

Plastics in regurgitated Flesh-footed Shearwater (*Ardenna carneipes*) boluses as a monitoring tool

Alexander L. Bond^{1*}, Ian Hutton², and Jennifer L. Lavers³

¹*Bird Group, Department of Life Sciences, The Natural History Museum, Tring, Hertfordshire, United Kingdom, HP23 6AP*

²*Lord Howe Island Museum, P.O. Box 157, Lord Howe Island, New South Wales, 2898 Australia*

³*Institute for Marine and Antarctic Studies, University of Tasmania, 20 Castray Esplanade, Battery Point, Tasmania, 7004 Australia*

*Corresponding author. E-mail address: Jennifer.Lavers@utas.edu.au (J.L. Lavers)

ABSTRACT

Plastic production and pollution of the environment with plastic items is rising rapidly and outpacing current mitigation measures. Success of mitigation actions can only be determined if progress can be measured reliably through incorporation of specific, measurable targets. Here we evaluate temporal changes in the amount and composition of plastic in boluses from Flesh-footed Shearwaters during 2002-2020 and assess their suitability for measuring progress against national and international commitments to reduce plastic pollution. Plastic in the shearwater boluses showed a generally decreasing pattern from 2002-2015 and increasing again to 2020. The colour and type of plastics in boluses was comparable to items recovered from live and necropsied birds, but a much smaller sample size (~35 boluses/year) was required to detect changes in plastic number and mass over time. We therefore suggest shearwater boluses are a low-effort, high-statistical power monitoring tool for quantifying progress against environmental policies in Australia.

Keywords: Long-term trends; Marine debris; Monitoring methods; Regurgitated pellet; Tasman Sea

1. Introduction

The annual production of plastics from fossil fuels is rising rapidly (PlasticsEurope, 2019), and leaks in global waste management streams mean that 19-23 million metric tons of plastic waste enters the oceans each year, far outpacing current mitigation measures to reduce plastic pollution of the oceans (Borrelle et al., 2020). Consequently, pollution of the environment with plastic has already exceeded two of the criteria of a planetary boundary threat, irreversibility and global ubiquity (Villarrubia-Gómez et al., 2018), and the 8 Gt of plastics in the world today are twice as much as the biomass of all animals (Elhacham et al., 2020). Global fossil fuel subsidies are in excess of \$5.3 trillion annually (2015 USD), and with 4-8% of fossil fuel extraction used for plastics manufacturing, this results in a subsidy of ca. \$200-400 billion (2015 USD) from extraction alone (Hopewell et al., 2009; Coady et al., 2017).

Global governments are taking increasing notice of the environmental, financial, and societal impacts of plastic pollution, with more international initiatives and aspirations, however these fall short of binding targets (Borrelle et al., 2017; Haward, 2018; Karasik et al., 2020), and governance of a largely self-regulated industry remains problematic (Dauvergne, 2018; Tessnow-von Wysocki and Le Billon, 2019). Primary among these global environmental targets are the United Nations Development Programme's Sustainable Development Goals (SDGs), which includes Goal 14.1, the aspiration to "by 2025, prevent and significantly reduce marine pollution of all kinds, in particular from land-based activities, including marine debris and nutrient pollution" (United Nations, 2015). Success can only be determined if progress towards the SDGs can be measured reliably (Butchart et al., 2016), which includes knowledge of temporal trends, and the current state of plastic pollution in marine ecosystems.

Regurgitated pellets (boluses) are a non-invasive and low-effort sampling approach for studying plastic pollution in seabirds (Provencher et al., 2019), and they are often used to monitor debris in both the terrestrial and marine environments (e.g., Ryan and Fraser, 1988; Hammer et al., 2016; Stewart et al., 2020; Winkler et al., 2020). On Lord Howe Island, Australia, Flesh-footed Shearwaters (*Ardenna carneipes*) are severely impacted by plastic pollution (Lavers et al., 2019a, in press), where it results in reduced chick growth, altered blood chemistry, and chemical contamination (Lavers et al., 2014; Lavers et al., 2019b). Adults provision nest-bound chicks for 90 days, including feeding them an array of plastic sizes (Warham, 1958; Lavers et al., 2019c), and when chicks emerge from their burrows, they will often cast a bolus of indigestible matter, including plastics, pumice (volcanic rock), and prey hard parts.

