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Evaluation of meso- and microplastic ingestion by the northern fulmar through a non-lethal sampling method

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ABSTRACT

An increasing number of organisms from the polar regions are reported contaminated by plastic. Rarely a non-killing sampling method is used. In this study we wanted to assess plastic levels using stomach flushing and evaluate the method suitability for further research and monitoring. The stomach of 22 fulmars from Bjørnøya, Svalbard, were flushed with water in the field. On return to the laboratory, the regurgitated content was digested using potassium hydroxide. The extracted plastics were visually characterised and analysed with spectroscopy. Only three birds had plastics in their stomach, totaling 36 particles, most of them microplastics (< 5 mm). The plastic burdens are much lower than previously reported in Svalbard. The stomach flushing is assumed not to allow the collection of the gizzard content. This is a major limitation as most of the plastics accumulate in the fulmar's gizzard. However, the method is still useful for studies investigating plastic ingestion dynamics, allowing to sample the same individuals over time.

1. Introduction

Plastic pollution is a global problem, even in remote regions such as Antarctic and Arctic waters (reviewed in Bergmann et al., 2022; Caruso et al., 2022). Increased research into plastic pollution in the polar regions shows plastic contamination in organisms and compartments previously thought uncontaminated (Benjaminsen et al., 2022; Collard and Ask, 2021; Grøsvik et al., 2023; Meyer et al., 2023; Technau et al., 2022; Tekman et al., 2020). Despite the increasing number of scientific articles on plastic pollution, there is only one species used as a bio-indicator for plastic pollution in a legislative framework, the northern fulmar *Fulmarus glacialis* (OSPAR Commission, 2021), hereafter called fulmar. Several characteristics such as feeding ecology and gut anatomy, make the fulmar ingest -and retain- a lot of plastics in their stomach. That stomach is divided in two parts, the proventriculus (or forestomach) and the gizzard (or ventriculus), where hard particles accumulate until they are small enough to pass through the pylorus and reach the intestine to be evacuated. Fulmars have been defined as a bio-indicator for the North Sea region in 2008 by the Oslo-Paris Convention (OSPAR Commission, 2008) and for Iceland more recently, in 2018 (Snæþórsson, 2021), and are recommended for biomonitoring in the Arctic (Arctic Monitoring and Assessment Programme, 2021) The OSPAR convention established the Ecological Quality Objective (EcoQO) based on beached fulmars where the proportion of birds having 0.1 g of plastic in their stomach should not exceed 10 % (OSPAR Commission, 2010).

Evidence of harm in seabirds caused by the interaction with plastic debris has been known for a long time (e.g. Pettit et al., 1981) and new impacts on health have been recently discovered, on fulmars but also on other species (Charlton-Howard et al., 2023; Fackelmann et al., 2023; Rivers-Auty et al., 2023; Tulatz et al., 2023) which supports the need for a more extended biomonitoring program on seabirds, for example in the Arctic where beached birds are difficult to obtain because of the remoteness of the region and presence of scavengers. Also, to sample

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healthy birds is often a prerequisite for the evaluation of the effect of microplastic (MP, < 5 mm) ingestion on the body condition and the exposure to plastic-related chemicals. It is therefore important to assess other ways to investigate plastic ingestion. Regardless of the context of the study, the stomach -or the gastrointestinal tract content- is most of the time the only matrix used to assess plastic pollution in many animals. Studies on seabirds, including fulmars, are no exception and alive birds are therefore sacrificed to collect the stomach content for further analyses, if no beached birds are used. Rarely a non-lethal sampling method is used, such as stomach flushing, regurgitates or scanning technologies. Scanning technologies, such as magnetic resonance imaging (MRI) scanning, are not adapted to be used in the field even though promising from tests in the laboratory (Anderssen et al., 2022). Regurgitates provide important information on recently ingested plastic but do not provide with certainty a complete sample and they cannot be collected from birds which are unable to regurgitate (Provencher et al., 2019). The stomach flushing method was already used in the 1970s to study the diet of reptiles (Legler, 1977) and birds (Moody, 1970). The stomach flushing method has over the years improved (Wilson, 1984) and some studies are using this for plastic pollution research (e.g. Lavers et al., 2014; Verlis et al., 2013). Even though this technique shows some limitations, it at least avoids euthanizing the studied animals, a factor of importance especially when the studied species is considered endangered or rare, and the sacrifice of many individuals is not desirable (Randall and Davidson, 1981). Euthanizing further prevents repeated sampling e.g. when studying the dynamics of a population where the same individuals are tracked and sampled for stomach content several times a year (Randall and Davidson, 1981). To our knowledge, the flushing method has not been used yet on the northern fulmar in the context of plastic pollution. The aim of this study is therefore to assess plastic ingestion in fulmars using stomach flushing and evaluate the method suitability for further research.

