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Recommended best practices for plastic and litter ingestion studies in marine birds: Collection, processing, and reporting

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Abstract

Marine plastic pollution is an environmental contaminant of significant concern. There is a lack of consistency in sample collection and processing that continues to impede meta-analyses and large-scale comparisons across time and space. This is true for most taxa, including seabirds, which are the most studied megafauna group with regards to plastic ingestion research. Consequently, it is difficult to evaluate the impacts and extent of plastic contamination in seabirds fully and accurately, and to make inferences about species for which we have little or no data. We provide a synthesized set of recommendations specific for seabirds and plastic ingestion studies that include best practices in relation to sample collection, processing, and reporting, as well as highlighting some "cross-cutting" methods. We include guidance for how carcasses, regurgitations, and pellets should be handled and treated to prevent cross-contamination, and a discussion of what size class of microplastics can be assessed in each sample type. Although we focus on marine bird samples, we also include standardized techniques to remove sediment and biological material that are generalizable to other taxa. Lastly, metrics and data presentation of ingested plastics are briefly reviewed in the context of seabird studies.

Key words: bird, bolus, diet analysis, marine debris, method standardization, necropsy, plastic debris, plastic ingestion

Introduction

Seabirds are particularly vulnerable to ingesting marine plastic pollution. Most seabird species feed at or near the ocean surface, the same zone in which marine plastics occur at a high density, along eddies and oceanic convergence zones, mistaking the plastics for prey items (Cadée 2002; Janinhoff et al. 2010;

Law et al. 2010; Song et al. 2014; Reisser et al. 2015). Ingestion of plastic was first reported in seabirds in the 1960s (Threlfall 1968; Kenyon and Kridler 1969), and there is a now a relatively comprehensive and widespread body of literature for seabirds compared with other marine megafauna groups (Provencher et al. 2017). Most of this work has focused on recording the type and number of items ingested by seabirds (i.e., frequency of occurrence; Provencher et al. 2017), with plastics reported in >200 species of seabirds (Kühn et al. 2015).

Currently the only species (of any biota) where a published, standardized protocol has been adopted to assess plastic ingestion is the seabird the northern fulmar (*Fulmarus glacialis* (Linnaeus, 1761)) (van Franeker et al. 2011; van Franeker and Law 2015; Provencher et al. 2017). These methods have been applied successfully in the broader North Atlantic region as well as in the North Pacific Ocean and have enabled some of the few, ocean-scale comparisons of marine plastic pollution over time and space (van Franeker et al. 2011; Provencher et al. 2017; Avery-Gomm et al. 2018). Although these methods of quantifying plastic pollution are often used elsewhere, most studies fail to report plastic ingestion results in a comprehensive way (O'Hanlon et al. 2017; Provencher et al. 2017).

Standardized reports of plastic ingestion (or lack of observed plastic ingestion) are important because they: (1) highlight the magnitude of the plastic pollution issue; (2) contribute to meta-analyses that report temporal, spatial, and taxonomic trends in plastic ingestion; and (3) could help elucidate the population level effects of plastic ingestion via correlative studies of load and demographic rates. Note that the absence of plastics in species is an important component of these standardized approaches (Provencher et al. 2017). Additionally, among species that are suitable as biological indicators of marine plastic pollution, reporting can provide a cost-efficient way to monitor the progress of waste management or plastic source reduction strategies that aim to reduce marine plastic pollution.

Unfortunately, a lack of standardized methods for sample collection along with comparable processing and reporting can undermine broad-scale assessments of the extent and impact of marine plastic debris on marine biota. The importance of lab processing methods was recently illustrated when two studies documenting plastic ingestion in birds from the same stranding event could not be compared because they followed different methods (Fife et al. 2015; Avery-Gomm et al. 2016). Additionally, some studies have not processed birds and presented data in the same way, such as reporting differences between sexes or among age classes (Provencher et al. 2014; Roman et al. 2016). Given that data on plastic and debris ingestion by seabirds form some of the most comprehensive datasets on marine pollution in terms of spatial, temporal, and phylogenetic coverage, there is a need to quickly standardize sample collection, processing, plastic quantification and reporting so as to improve the integrity of plastic ingestion research, and will facilitate statistically and scientifically credible large-scale comparisons in the future.

As plastic reduction policies are developed and implemented, directly comparable data are needed to test the efficacy of these policies at reducing plastic pollution in biota. Data from northern fulmars in the North Sea were used to test policy targets for plastic reduction, and have since helped policy makers and researchers evaluate if the policy goals were realistic and achievable (van Franeker et al. 2011; OSPAR 2015, 2017; Provencher et al. 2017). With a growing recognition of the importance of evidence-driven policy, consistent and repeatable monitoring methods and approaches are needed to evaluate reductions in the abundance of plastics in the world's oceans (Borrelle et al. 2017).

We provide a synthesized set of recommendations specific for seabirds and plastic ingestion studies that include best practices in relation to sample collection, processing methods, and reporting. We include standardized methods for assessing plastic ingestion by seabirds using necropsy, regurgitations, and pellets. Although each method has advantages and disadvantages (**Table 1**), each is

Table 1. Advantages and disadvantages of different methods used in seabirds to assess ingested and accumulated plastics.