Here we investigate temporal changes in the amount and composition of plastic in boluses from Flesh-footed Shearwater colonies on Lord Howe Island using data collected during 2002-2020.

Our objectives were to identify within- and among-year trends in the quantity of plastic ingested by this sentinel species, evaluate the effectiveness of shearwater boluses in reflecting ingested plastics assessed by other sampling approaches, and determine their suitability to measure progress against national and international commitments to reduce plastic pollution.

2. Methods

2.1. Sample collection and processing

Flesh-footed Shearwater boluses were collected by hand from the colony surface on UNESCO World Heritage-listed Lord Howe Island, New South Wales, Australia (31.55°S, 159.08°E) from 25 April until 30 May during 2002-2020 at the end of the breeding season when fledglings are departing the nest and after adults have departed. In the field, pellets that were structurally intact or mostly so (categories 1, 2, and 3 detailed by Provencher et al., 2019) were placed in individual paper envelopes and once in the lab, pellets were then gently separated into components using forceps. Plastic items > 1 mm were washed, dried, and weighed to the nearest 0.01 g using an electronic balance. Items were then sorted by colour (white, blue, green, red, yellow, and black) and type following the categories outlined by Provencher et al. (2017; industrial pellets, user plastic, foam, threads, sheet plastic, and other), with the exception of plastic strapping, which more closely resembled plastic fragments in our study system. Pumice was recorded in some boluses (Figure 1, panels C and D), but is not detailed in this study.

2.2. Statistical methods

We conducted all analyses in R 4.0.3 (R Core Team, 2020). We first examined variation in the number of pieces and mass of plastic in shearwater boluses over time using generalized linear models with Poisson and gaussian error distributions, respectively, followed by Tukey's Honest Significant Differences post hoc tests. Values are reported as the mean \pm SD, and parameters were considered significant when $p < 0.05$.

We conducted a power analysis to determine the sample sizes needed for future monitoring to detect 5-100% change in the number of pieces, and mass of plastic over time (van Franeker and Meijboom, 2002; Lavers and Bond, 2016) using the equation:

$$n = 2 \times \left\{ \frac{|z_{\alpha/2} - z_{\pi}| \times \frac{CV}{100} \times \mu_1}{\mu_1 - 100} \right\}^2 \quad (\text{equation 1})$$

where n is the required annual sample size, z is the t-value, α is the Type I error rate, π is the Type II error rate, μ_1 is the difference to detect (where a 5% change is $\mu_1 = 10$, a 10% change is $\mu_1 = 110$, etc.), and CV is the coefficient of variation (SD/mean) of the time series. We set $\alpha = 0.05$ and $\pi = 0.90$,

meaning $z_{\alpha/2} - z_{\pi} = 3.242$, which is a power of 80% to detect any trend (van Franeker and Meijboom, 2002; Provencher et al., 2015).

To compare the colour and type composition of boluses among years, we used Jaccard Similarity Index (Jaccard, 1912; Bloom, 1981), which assesses multivariate proportional composition of two samples in a pairwise fashion. The index ranges from $0 < J < 1$, with values of $J > 0.60$ indicating considerable overlap (Bond et al., 2012). Lastly, we compared the composition of boluses to concurrent data on plastic ingestion from 2005-2019 obtained from necropsies of dead birds, and stomach flushing of live birds (Lavers et al., in press) to understand whether boluses can provide a similar degree of information. All analyses were done using the R package *vegan* (Oksanen et al., 2020), *tidyverse* (Wickham et al., 2019), and *agricolae* (de Mendiburu, 2020).

3. Results

We collected 315 boluses from 2002-2020 (range: 2-115 per year). All boluses contained plastics, but the number of pieces varied among years ($z = -12.84$, $p < 0.001$; Table 1), with five distinct groups, and showed a generally decreasing pattern from 2002-2015, and increasing again to 2020 (Figure 2).

