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Effectiveness of emetics to study plastic ingestion by Leach's Storm-petrels (*Oceanodroma leucorhoa*)

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A R T I C L E   I N F O

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A B S T R A C T

Most plastic ingestion studies rely on dissection of dead birds, which are found opportunistically, and may be biased. We used Leach's Storm-petrels (*Oceanodroma leucorhoa*) in Newfoundland to study the effect of dose volume, and the efficacy of emesis using syrup of ipecac as an emetic. Ipecac is a safe method of non-lethally sampling stomach contents, and recovered all ingested plastic. Almost half the storm-petrels sampled had ingested plastic, ranging from 0 to 17 pieces, and weighing 0.2–16.9 mg. Using the Ecological Quality Objective for Northern Fulmars, adjusted for storm-petrels smaller size, 43% exceeded the threshold of 0.0077 g of plastic. Many adult seabirds offload plastic to their offspring, so storm-petrel chicks likely experience a higher plastic burden than their parents. The ability to study plastic ingestion non-lethally allows researchers to move from opportunistic and haphazard sampling to hypothesis-driven studies on a wider range of taxa and age classes.

1. Introduction

Seabirds' ingestion of plastic marine debris is a global phenomenon that has severe negative consequences on individuals (Laist, 1997; Ryan et al., 2009), including behavioral, demographic, and physiological effects (Sievert and Sileo, 1993; Hutton et al., 2008; Carey, 2011). Rates of plastic ingestion by seabirds have been used as indicators of the quality of the marine environment and risk to seabird health. For example, long-term data on ingestion rates in Northern Fulmars (*Fulmarus glacialis*) in the North Sea led to the development of the Ecological Quality Objective (EcoQO), where a certain level of plastic ingestion was deemed detrimental to the birds. For Northern Fulmars, this was set at no more than 10% of birds with 0.1 g of plastic (van Franeker et al., 2005).

There is a long history of plastic ingestion in Leach's Storm-petrels (*Oceanodroma leucorhoa*) (Rothstein, 1973; Day, 1980; Watanuki, 1985), but there has been no systematic assessment to date. Despite the high frequency of ingestion identified in earlier studies (>50%, e.g., Furness, 1985b), storm-petrels have received relatively little attention compared with other species (but see van Franeker and Bell, 1988; Ainley et al., 1990; Spear et al., 1995), likely because they are not encountered frequently on beached-bird surveys. Furness (1985b) suggested that Leach's Storm-petrels would be affected more per gram than Northern Fulmars because of their smaller gizzard. Their wide distribution in the North Atlantic and North Pacific Oceans, long lifespan, and overall abundance (millions of individuals) make Leach's Storm-petrels a useful indicator of plastic pollution.

Most studies of seabirds' plastic ingestion, however, rely on dead individuals (e.g., Furness, 1985a; Robards et al., 1995; Spear et al., 1995; Provencher et al., 2010). The ability to study plastic ingestion non-lethally would greatly increase the range of species and age classes studied, and allow for repeated sampling of the same individuals over time.

Emetics (pharmaceutical agents that induce regurgitation), and lavage (pumping the stomach with water until the individual regurgitates), have been used with varying degrees of success to study avian diet (Kadochnikov, 1967; Prys-Jones et al., 1974; Montague and Cullen, 1985; Poulin et al., 1993). Compounds such as apomorphine and tartar emetic (antimony potassium tartarate) can be toxic to wildlife (Prys-Jones et al., 1974; Carlisle and Holberton, 2006), and their use is discouraged. For example, a bird treated with too low a dose of tartar emetic will not regurgitate, and the compound will be absorbed into the blood stream, requiring euthanasia of the bird (Herrera, 1976).

Syrup of ipecachuana (an extract of the root of *Cephaelis ipecachuana* and *C. acuminate;* hereafter "ipecac") is rarely toxic, is used for emesis in human children, and is currently the recommended emetic for wild birds (Manno and Manno, 1977; Ornithological Council, 2010). Though it has been used to study avian diet since the 1960s (Kadochnikov, 1967; Radke and Frydendall, 1974), the

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administered doses were generally small and ineffective (Diamond et al., 2007).

Our goals were to (1) use ipecac to study plastic ingestion in Leach's Storm-petrels, (2) assess the effect of dose volume on the probability of regurgitation, and the effectiveness of ipecac in completely emptying the upper gastrointestinal tract, and (3) assess plastic ingestion by Leach's Storm-petrels globally.