	Pros	Cons
Necropsy—carcass examination (dissection)	- Can determine entire plastic burden - Tissues can also be easily taken for plastics-associated contaminants analysis - Sex and age, health status, and cause of death can be determined - In case of beach-cast birds: non-invasive	- Does not always allow for consistent repeated sampling - Is usually opportunistic without options for preplanned sampling in relation to season, sex or age - In the case of hunting specifically for research samples this technique is invasive
Regurgitations	- Can be repeated on the same individuals to compare plastic load between seasons and years - In comparison to hunting birds for research, regurgitations are relatively less invasive	- Not all species can be made to regurgitate - Does not guarantee a complete sample - Low but real possibility of injury occurring to the bird - The individual may lose a meal that was costly to acquire
Pellet collection	- Easily collected - Non-invasive collections - Can be repeated between seasons and across years - Can be regularly sampled - Can be implemented easily and at low cost	- Cannot be used to assess microplastics - Depending on the species, may be difficult to attribute pellets to individuals - Does not guarantee a complete sample - Typically only useful during the breeding season when birds are occupying nesting sites on land

appropriate in some scenarios. Adopting this approach will move the global plastic research community towards addressing some of the key questions about how or why species are affected by plastics, and other global research objectives that extend beyond the jurisdiction of a single species or study.

Methods

We define seabirds to include gulls, terns, skimmers, skuas, auks, and phalaropes (Charadriiformes), tropicbirds (Phaethontiformes), penguins (Sphenisciformes), petrels, shearwaters, and albatrosses (Procellariiformes), cormorants, frigatebirds, boobies, and gannets (Suliformes), and pelicans (Pelecaniformes) consistent with the definition proposed by Gaston (2004), with some updated classifications (Boesman et al. 2017). Although we focus on marine birds, these methods are applicable to all birds, particularly other aquatic species such as loons (Gaviiformes) and waterfowl (Anseriformes), in which plastic has been recorded (Avery-Gomm et al. 2013; Provencher et al. 2014; English et al. 2015; Holland et al. 2016; Gil-Delgado et al. 2017), and much is transferable to studies of ingested plastics in other vertebrates (e.g., marine mammals, turtles, and fish).

Sample collection in the field

Collection of intact carcasses

Dissection of carcasses is the most commonly used technique for assessing ingested plastics in seabirds (Provencher et al. 2017), and has several advantages over the other methods described here, including the ability to determine age/sex of individuals, potential internal pathology related to plastic burdens, and the most accurate measurement of plastic particles (van Franeker et al. 2011; Acampora et al. 2014; Jiménez et al. 2015; Box 1; Table 1). Several different measures of body condition can be examined, which is useful when exploring the potential implications of plastic ingestion on the health of biota including pectoral muscle size, body mass, and subcutaneous fat (Fry et al. 1987; Harper and Fowler 1987; van Franeker et al. 2011; Donnelly-Greenan et al. 2014). Carcasses can also readily permit sampling of internal tissues for subsequent pathological or chemical analyses (Lavers et al. 2014; Fife et al. 2015; Herzke et al. 2016), and in some jurisdictions permits and licenses are easier to obtain for salvaging carcasses than working with live birds.

Box 1. Key metrics to measure and report in seabird plastic ingestion studies.

- Species

- Age

- Sex

- Body condition metrics: pectoral muscle size, body mass (when the bird is dry and clean), and subcutaneous fat

- Cause of mortality (with necropsy, and where possible)

- Report zeros

Carcasses can come from a variety of sources, including beached birds, fisheries bycatch, ship strikes, rehabilitation centres, collaboration with hunters, as well as collection for scientific research (Provencher et al. 2017). The disadvantage of carcass collection is that it is generally opportunistic. Carcasses can often only be collected at irregular time intervals, which may be limited to species that are most likely to be beach-cast or hunted (Avery-Gomm et al. 2012; Provencher et al. 2014; Seif et al. 2018).

Although all carcasses should be treated in the same way when examined for ingested plastics, it is important to consider the source of mortality as it can influence the levels of plastics reported. The cause for mortality may or may not represent a true sample of the population and, thus, may affect how the results are interpreted. Plastic ingestion data may be biased in beached birds or those that died of unknown causes, as plastic may have contributed to the death of the animal (Auman et al. 1998) or have been a consequence of its poor condition. Although there were no significant differences in plastic burdens between beached northern fulmars and those that died in good condition (as a consequence of e.g., drowning in nets or by collision with ship cables, lights and antenna cables, etc.; van Franeker and Meijboom 2002), Short-tailed shearwaters (*Ardenna tenuirostris* (Temminck, 1835)) differed in their plastic burden depending on how they died (Rodríguez et al. 2018).