The mass of plastic also varied over time ($F_{1,317} = 12.37$, $p = 0.001$), decreasing from 2002-2020 ($\beta \pm SE$: -0.103 ± 0.029), although the mass of plastic showed the same general pattern of decreasing from 2002-2015, and increasing thereafter (Figure 3).

The colour of plastic items was broadly similar among all years ($J = 0.777 \pm 0.128$), however 2010 differed from most years with larger proportions of blue and red, however this was from only $n = 3$ (Table S1). Plastic type did not vary among years ($J = 0.932 \pm 0.039$; range: 0.8702-1.000; Table S2) and was predominantly fragments (97.9%), with <1% industrial pellets, sheet, foam, thread, or other. Compared to samples from live and necropsied birds, boluses had nearly identical colour ($J = 0.890$ - 0.901) and type composition ($J = 0.864$ - 0.882).

With a mean sample size of 24 (Table 1), the power analysis indicated that we would be able to detect a 70% change in the number of plastic pieces, and a 60% change in the mass of plastic in boluses (Figure 4).

4. Discussion

4.1. Bolus composition

All the Flesh-footed Shearwater boluses examined contained one or more pieces of plastic, with an average of 21.8 items (4.0 g) per bolus (Table 1). This greatly exceeded the quantities of plastic reported concurrently for the same species and site examined using lavage (frequency of

occurrence: 0.59, 9.17 items/bird, 0.86 g/bird) and necropsy (frequency of occurrence: 0.86-0.91; 9.33-32.22 items/bird, 1.29-4.81 g/bird; Lavers et al., in press). Despite these differences, the composition of boluses, in terms of plastic colour and type, was similar to ingested plastics from live and necropsied birds, which often had a larger sample size. This suggests that monitoring the nature of individual pieces of plastic ingested by Flesh-footed Shearwaters could be done using boluses, while data from the whole bird unit of replication (such as total number and mass) can be obtained through lavage and necropsy (Lavers et al., in press).

Compared to necropsied birds or lavage samples, a much smaller sample size was required to detect changes in the number and mass of plastics. For example, to detect a 50% change in the number or mass of plastics requires 38 and 32 boluses, respectively, but requires 211 and 281 lavage samples, and 230-437 and 381-685 necropsied birds annually (Lavers et al., in press). While boluses may be useful for monitoring changes in plastics over time (Stewart et al., 2020), they cannot be linked with individual birds, and therefore the physical or chemical effects of plastics on individual organisms, a key research gap in plastic pollution research more broadly (Provencher et al., 2020).

Fragments, and in particular white fragments, are the most common plastic debris type found in many birds, as these can result from both inherently white fragments, but also the breaking up of larger pieces of debris and bleaching (Shaw and Day, 1994). They are the most common debris type ingested by all species of shearwaters in eastern Australia (Verlis et al., 2013; Lavers et al., 2014; Lavers et al., 2018; Puskic et al., 2020) whereas scavenging species, like Pacific Gulls (*Larus pacificus*) ingest more sheet and foam plastics because of their access to terrestrial sources of anthropogenic waste (Lindborg et al., 2012; Stewart et al., 2020). Adult Flesh-footed Shearwaters from Lord Howe Island provision their chicks with fish and squid from the Tasman Sea (Reid et al., 2012), primarily from the upper 5 m of the water column (Rayner et al., 2011). Plastics are most abundant in the upper 1 m of the water column, where they comprise the majority of marine debris, and items are mostly 1-5 mm (Reisser et al., 2015; Rudduck et al., 2017), and in the offshore, white fragments are the most common type (Shaw and Day, 1994; Reisser et al., 2013) suggesting that Flesh-footed Shearwaters could indeed be an effective sentinel or indicator species for marine plastic pollution in eastern Australia.

4.2. Uncertainties & biases

Boluses are likely equivalent in detection probability on the colony surface, as they also contain feathers, vegetation, pumice, and prey hard parts meaning the presence of plastics is not a prerequisite for collection, and boluses that contain small amounts of plastic are just as likely to be detected.