2. Methods

We captured 63 adult Leach's Storm-petrels at Gull Island, Witless Bay, Newfoundland and Labrador, using either mist-nets ($n = 25$) or by spotlighting ($n = 38$) birds at night. Birds caught by mist-net were removed from the net within 25 min of capture, and birds caught by spotlighting were processed immediately upon capture. All birds were weighed (±2 g), measured (flattened straightened right wing length (±1 mm), culmen (±0.1 mm), bill depth at gonys (±0.1 mm), and head + bill length (±0.1 mm)), and banded prior to administration of ipecac by inserting a feeding tube into the stomach (past the pyloric sphincter), and using a 5 ml syringe. Our ipecac formulation was obtained from commercial pharmacies in Canada, and was 1.4 mg/ml syrup of ipecac.

We alternatively administered 2.0 ml ($n = 13$) or 3.0 ml ($n = 19$; representing, on average, 0.04–0.06 ml/g body mass) to adult storm-petrels. As we experienced a low rate of regurgitation, we then increased the dose to 0.1 ml/g body mass (Diamond et al., 2007), up to a maximum of 5.0 ml ($n = 31$). We held birds individually on elevated metal grates inside plastic tubs for up to 25 min after dosing with ipecac (Montague and Cullen, 1985). Birds were released immediately upon regurgitation, or after 25 min, by placing them outside on a dry surface until they flew away (which occurred in <10 min). We recorded the time from dosing to regurgitation, and general condition (e.g., if the bird appeared impaired or lethargic). Regurgitated plastic and prey remains from each bird were collected and stored in individual sterile plastic bags.

2.1. Efficacy of emesis

Because we experienced high mortality due to capture and processing times, we abandoned mist-netting, and captured birds by spotlighting (see Section 4). We examined 12 birds that were dosed with ipecac that were subsequently euthanized by cervical dislocation to determine the efficacy of emesis on removing all plastic from the upper gastrointestinal tract, including the gizzard.

2.2. Plastic ingestion

Data on plastic ingestion were analyzed following van Franeker et al. (2005). Briefly, each regurgitated sample was examined for plastic under a dissecting microscope, and plastic debris was removed, washed in water, dried, and weighed to the nearest 0.0001 g. We also recorded the color and type of debris (plastic pellets, sheet, foam, fragment, thread, or other; van Franeker et al., 2005). The frequency and mass of ingested plastic are presented as population means ± S.D. (i.e., using both birds that had ingested plastic, and those who had not).

2.3. Statistical analysis

To test for differences in the proportion of birds that successfully regurgitated by dose volume in our field study, we used a generalized linear model with a binomial error structure. We also tested differences in the time to regurgitate by dose volume using a general linear model, and Tukey's honest significant difference (HSD) post hoc test. We used linear regressions to examine relationships between the mass of plastic ingested and body condition (mass, and mass corrected for size using residuals from a linear regression of body mass and head + bill). Data are presented as mean ± SD, and all analyses were conducted in R 2.15.1 (R Development Core Team, 2012); terms with $p < 0.05$ were considered significant.

2.4. Ethical statement

Under our initial experimental design, we experienced unacceptable levels of mortality (see Section 3). We believe these mortalities were caused by long handling times, and we therefore changed to spotlighting only (where we had three mortalities attributable to the emetic). This mortality rate, though undesirable, is low (8%), and far superior to the existing method of studying seabird plastic ingestion, in which individuals are salvaged, or the mortality rate is 100%. Ipecac can cause mortality in penguins with stomach ulcers (Montague and Cullen, 1985), a condition that would be impossible to detect without a detailed post mortem. This mortality rate is also much lower than other emetics (Randall and Davidson, 1981; Carlisle and Holberton, 2006). We took immediate steps to identify the cause of mortality (including varying the dose and holding conditions), and ceased activities that resulted in adverse effects to the birds. The 12 birds that died as a result of our study could not be identified *a priori* as stressed, weakened, or different in any other measured variable (t-tests between euthanized and released birds for body mass, flattened straightened wing length, culmen, bill depth, and head + bill length, all $t > 0.96$, all $p > 0.34$).

Rather than exclude these cases, we have included them in our analysis. We have also made use of the birds that died by performing necropsies to determine the efficacy of emesis. While any mortality due to scientific inquiry is undesirable, it is important that researchers make complete use of specimens that result from such mortalities. We believe that researchers have an ethical and moral responsibility to make the scientific community aware of unintentional mortality, and also that the data derived from those individuals is scientifically valid, and useful.

The Canadian Wildlife Service (permit SC 2783) and University of Saskatchewan Animal Research Ethics Board (protocol 20120008) approved this research.

3. Results

Birds dosed with 2.0 ml ipecac regurgitated 54% of the time (7/13 birds), while those given 3.0 ml ipecac regurgitated 84% of the time (16/19 birds), and those given 0.1 ml/g body mass regurgitated 100% of the time (31/31 birds). Birds given 0.1 ml/g body mass were more likely to regurgitate than the 2 ml dose (generalized linear model, $p = 0.05$), but not different from a 3 ml dose ($p = 0.15$).