Regurgitations from birds

Some species can be made to regurgitate items in the field (**Fig. 1**), and 17% of published papers reported data on ingested plastics in seabirds using regurgitations, water-offloading (lavage or flushing), or emetics (Provencher et al. 2017). An advantage of regurgitations is that they provide a sample of items consumed very recently. Regurgitations also allow targeted sampling of species that are not commonly found as carcasses (i.e., non-hunted species and those not affected by fisheries bycatch or rarely found beached), and can provide particularly valuable insights into individual behaviour or preferences. Targeted sampling allows dedicated study designs with pre-determined sample sizes, focusing on particular age classes, and repeated sampling of individuals.

The main challenge with this suite of sampling techniques is acquiring a complete sample. Fijn et al. (2012) found considerable differences between diet data obtained from birds that had first regurgitated spontaneously, and were subsequently lavaged. Lavage likely returns the complete proventricular contents (**Fig. 2**), but it is less clear whether the entire contents of the gizzard are flushed out. In Procellariiformes, gizzard contents may not be regurgitated (Furness 1985; Ryan and Jackson 1986), whereas gizzard contents were regurgitated completely when using emetics (Bond and Lavers 2013). Between 6% and 22% of plastic items remained inside shearwater
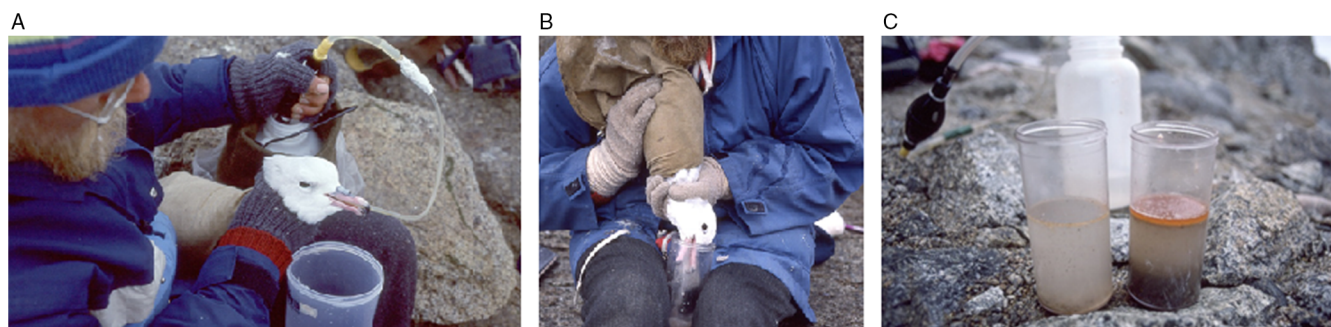
Fig. 1. A southern fulmar (*Fulmarus glacialoides*) being lavaged in the field. (A) using a simple hand pump, hand-warm water is pumped into the stomach of the bird until fully filled; (B) the bird is then held over a sample container to collect the regurgitation, this is done at least twice to check for completeness of the sample; and (C) the regurgitate is then collected for examination back in the laboratory (right first flush, left second flush). Photos courtesy of Jan van Franeker.
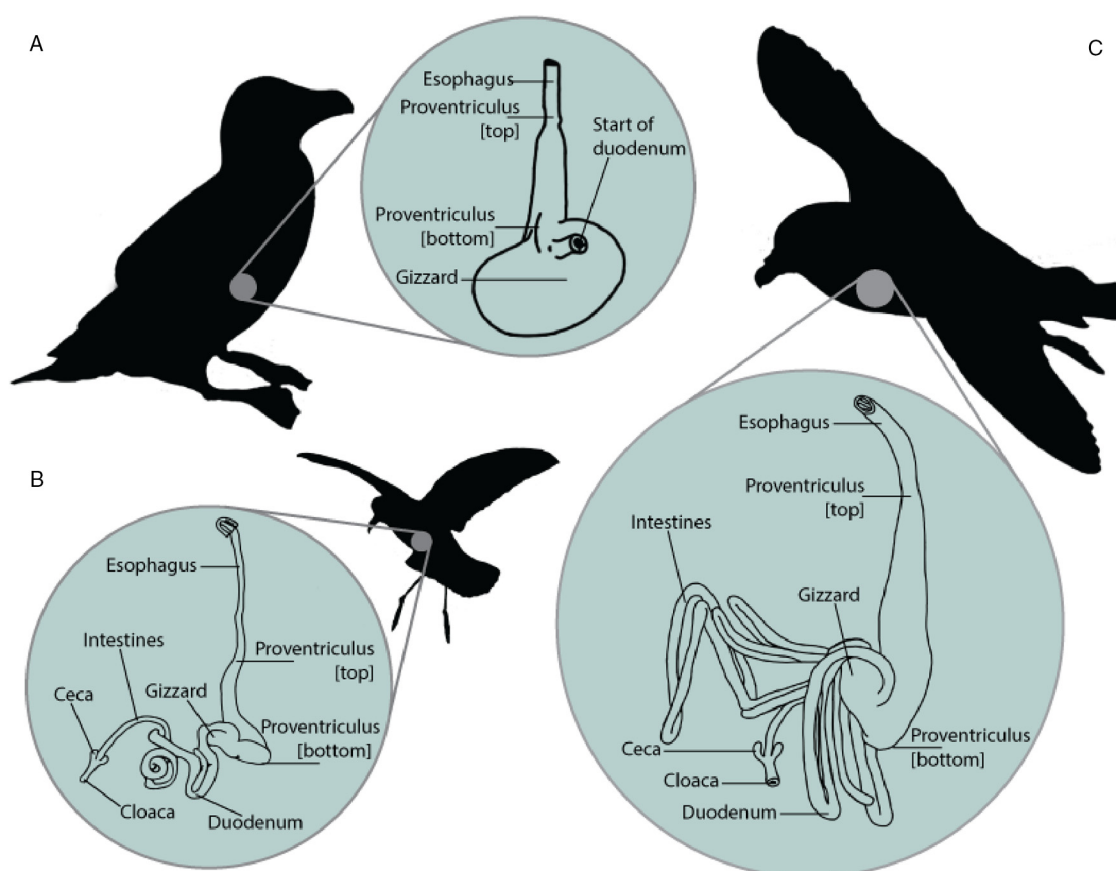