2. Methods

2.1. Sampling

In July 2018 and July 2019, the stomach contents of 10 and 12 breeding adult fulmars, respectively, were sampled in Herwighamna, Bjørnøya, Svalbard. The birds were captured by using a snare attached to a pole. The stomach flushing method described by Wilson (1984) was used. The bird was contained in a bird bag with the head sticking out and the beak was held open, while a rubber tube was gently guided down the throat. Lukewarm water was pumped into the bird, until it started flowing out. The tube was then removed, and the bird turned upside down over a tray, the throat was massaged gently to induce regurgitation. If the bird regurgitated after a small amount of water was pumped, the process was repeated. The bird was then released, the sample rinsed from the tray into a container which was marked and frozen and sent to the Norwegian Polar Institute, Tromsø, Norway, for analysis.

2.2. Plastic extraction

For the samples collected in 2019, the stomach content was first sieved through a stainless-steel sieve with a 1-mm mesh size. The material on the sieve was then rinsed off with a 10 % KOH solution into a glass beaker. More KOH was then added to reach a ratio of 3:1 (*v*/v, Rochman et al., 2015). The mixture was then left for 24–48 h to digest. Afterwards, the mixture was sieved through the same sieve mentioned previously. The hard particles were then rinsed off with milliQ water into a filtration unit containing a filter membrane in cellulose acetate (5 μm mesh size, Sartorius). For the samples collected in 2018, the content was sieved through two sieves, 100 μm and 20 μm. the procedure is then the same as described hereabove. The filter membrane was rinsed with ethanol and all hard particles were stored in a 15-ml tube. In this study we focused on the larger size class (> 100 μm), the smaller being stored for future studies. For the samples collected in 2019, the filtering membranes were stored for further analyses.

2.3. Spectroscopic analyses

The particles from the two sample sets (2018 and 2019) were analysed with two different instruments. The samples collected in 2018 were analysed by Raman spectroscopy and the ones collected in 2019 were analysed by FTIR spectroscopy.

Prior to the Raman analyses, the 15 ml tubes, containing the particles, were centrifuged at 5000 rpm for 5 min (Collard et al., 2015). The bottom of the tube was collected (around 2 ml) and spread onto a stainless-steel plate (Collard et al., 2021). Spectra were obtained using a LabRam 300 spectrometer (Horiba Jobin-Yvon) equipped with an Olympus BX 40 confocal microscope and an Andor BRDD iDus CCD detector cooled at −70 °C. Depending on the particle, two excitation sources were used: a 532-nm or a 785-nm diode laser. The maximal beam laser powers at the particle surface were 5 mW and 30 mW, respectively. A 600 or a 1200 lines/mm grating was used with the 532 nm and 785 nm lasers, respectively. Obtained spectra were matched with reference spectra with Omnic Specta software (Thermo Fisher Scientific, U.S.A.). All the hard particles were checked for composition.

For the samples collected in 2019, a portable Fourier-transform infrared (FTIR) spectrometer “Cary 630” (Agilent Technologies, Santa Clara, USA) with a Diamond Attenuated Total Reflectance (ATR) sampling accessory was used to identify the composition of the particles. The spectral resolution was set at 8 cm^{-1} and spectra were collected between 650 and 4000 cm^{-1}. Thirty-two scans were accumulated for each analysis. After each sample, the crystal was cleaned with 2-propanol on a wipe. Acquired spectra were compared to those in the ATR Demo reference library. In both cases, only the polymers showing a match equal or superior to 0.7 (Joint Research Centre, 2013) were included in the results.

All the particles were photographed to measure their maximum length with the ImageJ software (v1.52). Unfortunately, 5 plastic particles could not be measured as they were lost during manipulation. Four of them were red fibres in sample 2018–09. The colour was also recorded using the eight categories (off/white–clear; grey–silver; black; blue–purple; green; orange–brown; red–pink, or yellow) defined by (Provencher et al., 2017) and the type was determined following the “Save the North Sea” protocol (van Franeker et al., 2005) from 5 categories: fragment, sheet, foam, thread (all user plastics) and pellet (industrial plastic), with one more category, fiber, as all particles were included in the results. The plastic particles extracted from the stomach contents of 2019 were weighed (Quintix64-1S, Sartorius AG, Göttingen, Germany, precision 0.0001 g).