Birds given 0.1 ml/g body mass regurgitated in 7.3 ± 8.0 min (range: 1–23 min), which was significantly faster than birds given 3 ml (17.1 ± 6.4 min, range: 2–25 min), or 2 ml (20.3 ± 6.8 min, range: 8–25 min; $F_{2,60} = 18.9$, $p < 0.001$, Tukey's HSD, $p < 0.0001$).

Eight birds caught by mist-net required euthanasia ($n = 25$ captures over three nights). Of these, two were females with an egg in their oviduct. Processing time (from earliest possible capture to latest possible release) was between 60 and 90 min, suggesting that stress from both reproductive activities, and prolonged captivity contributed to mortality. These birds were held significantly longer than average (22.6 ± 3.3 min vs. 18.3 ± 6.0 min; $F_{2,60} = 5.3$, $p = 0.007$). Using spotlighting, only 4/38 birds required euthanasia, one of which was the result of a window strike.

3.1. Efficacy of emesis

Of the 12 birds examined, 10 had regurgitated prior to euthanasia, and six contained 5.5 ± 6.1 pieces of plastic prior to euthanasia. No plastic was found in any individual during dissection.

3.2. Plastic ingestion

Of the 63 birds captured, 30 (48%) contained at least one piece of plastic debris. Leach's Storm-petrels contained 1.9 ± 3.4 pieces (range: 0–17 pieces) of debris, all of which were fragments of industrial or commercial plastic. Individual pieces weighed 3.1 ± 2.5 mg (range: 0.2–16.9 mg; $n = 118$ pieces), and each bird carried 5.7 ± 12.2 mg (range: 0.0–61.1 mg) of plastic debris, which represented 0.012 ± 0.025% of the birds' body mass (range: 0.000–0.136 %). There was no relationship between the mass of plastic ingested and body mass (only birds with plastic; $F_{1,28} = 0.28$, $p = 0.60$) or size-corrected mass ($F_{1,28} = 0.71$, $p = 0.41$).

White or off-white was the most frequent color ($n = 71$, 60%), followed by brown ($n = 16$ 14%), gray ($n = 10$, 8%), red ($n = 9$, 8%), green ($n = 8$, 7%), and yellow ($n = 4$, 3%). White comprised the majority of the mass of plastic (61%), followed by brown (13%), yellow (8%), red (8%), gray (7%), and green (4%).

4. Discussion

We have shown that ipecac is a relatively safe and effective method for emesis in storm-petrels to study plastic ingestion, provided the correct dose is administered, and handling time is kept to a minimum. Levels of expected mortality (3/38, or about 8%) are comparable with other invasive techniques to study diet, such as stomach lavage (Gonfriddo et al., 1995), which can have varying success, depending on the species (Wilson, 1984; Arnould and Whitehead, 1991), and the size of prey (Croxall and Prince, 1980). We are confident that the observed mortality in our study was caused by capture stress and not the emetic itself, since a reduction in handling time (switching from mist-netting to spotlighting) greatly reduced mortality. Ipecac may be fatal when the regurgitate is aspirated (Arnold et al., 1959), but this can be avoided by inserting a feeding tube past the pyloric sphincter, as we did. We were able to confirm complete emesis in the 12 euthanized birds, consistent with literature on human studies (Vasquez et al., 1988), and the first such assessment in wild birds.

Reviews by Duffy and Jackson (1986) and Barrett et al. (2007) both recommended water offloading (lavage, or stomach flushing) as a preferred method for inducing regurgitations, but their conclusions regarding emetics were not based on ipecac, but more harmful compounds, such as tartar emetic (Diamond et al., 2007). Both methods can be useful, and the choice will depend on the researchers' experience and species or age class of interest. Auks, for example, may not respond well to stomach flushing (Barrett et al., 2007), and chicks may experience damage to their sensitive stomach wall. We do not advocate abandoning lavage entirely, but point out that both methods can be safe (Diamond et al., 2007; Fijn et al., 2012), or experience problems (Randall and Davidson, 1981; Montague and Cullen, 1985). Ipecac is simply another tool that had not previously been assessed in the context of seabirds' ingestion of plastic debris.

4.1. Plastic in Leach's Storm-petrels

Our assessment (the first in more than 20 years) shows that Leach's Storm-petrels continue to ingest plastic debris, and are likely to do so with increasing frequency as plastic in oceanic waters increases (Law et al., 2010). Furthermore, we show that simply mist-netting birds to examine regurgitated oil or chick meal deliveries vastly underestimates the true frequency of plastic ingestion – none of the birds in our study regurgitated plastic until treated with emetic.