Fig. 2. Gut morphology of several seabird groups that can be examined for accumulated plastic pieces. Although many papers refer to plastics in the gastrointestinal tract (GIT), often only portions of the GIT are examined. Most seabird studies examine the gizzard and proventriculus, but the portion examined depends on the species and the research questions of interest. For studies examining plastic or microplastic excretion, the entire intestinal tract may be of interest. (A) a simplified auk GIT, (B) a storm-petrel GIT, and (C) a fulmar GIT. Adapted from Matthews (1949).

fledglings after lavage (Hutton et al. 2008; Lavers et al. 2014), particularly in individuals with large amounts of plastic, and therefore mass and frequency data collected using this technique should be considered underestimates in some species (Bond and Lavers 2013). Lavage and emetics may be ineffective in some groups of seabirds, such as those that do not regurgitate food to offspring (e.g., auks, but see Wilson et al. 2004). The completeness of the sample likely depends on the species' stomach morphology, as different species have differently shaped gastrointestinal tracts (GITs; Fig. 2), and the methods applied.

The impacts of stomach lavage on seabirds are species specific. In some penguins, there were no negative effects of stomach lavage on adult survival, chick growth, or breeding success, and the procedure did not alter the foraging behaviour of adults (Robertson et al. 1980; Goldsworthy et al. 2016). However, in diving petrels there was 2.3% mortality (5 out of 220 handled birds; Jahncke et al. 1999) following lavage, and in Leach's storm-petrels (*Oceanodroma leucorhoa* (Vieillot, 1818)), 8% mortality occurred when emetics were used (Bond and Lavers 2013). In 323 stomach flushings of four species of Antarctic fulmarine petrel and one southern fulmar (*Fulmarus glacialoides* (Smith, 1840)), 0.3% died because a large prey item became stuck in the throat and caused shock. In breeding pairs with chicks, sampling was restricted to one parent during the season, which had no detectable effect on adult foraging behaviour or chick growth until fledging (van Franeker et al. 2001). Although lavage and emetics both require training and have an associated risk of harm to the animal if not done correctly (e.g., aspiration of fluid into the lungs), both can be useful methods in specific cases (Gaunt and Oring 1997; Bond and Lavers 2013).

Many birds regurgitate immediately upon being captured and, therefore, can be sampled using this technique relatively easily (Bond et al. 2010). However, not all individuals will regurgitate, or they will do so only partially, which makes distinguishing individuals with a true absence of plastic from those who contain plastic but did not regurgitate it difficult. For these species lavage or administering emetics may be preferable. For example, spontaneous regurgitations by gull chicks were incomplete and, therefore, underestimated the amount of ingested plastic to an unknown extent (Hunt 1972). Plastic burden may also be underestimated in spontaneous regurgitations if samples are collected from the ground, or plastic sheets placed below mist-nets, where small pieces of plastic may not be detected. For example, in a sampling of 973 Leach's storm-petrels using spontaneous regurgitation between 1987 and 1988 and 2002 and 2006 from a single site in Newfoundland, 5%–6% contained plastic (Provencher et al. 2014). However, the same population sampled in 2012 using emetics ($n = 63$) was found to have a 48% frequency of occurrence of plastics, with all pieces <17 mg (Bond and Lavers 2013).

Collection of pellets

Several species regularly regurgitate pellets composed of indigestible items. These pellets or boluses can be found around nesting sites (Fig. 3), and 10% of plastics studies between 1965 and 2016 used this technique (Provencher et al. 2017). Examining pellets is a useful, non-destructive sampling technique (Lindborg et al. 2012; Hammer et al. 2016), and has been used most frequently for scavenging species, such as gulls and skuas. It can also be suitable for other seabirds such as cormorants, shags, and terns that produce pellets (Barrett et al. 2007; Álvarez et al. 2018). However, some species known to have the highest level of plastic accumulation (e.g., Procellariiformes) do not typically produce pellets, and this method is not suitable for those species. Furthermore, this technique does not allow researchers to quantify how much plastic is left in the bird—unlike lavage, emetics, or gastrointestinal analysis of carcasses. As this technique is used with a variety of species, it is important to consider species-specific differences in the amount of plastic ejected in pellets and retained in the bird.

