrievable because of the northerly winds that drove them onto the beach. It is therefore possible that prior to wrecking, our Dovekies may have ingested local-source plastic that they would not otherwise have encountered on the Grand Banks. To address this possibility, we present a comparison of our plastic ingestion results both with and without the inclusion of burned and melted plastics in Table 1. Although excluding burned and melted plastics did little to reduce the prevalence rate (27.5%) or the average mass and number of plastics ingested, this finding raises the possibility that utilizing beach cast birds in plastic ingestion studies may inflate plastic ingestion results, relative to the overall population. For other species, such as Northern Fulmars, it has been established that the levels of plastic ingested by wrecked birds are representative of non-wrecked birds (van Franeker and Meijboom, 2002), but this has not been established for other species. Future studies could test the hypothesis that levels of plastic ingestion in wrecked birds are representative of healthy populations by coordinating the collection of healthy birds at sea with wrecking events.

The motivation for many plastic ingestion studies is to provide a baseline against which future comparisons can be made, and adoption of standardized methods is a crucial part of this. A comparison of our study results with Dovekies collected from nearby White Bay during the same wrecking event (Fife et al., 2015), illustrates the problems that arise when standardized methods are not used. As part of a broader study on Dovekie foraging ecology and contaminant profiles, Fife et al. (2015) examined 65 beach cast birds and reported a 13.8% prevalence rate (Table 1). Surprisingly, the prevalence of plastic ingestion among the Dovekies we collected in Holyrood, Newfoundland was more than twice as high (30.4%). Despite the fact that both studies examined emaciated Dovekies collected from beaches only 350 km apart at the same time of year, key differences in the methodology undermine our ability to directly compare the results. First, although both studies examined stomach contents visually under a microscope, Fife et al. (2015) examined only the gizzard, not the proventriculus, whereas we examined both, as per standardized protocols (van Franeker et al., 2011). By only quantifying plastic in the gizzard, the previous study may have underestimated the prevalence rate, mass and number of plastics ingested by Dovekies. Second, we rinsed our samples over a 1 mm sieve to achieve a standardized smallest particle size, whereas Fife et al. (2015) did not. We suggest that the disparity in prevalence rate between these two otherwise similar studies was more likely due to a difference in methodology than regional differences, highlighting the importance of adopting standardized methods (van Franeker et al., 2011).

Over the past decade, two other studies have examined Dovekie stomach contents in Newfoundland (Table 1), although not for the express purpose of documenting plastic. One involved an examination of the proventricular and gizzard contents of beached birds in December 2003 following an oil spill (Robertson et al., 2006), while the second involved at-sea collection of Dovekies in March 2011 and subsequent examination of the proventricular content as part of dietary analysis (Rosing-Asvid et al., 2013). In both cases the prevalence of plastic ingestion observed were low (1.40% in 76; Robertson, *unpub data*) or non-existent (0% in 50; Rosing-Asvid et al., 2013). Unfortunately, the differences in analytical technique obscure whether or not there has actually been an increase in frequency of plastic ingestion, or whether this was an artifact of methodological differences between studies. Plastic load may also be influenced by the interaction between body condition and collection method (healthy birds shot at sea versus beach cast, oiled or emaciated birds), but investigations into this were beyond the scope of the present study.

Researchers working with diet samples, have a unique opportunity to contribute to our understanding of seabird plastic ingestion - but to enable future spatial and temporal comparisons, we strongly recommend that researchers follow standardized methods (i.e., van Franeker, 2004; van Franeker et al., 2011). The key elements of the standardized method are: 1) examine both the proventriculus and gizzard, 2) wash the stomach over a 1 mm sieve to achieve a common minimum particle size, and 3) separate plastics from non-plastics under a dissecting microscope, and classify the plastics under a compound microscope. Where possible, FT-IR spectrophotometry should be used to positively confirm type of plastic polymers and histological methods should be used to verify the presence of ulcers or lesions. A call to standardize methodologies for the detection of marine plastics is not new; the European Union Technical Subgroup on Marine Litter (TSG-ML) has proposed a standardized monitoring strategy across water, shoreline, sediment, and biotic monitoring techniques (Hanke et al., 2013), following similar calls for standard operation protocols for plastic pollution sampling and detection (Hidalgo-Ruz et al., 2012; Löder and Gerdts, 2015).

5. Conclusion

In addition to rigorously quantifying the physical and chemical characteristics of plastic ingestion in Dovekies, the most abundant seabird in the North Atlantic, our study demonstrated the importance of using standardized methods. We detected no adverse impact of plastic ingestion in our study of beach-cast emaciated carcasses and have underlined the importance of using histological techniques to rigorously identify internal injuries and other lesions. In this study we have documented a previously unreported source of ingested plastic (partly incinerated waste). Even though rapidly developing countries are credited with the lion's share of responsibility for marine plastic pollution (Jambeck et al., 2015) our results suggest that remote communities in developed countries are also contributing to marine plastic pollution. Finally, we suggest that caution be applied when using emaciated birds collected from wreck events to establish representative rates of plastic ingestion for a species or region.

Acknowledgements

We thank Dr. Y. Wiersma at for use of her research laboratory, Brettney Pilgrim for assistance with genetic analysis, Maryelle Nyeck for assistance with plastic polymer analysis. G. J. Robertson, P. D. O'Hara and S. P. Blomberg for providing helpful comments that improved this paper. Special thanks to G. J. Roberston and A. Rosing-Asvid for shared access to unpublished data and to L. Roberts and H. Whitney at the Animal Health Division of the Newfoundland Forestry and Agrifoods Agency for cause-of-death determination. Adam D. P. Cross generously donated his artistic talent to design the graphical abstract. Our study was made possible by funding from the National Science and Engineering Research Council (NSERC Discovery Grant #200335) to ILJ and SAG, Endeavour Postgraduate Scholarship support to SAG, support to MLV from Memorial University of Newfoundland (MUCEP program), early career funding from the Faculty of Humanities and Social Sciences, Memorial University of Newfoundland to ML, and in-kind support from the Wildlife Research Division of Environment Canada.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2016.08.062.

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