In this study, all plastic particles visible with the naked eye, including fibres, were included, with a lower detection limit of 100 μm and 1 mm for the samples collected in 2018 and 2019, respectively. However, to improve the comparability with studies following the OSPAR protocol (OSPAR Commission, 2008), results are also given only for particles larger than 1 mm and with fibres excluded. The type was therefore categorized following the “Save the North Sea” protocol (van Franeker et al., 2005): fragment, sheet, foam, thread (all user plastics) or pellet (industrial plastic). Since we focused on visible particles, our samples were less prone to contamination. To ensure the absence of contamination, we performed one blank with the same water than used to flush the birds' stomach to check for any procedural contamination which might have come from the equipment or consumables. No plastic piece was found in the blank.

3. Results

In total, 36 plastic particles were extracted from the stomach contents of 22 adult fulmars (Table 1). Only three birds had plastics in their stomach after applying the flushing technique (percent of occurrence of

Table 1
Overview of ingested microplastics (MP), ND: not determined, PA: polyamide, PE: polyethylene, PET: polyethylene terephthalate, PP: polypropylene.

Sample ID	Number of plastic pieces	Polymer	Shape	Colour	Length (mm)
2018-Blank	0				
2018-1	0				
2018-2	2	PE	Fragment	White	5.65
					3.16
2018-3	0				
2018-4	0				
2018-5	0				
2018-6	0				
2018-7	0				
2018-8	0				
2018-9	24	PET	Fiber	Red	0.76
					1.42
					0.72
					0.39
					0.45
					1.04
					1.79
					0.96
					0.76
					0.81
					0.52
					0.22
					0.41
					0.55
					0.87
					0.70
					0.93
					1.32
					0.77
					1.43
					ND
					ND
					ND
	1	PA	Pellet	Off	ND
2018-10	0				
2019-01	0				
2019-02	0				
2019-03	0				
2019-04	0				
2019-05	0				
2019-06	0				
2019-07	0				
2019-08	0				
2019-09	1	PE	Fragment	Yellow	4.14
	2	PP	Fragment	Off	4.20
	3	PP	Sheet	White	3.23
	4	PA	Pellet	Off	4.03
	5	Rubber	Fragment	Black	4.96
	6	PP	Fragment	Grey	4.29
	7	PP	Fragment	Yellow	6.45
	8	PP	Thread	Green	5.20
	9	PP	Sheet	Off	4.86
2019-10	0				
2019-11	0				
2019-12	0				

13.6 %). On average, 1.6 particle (± 5.5 SD) of plastic was found per stomach. The variability was high as the maximum of plastic pieces in one bird was 25, i.e. almost two thirds of the total number of plastic pieces, with 23 being fibres. Those fibres were made of polyethylene terephthalate (PET) but other polymers were also found (Table 1). The average length was 2.16 mm (± 1.89 SD) and 77.8 % of all plastics were microplastics (≤ 5 mm). Several colours were reported but red was the most numerous due to the high number of similar red fibres in a stomach sampled in 2018. The plastic particles from the 2019 samples weighed 52.3 mg altogether.

According to the OSPAR guidelines, only 12 particles should have been included in the results, once the fibres and the small particles (< 1 mm) are excluded leading to an average of 0.5 particle per stomach. The percent of occurrence would though remain the same (13.6 %). The average length, however, would then be 4.56 mm (± 1.02) as most of the red fibres were smaller than 1 mm, increasing the average length. The total weight of the plastics from the samples collected in 2019 (Fig. 1) would still be 52.3 mg and no bird would exceed the EcoQO performance threshold of 0.1 g (OSPAR Commission, 2008).