Different types of pellets have different production rates (Votier et al. 2001). Single pellets may not represent single meals, but an approximate conversion rate can be calculated based on the species of

Fig. 3. A regurgitated pellet collected from a great skua (*Stercorarius skua*). Photo courtesy of Sjúrður Hammer.

interest (Ryan and Fraser 1988; Votier et al. 2001; Hammer et al. 2016). In contrast to the accumulated plastics found during stomach dissections, pellets, like regurgitations, represent a snapshot of only a short period of time. Most pellet collections are also limited to the breeding season when marine birds are at terrestrial nesting sites, limiting seasonal comparisons in many species.

When collecting pellets, their condition should be graded. The type of prey that constitute the pellets may determine how long they will last on the ground before they disintegrate, which may bias the amount of plastic found in one type of pellet over another. To overcome this, the integrity of the pellet should be recorded as: (1) fresh/wet (not to be mistaken for wet due to precipitation) and structurally intact, (2) dry and structurally intact, (3) partially broken down, and (4) completely broken down (individual fragments found). The likelihood of finding plastic decreases as the integrity of the pellet decreases, and it is impossible to associate any plastic found with completely broken down pellets. In some species, like the flesh-footed shearwater (*Ardenna carneipes* (Gould, 1844)), chicks often cast a pellet when they emerge from their burrow to fledge (Lavers et al. 2014). These pellets are not often well defined, but instead appear more like a "spray" of plastic on the ground (**Fig. 4**). Unlike pellets from gulls and skuas, those from shearwaters are more difficult to identify and delineate, particularly when they contain pieces of pumice, which resembles the colony substrate (**Fig. 5**). Ideally, all pellets should be individually stored in clean bags or jars. If this is not possible, pellets can be stored based on their decomposition status, with intact pellets bagged together, and all partially or completely degraded pellets bagged individually.

Processing methods

Necropsies

The purposes of necropsy are to determine cause of death, collect data about the bird (morphometrics, condition, etc.), and collect the gastrointestinal tract (GIT) and any tissues of interest. Regardless of the source, all carcasses destined for plastics examination should be handled carefully to ensure that the GIT is complete upon examination (**Fig. 2**). If carcasses have been opened by decomposition or scavengers, ingested debris assessments may be either incomplete due to pieces

Fig. 4. Example of a sprayed regurgitation from a flesh-footed shearwater (*Ardenna carneipes*) from Lord Howe Island. These pellets are not intact, and thus of limited use to assess small microplastics that are easily lost to the environment. Photo courtesy of Jennifer Lavers.

Fig. 5. Plastic items and pumice found in a pellet from a flesh-footed shearwater (*Ardenna carneipes*) from Lord Howe Island. Photo courtesy of Silke Stuckenbrock.

being lost to the environment or inflated because of additional plastic pieces from the environment. The GIT may be preserved (e.g., freezing or submersion in ethanol) so that it can be processed under controlled laboratory conditions at a later date.

When examining GITs, the appropriate location within the GIT must be identified and noted explicitly (**Fig. 2**). Although "GIT" is often used when referring to ingested debris in seabirds, often only portions of the GIT are examined (**Fig. 2**). Most seabird studies examine the gizzard and proventriculus (van Franeker et al. 2011), some studies report separate values for these two compartments (Mallory 2008), and others report plastic burdens from the entire length of GIT, including the intestines (Provencher et al. 2010). This often varies due to the original purpose of the study. For plastic accumulation studies, the gizzard and proventriculus are where plastics are retained. Plastic or debris items found in the intestines are likely small enough to be passed through the GIT, and will be excreted by the individual (Provencher et al. 2018).

When possible, debris contents for different sections of the GIT should be recorded separately. In monitoring of northern fulmars, contents of the proventriculus and gizzard (Fig. 2) are combined for cost efficiency of the monitoring program (OSPAR 2017), but in the parallel monitoring of sea turtles in other areas of the European Union, esophagus, stomach, and gut contents are all quantified separately as these different GIT sections all regularly contain plastics in highly variable quantities within and among species, which may be an issue for separate reporting in the future (Matiddi et al. 2017). This is important to consider within the context of birds as well, because of physiological differences among species and detection differences among sampling techniques. Ideally, first descriptions of plastic ingestion in any species will report plastic loads for each GIT compartment separately, but to ensure comparability of results across time and space, plastic ingestion data for monitoring species should conform to established procedures relevant to the monitoring program.

The GIT walls should also be examined during the dissection. All compartments of the GIT should be flushed with water while manipulating the GIT to ensure that all cracks and crevices are thoroughly washed. This also allows for examination of any embedded litter or plastic in the wall or lining of the GIT (e.g., Seif et al. 2018), or the presence of other non-food items such as pumice. Reporting such occurrences may help to document the possible effects of both plastics and other debris items. Often regular tap water is sufficient as long as the purpose of the study is not focused on microplastics that could contaminate the sample (Carrington 2017). When assessing microplastics (size class <5 mm), a clean laboratory setup is necessary during the dissection to avoid contamination of GIT samples by airborne microfiber pollution (Torre et al. 2016). This includes working surfaces that are easily cleaned and maintained, and proper personal protective equipment used at all times in the lab to prevent cross-contamination of plastics from lab personnel.