The highest rate of plastic ingestion (59%) in Leach's Storm-petrels was in Scotland in 1983 (Furness, 1985b), but included only 17 individuals. Three other studies had sample sizes >10, and reported similar (48.4%, Robards et al., 1995) or lower rates of ingestion (6.5%, Watanuki, 1985; 29.1%, Ainley et al., 1990). The frequency of plastic ingestion by Leach's Storm-petrels has been relatively well studied compared with many seabirds (Fig. 1). Of these, most studies used dead birds (Rothstein, 1973; Day, 1980; Furness, 1985b; Ainley et al., 1990; Robards et al., 1995), and one used a stomach pump (Watanuki, 1985), which may be an underestimate of the actual frequency of plastic ingestion, especially when the stomach is very full (Lishman, 1985; Ryan and Jackson, 1986). We are the first to report on the mass of ingested plastic, critical to assessing the impact of plastic on seabirds (van Franeker et al., 2011). In general, we found an increase in the proportion of birds and number of particles ingested at Gull Island between 1962 and 2012 from 14.3% to 47.6%, and from 0.3 to 1.9 pieces/individual at the population level (Rothstein, 1973; Fig. 1). Plastic ingestion also increased in Leach's Storm-petrels in Alaska from 25% in 1969–1977 (Day, 1980) to 48.4% in 1988–1990 (Robards et al., 1995). Our sampling was during pre-laying and incubation, and Leach's Storm-petrels apparently offload some plastic to chicks (Day, 1980), but the degree of this intergenerational transfer is unknown, and must be considered when comparing studies. Nearly all Flesh-footed (*Puffinus carneipes*) and Short-tailed Shearwater (*Puffinus tenuirostris*) chicks contain significant amounts of plastic (Hutton et al., 2008; Carey, 2011), but adults are largely unaffected during the breeding season, likely due to the offloading of plastic to their chicks (JLL unpublished data). It is therefore likely that Leach's Storm-petrel chicks experience a higher plastic burden than their parents, and that the plastic recovered from adult storm-petrels represents local accumulation during the pre-laying and incubation periods.

If the EcoQO for plastic ingestion by Northern Fulmars (10% of birds with 0.1 g of plastic) were applied to Leach's Storm-petrels, adjusting the value to 0.0077 g (assuming a 650 g fulmar, and 50 g storm-petrel), then 13/30 birds in our study (43%) fall above this threshold. This is higher than fulmars' plastic ingestion EcoQO performance from the Canadian Arctic and Iceland, but lower than those from the northeastern Pacific or the North Sea (Avery-Gomm et al., 2012; Kühn and van Franeker, 2012). The development of

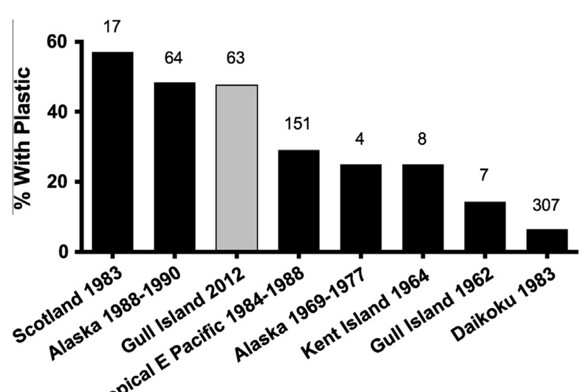

Fig. 1. The frequency of occurrence of plastic in Leach's Storm-petrels from Gull Island, Newfoundland in 2012 (this study), and other published reports (Rothstein, 1973; Day, 1980; Furness, 1985b; Watanuki, 1985; Ainley et al., 1990; Robards et al., 1995). Numbers above bars indicate sample sizes.

metrics to compare plastic ingestion among diverse species and regions should be a management priority.

Global plastic production is increasing rapidly (Hammer et al., 2012), but local conditions can influence some species' exposure (e.g., proximity to oceanic gyres, Galgani et al., 1995). Wide-ranging pelagic seabirds, like storm-petrels, can forage hundreds of km from breeding colonies, and are therefore exposed to plastic debris on a larger scale. Recent studies indicate the Atlantic Ocean has some of the highest densities of micro-plastic particles (<5 mm), and plastic density in the Atlantic is increasing in some areas (Thompson et al., 2004; Barnes and Milner, 2005; Law et al., 2010; Morét-Ferguson et al., 2010).

Necropsies of dead individuals, or the assumption that regurgitated meals were complete, and no plastic remained after inducing regurgitation (e.g., by stomach lavage or spontaneous regurgitation during capture) may not be the most suitable way to study plastic ingestion, especially when testing hypotheses. Ipecac is another method for sampling gastric contents of wild birds, results in complete emesis, and is easily administered. By adding safe emetics to their arsenal of tools, researchers can greatly expand the range of species available for study, and move from opportunistic and haphazard sampling to focusing on hypothesis-driven questions at individual, population, and community levels.

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