4. Discussion

Compared to previous studies performed in Svalbard, the plastic burdens found in fulmars in this study using the flushing technique were quite low. The percent of occurrence, the average number of plastic pieces and the average mass of plastic in the fulmars' stomach are all much lower than previously reported on the same species in other parts of the Svalbard region (Table 2). Several factors could explain this difference, for instance the occurrence of regurgitation as a defence mechanism while manipulating the bird for sample collection. The latter did happen a few times in our fieldwork but the regurgitated volumes seemed non-significant compared to the stomach contents we were able to collect through stomach flushing. A more important factor is the effectiveness of stomach flushing to collect plastic pieces ingested by fulmars. The stomach flushing is assumed to allow the collection of the proventriculus content only due to the isthmus juncture, the gizzard remaining unavailable (Ryan and Jackson, 1986; Trevail et al., 2015; Verlis et al., 2013). This is a major limitation when investigating plastic pollution as most of the plastic pieces ingested by fulmars accumulate in the gizzard and the number of pieces is much higher there than in the proventriculus (Collard et al., 2022a; Mallory, 2008; Terepocki et al., 2017). Plastics do accumulate in the gizzard where they, together with other hard particles, will be ground up into smaller particles. The retention time of those particles can consequently be very long, from several weeks up to a year according to some studies (Nania and Shugart, 2021; Ryan, 1988; Ryan and Jackson, 1987; Terepocki et al., 2017; van Franeker and Law, 2015). It can however be useful if a snapshot of the most recently ingested plastic particles is needed, the main limitation being that very small particles may have reached the gizzard already. To ensure a more reliable collection, a particle size limit should be set, fitting the isthmus juncture diameter. Sampling the gizzard content, where plastics accumulate, is of high importance as plastics may negatively impact the bird's health through both physical and toxicological effects, such as puncture, fibrosis and pollutant leaching (Charlton-Howard et al., 2023; Tanaka et al., 2020; Tulatz et al., 2023). Another factor is the sampling season. In this study the birds were sampled late July, when the chicks have hatched and are fed by the parents. Previous studies reported a parental transfer of plastic through regurgitation during the chick-rearing period (Rodríguez et al., 2012; Ryan, 1988; Tulatz et al., 2023). In the case of Svalbard, the comparison with other studies is challenging as some of them included several age classes (Collard et al., 2022a; Trevail et al., 2015; Tulatz, 2021). Those which included adult birds did not sample in July but rather in March (Collard et al., 2022a) or September (Trevail et al., 2015; Tulatz et al., 2023). The only other study focusing on Bjørnøya did not mention what time of year the fulmars were collected (van Franeker, 1985). Therefore, it is impossible to evaluate the impact of the parental transfer in fulmars from Svalbard. We recommend that future studies on plastic ingestion in fulmars, and seabirds globally, report the timing of sampling and breeding conditions (Provencher et al., 2017).

From the several studies performed in Svalbard, only one was carried out with fulmars sampled in Bjørnøya (van Franeker, 1985) and our study is the only one which used that method among all the published articles on plastic pollution in fulmars from Svalbard. Although the plastic levels in those birds were also quite low compared to other places in Svalbard, the percent of occurrence was still high: 82 % of the sampled birds ($n = 22$) had plastic in their stomach. This supports the assumption that the full stomach content, i.e. the gizzard content, is not

Fig. 1. Example of plastic pieces found in fulmar 2019-09. Scale bar: 5 mm.

Table 2
Summary of data on plastic ingestion by fulmars in Svalbard (modified from Collard et al., 2022a). PO: percent of occurrence, OSPAR: results according to OSPAR guidelines.

Sampling year	Sampling method	PO (%)	Plastic burden	Region	Study
1980	Dissection	82	4–5 items/ind.	Bjørnøya	van Franeker, 1985
1997	Dissection	91	10.3 items/ind. 0.07 g/ind.	Kongsfjorden	Collard et al., 2022b
2013	Dissection	87.5	15.3 items/ind. 0.08 g/ind.	Isfjorden	Trevail et al., 2015
2018–2019	Flushing	13.6	1.6 item/ind.	Bjørnøya	This study
2018–2019 (OSPAR)		*13.6*	*0.5 item/ind.*		
2020	Dissection	95	36.1 items/ind. 0.21 g/ind.	Kongsfjorden	Tulatz et al., 2023
2021	Dissection	100	45.5 items/ind. 0.31 g/ind.	Kongsfjorden	Collard et al., unpublished data

recovered from the stomach flushing, leading to an underestimation of the plastic levels in fulmars collected for this study. In addition, there are almost 40 years between the sample collections. Back in 1980, one can think that, although present, the marine environment was less polluted in plastic. The global production of plastic was 65 million tonnes against 368 million tonnes in 2019, between 5 and 6 times more than 40 years ago. It is estimated that approximately 7 billion of the estimated 9.2 billion of cumulative plastic production between 1950 and 2017 became plastic waste, three-quarters of which was placed in landfills, were mismanaged or ended up in the environment (United Nations Environment Programme, 2021). Despite this, more plastic was found in fulmars collected in 1980 than in our study, in 2018 and 2019. The stomach flushing method does not provide a complete overview of all the plastics ingested in the recent history of the bird, but it can still be useful for studies which want to investigate the dynamics of plastic pollution in fulmars, or other organisms, over time allowing to sample the same individuals if marking the bird is possible.