Regurgitations from birds

Unlike carcasses, regurgitation samples require less processing between collection and examination for plastics. Preservation of the sample is necessary to minimize decomposition of likely associated food items in the samples if diet questions are of interest as well. The entire regurgitation (solids and liquids) should be stored in a clean container and preserved in alcohol or frozen prior to laboratory examination.

Pellet examination

Unlike for carcasses, little processing is needed for pellets between field collection and plastic examination. After pellets have been collected in the field they should be dried as quickly as possible to prevent fungal growth, and stored in a dry and dark condition before dissection. Samples should be dried either at room temperature or in an oven at 55 °C for 48–72 h. Pellets should be weighed prior to drying and then during the drying process; drying is complete when the pellet has a constant mass. The pellets can then be stored in a dry place until examined for plastic.

Cross-cutting ingested plastics assessment methods

Separation of liquids and solids

For all samples that need to separate solids from liquids (e.g., gut contents from necropsies and regurgitations), a series of stainless steel or brass sieves can be used. Stacked sieves (e.g., 5, 1, and 0.3 mm) can be used to separate different fractions. The 5 mm sieve splits meso/macro plastics (>5 mm) from microplastics (<5 mm). Further sieve sizes will depend on the plastic size of interest, but for most birds a 1 mm sieve should be used as pieces smaller than this are likely passed along through the pyloric sphincter and do not accumulate within the bird. A smaller sieve size (e.g., 0.3 mm) can be used when particularly small species are being examined (e.g., storm-petrels). Most microplastic studies based on net sampling at sea use a mesh size of 0.3 mm. Importantly, if microplastics are of interest

special laboratory set-ups are needed to assess microplastics in these smaller size classes accurately (see *Examining samples for <1 mm plastics*, below). If diet composition is also of interest, this method can also be used to separate prey items. In most cases, once the plastics have been rinsed using sieves the liquid can be discarded. All items in the sieve can be examined. Once diet items of interest are removed, the sample can be further processed to remove any biological material from the sample (see the *Removal of organic matter*, below).

Removal of sediment

For species that consume shelled organisms, large amounts of sand and hard shells may be present in their guts (e.g., bottom feeding seaducks). Once ingested items are ready to be processed, density separation can be used to separate sand and grit from plastic items and prey items. This can include density separation of solids using a simple 5 mol/L NaCl (density = 1.15 g/mL) solution. Without introducing additional chemicals, the saturated NaCl solution will separate the most common plastic polymers found in seabirds (i.e., polyethylene (d = 0.91–0.97 g/mL), polypropylene (d = 0.94 g/mL), and polystyrene (d = 1.05 g/mL)) from sand and grit. To obtain a higher certainty that all polymer particles will float (e.g., including polyvinyl chloride d = 1.4 g/mL), other solutions may be needed. For example, an aqueous 5.4 mol/L lithium metatungstate solution (d = 1.6 g/mL) may be used (Masura et al. 2015), but these have the risk of introducing unwanted chemicals to the plastic material and are not recommended in cases where the chemical analysis of the ingested plastics are of interest without further testing of how these methods may affect the chemical composition of plastics. After density separation, floating items can be collected using sieves as appropriate. If needed, additional techniques to separate organic matter from plastics can be applied (see the next section).

Removal of organic matter

If samples are cluttered with biological material, a digestive treatment may be needed to separate plastics. This may be the case if carcasses are collected when the birds are feeding, or regurgitations are collected when adults are returning to colonies with large quantities of prey to feed to their chicks. Most digestion protocols have been developed to study microplastic ingestion by fish, invertebrates, and marine birds (Besseling et al. 2015; Courtene-Jones et al. 2017; Kühn et al. 2017).

Digestion protocols consist of four main groups: acidic, alkaline, oxidizing, and enzymatic dissolution (Dehaut et al. 2016). Acidic solutions tend to dissolve some polymer types (Dehaut et al. 2016; Lusher et al. 2017), and may degrade plastics, which can reduce their mass or lead to yellowing (which is important to note in relation to reporting the colour of plastics; Dehaut et al. 2016). Potassium hydroxide (KOH) in 1 mol/L concentrations with heat (60 °C) is generally preferred as the least destructive treatment to the plastics (except cellulose acetate), but experimentation is still needed under a range of conditions and tissue types (Kühn et al. 2017). Enzymatic digestion can also be used for digestion of organic material in samples. Enzymes such as trypsin, papain, and collagenase (Courtene-Jones et al. 2017), and off-the-shelf mixtures such as enzymatic washing detergent (Bravo Rebolledo et al. 2013; Besseling et al. 2015) have been tested on mussels, seal scat, and cetacean intestines. Proteinase-K has also been effectively used to isolate microplastics from biotic samples (Cole et al. 2015; Löder et al. 2017). Cole et al. (2015) found that >97% of the biological material from a sample could be reduced using this technique. Even though the effects of enzymes on plastics have been observed, enzymatic approaches required less time and effort (Lusher et al. 2017). Importantly, however, the concentrations required and the saturation point of enzymatic activity have not been determined for many tissues and sample conditions.

Digestion protocols should only be used when necessary, and the solution and potential impact on the integrity of the sample (i.e., impacts on colour, mass, or degrading of certain polymer types) should be reported. Typically, plastics >1 mm can be identified without digestion protocols by an experienced

observer. However, a growing interest in quantifying plastics <1 mm might lead to future research questions regarding smaller plastic particles in seabirds.

Alternative to separation techniques

Where samples are likely to contain microplastic pieces and have high amounts of sediment or biological material that cannot be removed easily, there are approaches such as staining with Nile Red that may be useful. This approach uses the selective staining properties of Nile Red onto plastics surfaces, which then makes plastic pieces fluoresce under blue light (Maes et al. 2017). This technique has been employed in environmental samples, but not in seabird studies to date. This may be a particularly useful method where large sample sizes prohibit detailed examination of the samples as it can be done quickly. The disadvantage of this technique is that it only works to detect plastics, not other debris types, which could be of interest in some studies (Seif et al. 2018). It may also stain the plastics, which may be a disadvantage if colour is a metric of interest.

Drying plastics

Once plastics and other debris items are removed from the sample, all plastics and other items should be thoroughly cleaned and dried before weighing. It is important to note that aggressive rinsing or scrubbing could remove adhered pollutants that may also be of interest in the study; therefore, the goals of the study will determine how well debris items should be rinsed or scrubbed. All debris should be air dried for at least 24 h at room temperature or for a minimum of 12 h in a drying oven at 40 °C on a covered stainless steel or glass plate to prevent any cross-contamination. If porous or foam-based material is involved, drying may require more time. Once plastics are dry, pieces should be weighed individually using an analytical balance, ideally to 0.001 g precision.

Categorization of ingested plastics and other debris

Suggested best practices for categorizing debris and reporting results have been detailed by Provencher et al. (2017).

Other aspects to consider in plastics studies of seabirds

Polymer identification

In addition to visual examination, Raman spectrometry or Fourier-transform infrared spectroscopy (FTIR) can be used to identify the polymer types of plastic (Avery-Gomm et al. 2016). Such polymer identification may also be important when assessing which plastic-associated contaminants may be absorbed by wildlife as a result of accumulated plastics in the gut (Rochman et al. 2014; Koelmans et al. 2016).

Some of the digestion procedures outlined above may alter the chemical composition of some polymers, leading to challenges in accurate polymer identification. For example, the alkaline solution NaOH degraded cellulose carbonate, polycarbonate, and polyethylene terephthalate (Karami et al. 2017), whereas the oxidizing peroxide sulfate potassium did not harm polymer structure but was less efficient in dissolving organic material and had some handling issues that were of concern (Karami et al. 2017).

Contaminant analysis of plastics

For some research questions it may be useful to analyze the ingested plastics themselves for contaminants (Rochman et al. 2013). If ingested plastics are to be analyzed for intrinsic and adsorbed contaminants, separation of the plastics from organic matter may need to be done manually to ensure that the contaminant concentrations are those as they were found in the stomachs and not altered due to a chemical reaction in the sorting process. Plastics should then also be kept in chemically clean

containers (rinsed thoroughly with a solvent) between dissection and chemical analysis (Rochman et al. 2013).

Examining samples for <1 mm plastics

Regurgitated pellets are not appropriate to quantify ingested microfibers and other small plastics because of the high levels of environmental contamination. Plastic pieces that are <1 mm do not likely accumulate in most seabirds, and therefore seabirds are not likely to be useful indicators of the distribution of this small microplastic pollution in the environment. However, there may be research questions related to the plastic excretion by seabirds, or evaluating seabirds as vectors for plastics pollution through their guano (van Franeker 2011; Provencher et al. 2018).

If samples are being used to determine the ingestion of plastics <1 mm, there are cross-contamination procedures that must be put into practice to ensure that rigorous and robust findings are reported (Torre et al. 2016; Hermsen et al. 2017; Wesch et al. 2017). Samples are at risk from contamination by particles present in re-used sample containers, on the clothes of workers, airborne (clothing) fibres, and (or) by improperly sealed samples (Torre et al. 2016; Wesch et al. 2017). The use of filtered water may be necessary given that tap water may introduce microplastics to a sample (Vermaire et al. 2017). Personal protection equipment such as laboratory coats, gloves, and even hairnets or hats may be necessary to reduce fibres from lab workers from entering the sample during the entire dissection process (Torre et al. 2016). Protective clothing that is a bright or uncommon colour (i.e., pink or orange) can be used, as microfibers of these colours can then be discounted from the final tally of debris in a sample.