Fisheries are a likely source of plastic in the Arctic (Grøsvik et al., 2018), and has been mentioned in some previous studies focusing on marine organisms (Liboiron et al., 2019, 2016; Murray and Cowie, 2011). In this study, only one particle is suspected to find its origin from fishing gear, more specifically fishing net or rope. That particle is a green thread-like particle made of polypropylene, a polymer commonly used to produce fishing nets. The Barents Sea gather several fisheries area and therefore more thread-like particles were expected to be found in the stomachs compared to hard fragments. A possible sorting from the bird could explain this finding. Fragments might look more like usual preys of fulmars. Due to one individual, a lot of red fibres in polyethylene terephthalate (PET) were found. PET is a common polymer used in textile, which could originate from effluents, local or from a distant origin. Very few towns or settlements in the Arctic are equipped with wastewater treatment plants, leading to a direct release of used waters in the environment. Among others, each washing cycle can produce between hundreds to millions of fibres (De Falco et al., 2018; Pirc et al., 2016; Sillanpää and Sainio, 2017) that will be directly release to the environment if there is no mechanical process to retain them.

Bjørnøya is located in the western Barents Sea, and is the southernmost island in the Svalbard archipelago. Bjørnøya is surrounded by Arctic waters, but is also close to the Polar front constituting the border between the Atlantic water masses, coming for lower latitudes, and Arctic water masses. The fulmar, once the chick is a few days old, spends less time at the colony and increase their foraging distance (Hamer et al., 1997). Depending on the population, fulmars can have a foraging range of 245 km (Hamer et al., 1997). With such long trips, fulmars from Bjørnøya could feed themselves, or their chicks, both in Arctic and Atlantic water masses, Recent tracking conducted as part of the

SEATRACK program indicate that fulmars breeding on Bjørnøya forage in Arctic water masses late in the breeding season and during the post-breeding period (https://seatrack.seapop.no/map/). Due to the huge foraging range of fulmars, future studies of plastic pollution in this species should be combined with population specific tracking to reveal their area use at sea. This will make it possible to be more specific about where the fulmar gets contaminated and make the long-term monitoring more representative.

In conclusion, as expected, stomach flushing provides different results than necropsies. The main question to be answered is what proportion of ingested plastics is the stomach flushing collecting when performed on fulmars? After this question is answered, stomach flushing could be used for biomonitoring as it still has the main advantage of being non lethal. Depending on the scientific question to be answered and the ethics of future studies, stomach flushing should be considered to spare lives of birds.

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Ethical statement

The samplings were allowed by the Governor of Svalbard under the permits nr. 16/00657- 21 and 16/00657-26 for 2018 and 2019, respectively. The manipulations of the birds in the field were also approved by the Norwegian Food Safety Authority (Mattilsynet, file reference: 16297). The birds were handled by experienced staff and the utmost was done to avoid additional stress to the birds.

CRediT authorship contribution statement

France Collard: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Visualization, Funding acquisition. **Hallvard Strøm:** Resources, Writing – review & editing. **Marie-Océane Fayet:** Investigation, Writing – review & editing. **Fannar Þeyr Guðmundsson:** Resources, Writing - review & editing. **Dorte Herzke:** Resources, Writing – review & editing. **Ådne Hotvedt:** Investigation, Writing – review & editing. **Arja Løchen:** Resources, Writing – review & editing. **Cédric Malherbe:** Resources, Writing – review & editing. **Gauthier Eppe:** Resources, Writing – review & editing. **Geir W. Gabrielsen:** Investigation, Resources, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: France Collard reports financial support was provided by the Fram Centre. Hallvard Strøm reports financial support was provided by the Research Council of Norway and the Norwegian Polar Institute.

Data availability

Data will be made available on request.

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