Isolated spaces where plastics <1 mm will be examined can be effective at reducing cross-contamination. Creating an isolated stereomicroscope area for visual identification can reduce airborne contamination (Torre et al. 2016), and is commonly used in microplastic studies in other biota (Davidson and Dudas 2016). Sample blanks must be used to detect and correct for any cross-contamination that does occur (Vermaire et al. 2017). This can be done through opening a set of empty jars for the same amount of time for which a standard seabird sample is examined, and then using the same quantification techniques. If digestion of the samples is done, similarly clean jars should be subjected to the same processes and then examined. The maximum number of microfibers in the blanks can then be subtracted from each sample as a conservative approach to accounting for such cross-contamination (Vermaire et al. 2017; Provencher et al. 2018). On a smaller more portable scale, the use of glove boxes that create a confined air space around samples can be used to reduce environmental microfibers from being introduced into the samples (Torre et al. 2016). These methods may not be applicable to most seabird studies, which focus on plastics >1 mm, but are useful when examining microplastics.

Data processing and presentation

Variables related to debris ingestion to be reported

Many factors influence metrics of ingestion of marine debris in seabirds, including species, sex, age, and breeding stage. If these metrics are not of direct interest to the published manuscript containing the plastic ingestion data, they should still be recorded for each sample (e.g., each carcass) and reported in supplementary online material or in open data archives as they may be critical to future studies and comparisons (e.g., O'Hanlon et al. 2017).

Reporting of ingested plastic metrics in seabirds

Standardized reporting recommendations have been discussed extensively by Provencher et al. (2017). Briefly, as a minimum, the location of sampling, the type of sampling, sample size, and the frequency of occurrence of ingested plastics should be reported. If specific types of plastic items can

be identified, they should be reported. To allow for a full understanding of the data, the mean (with standard deviation and error), median, and range of mass of ingested plastics per individual, including all individuals sampled, and the mean (with standard deviation and error), median, and range of all plastics reported by debris category (see above) should be reported. In the context of reporting micro-plastics <1 mm, cross-contamination cannot be excluded in most cases (Wesch et al. 2017); therefore, fibres should always be reported separately and all measures taken to prevent contamination should be mentioned.

Although documenting and reporting ingested plastics is critical to understanding global patterns, it is also critical to use these standardized approaches when reporting "zeroes"; this is analogous to issues arising from meta-analyses and the importance of publishing non-significant results (Koricheva 2003; Dwan et al. 2013). When standardized protocols are applied and no plastics are found, these data should be published. Establishing the absence of plastics in species or individuals will enhance our understanding of the pervasiveness of plastic pollution, the potential impacts of plastic ingestion, and allow for the development of more robust predictive models for data-limited species.

Discussion

We detailed standardized approaches for the three main techniques for evaluating plastic ingestion by seabirds (necropsies, regurgitations, and pellets), including field collection, laboratory processing and data presentation. Creating a single protocol that will be widely applicable to hundreds of species from the emperor penguin (*Aptenodytes forsteri* Gray, 1844; 23 kg) to the least storm-petrel (*Halocyptena microsoma* (Coues, 1864); 25 g). Therefore, we have purposely presented an overview of harmonized best practices for assessing ingested plastics in marine birds and not a detailed protocol. There is room for the development of specific, step-by-step protocols for groups of species, or approaches that are used across a suite of sampling techniques, and we hope that this discussion will inform future efforts.

Given the breadth of plastic pollution information that can and has been gleaned from seabird studies, there is an immediate need to move beyond simply reporting plastic and litter ingestion by birds to allow the field to address larger, ecologically relevant questions. As any field of research matures, adoption of standardized methods becomes important. Moving forward, plastic ingestion studies employing standardized methods for sample collection, processing, plastic quantification, and report-ing will provide valuable data points that will make statistically and scientifically credible large-scale comparisons possible (Stewart 2010). This will enhance our understanding of the distribution of plastics in the environment, which species are most vulnerable to plastic ingestion, and the potential population-level impacts of plastic ingestion.

Acknowledgements

and Engineering Research Council of Canada. MLM was supported by a Natural Sciences and Engineering Research Council grant, as well as a Chair position from Fulbright Canada. Lastly, we thank the three anonymous reviewers who contributed constructive comments on this manuscript that helped to improve the work.

Author contributions

JFP, SBB, ALB, JLL, JAVF, SK, SH, SA-G, and MLM conceived and designed the study. JFP, SBB, ALB, JLL, JAVF, SK, SH, SA-G, and MLM performed the experiments/collected the data. JFP, SBB, ALB, JLL, JAVF, SK, SH, SA-G, and MLM analyzed and interpreted the data. JFP, SBB, ALB, JLL, JAVF, SK, SH, SA-G, and MLM contributed resources. JFP, SBB, ALB, JLL, JAVF, SK, SH, SA-G, and MLM drafted or revised the manuscript.

Competing interests

MLM is currently serving as a Subject Editor for FACETS, but was not involved in review or editorial decisions regarding this manuscript. At the time when he was offered the opportunity to handle the paper the handling editor (Dr. B. Favaro) acknowledged that he and Dr. Provencher are both Liber Ero Fellows and that they know each other, have worked with each other, and are preparing to co-supervise students. Dr. Favaro declared that he was nonetheless able to deliver an unbiased handling of the peer review of the paper to inform the eventual editorial decision.

Data availability statement

All relevant data are within the paper.

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