Most previous studies that have used regurgitated boluses or pellets to assess plastic pollution used those produced by post-fledging birds, either juvenile or adult (Bond, 2016; Hammer et al., 2016; Yorio et al., 2020), whereas bolus production, and therefore plastic retention, in chicks is poorly understood. Laysan Albatrosses (*Phoebastria immutabilis*) are assumed to produce a single bolus immediately prior to fledging which contains the majority of plastics fed to chicks by adults (Young et al., 2009; Awkerman et al., 2020). Hard parts from prey, including squid beaks or fish eye lenses, can be retained for more than a month, or perhaps even longer (Barrett et al., 2007). In albatrosses, shearwaters, and petrels (Procellariiformes), plastics can be retained for many months, particularly in nest-bound chicks (Ryan, 2015). The seasonal appearance of shearwater boluses on Lord Howe Island suggests regurgitation takes place only once, just prior to chicks fledging around 90 days old.

The timing of bolus production means that chicks that do not emerge from burrows to the colony surface (i.e., fail to fledge) do not likely produce a bolus, and so bolus sampling reflects a biased proportion of the chicks that hatch. The same biases are present in lavage and necropsied birds, which are only sampled once they emerge and are present at the colony surface (Lavers et al., 2014; Lavers et al., in press). As burrows can be 1-3 m deep, and birds may be prone to disturbance before emerging (Powell et al., 2007; Carey, 2009; Lavers et al., 2019a), sampling chicks that do not fledge is logistically challenging.

Collection of boluses is challenging and limited by the birds producing them. As such, the sample size in some years was low, reflecting the lack of intact boluses that could be located during the chick fledging period. With a mean sample size of 24, we were only able to detect a 70% change in the number of pieces and 60% change in the mass of plastic in boluses. This also reflects the highly stochastic nature of the boluses, with mean annual mass ranging over an order of magnitude, from 0.61 g (2015) to 5.73 g (2012; Table 1), and the mean annual number of pieces from 5.13 (2015) to 29.58 (2012).

4.3. Shearwater boluses and policy targets

Global and national policies aimed at measuring and reducing marine plastic pollution remain largely aspirational, non-binding, and lacking in specific targets (Borrelle et al., 2017; Borrelle et al., 2020). Perhaps the one exception to this is the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention) which has a policy target of < 10% of beached Northern Fulmars (*Fulmarus glacialis*) with < 0.1 g of plastic ingested (OSPAR, 2008; van Franeker et al., 2011).

In Australia, the main legislative instrument is the Environment Protection and Biodiversity Conservation Act (EPBC Act; Commonwealth of Australia, 2020), which requires identification of Key Threatening Processes, and the production of Threat Abatement Plans to address them. Injury and fatality of marine vertebrates from ingestion or entanglement in marine debris was listed as a Key Threatening Process in 2003, and the most recent Threat Abatement Plan is from 2018 (Department of Environment and Energy, 2018), which was recently informed by a report from the Australian Senate (Commonwealth of Australia, 2016). The same Key Threatening Process is also identified in state-level legislation (Government of New South Wales, 2020).

Despite this attention, no measurable objective for achieving the recommendations around plastic pollution exists in Australian Commonwealth or state legislation, which all concern identification and evaluation of the extent and nature of the problem, removal of existing debris, or public education initiatives. While all these are useful, and indeed necessary, they do not meaningfully contribute to Australia's commitment to Sustainable Development Goal 14 because progress cannot be measured robustly (Butchart et al., 2016), despite the Threat Abatement Plan specifically calling for, and directing funding towards, identifying vertebrate indicator species for long-term monitoring to measure the impact of plastic debris (Action 2.04; Department of Environment and Energy, 2018). Identifying and understanding policy tools that lead to a reduction in plastic pollution is an urgent research and policy gap (Provencher et al., 2020; Roman et al., 2021), which can only be addressed through time series of data, such as we present here.

Plastics are one of the potential markers of the geological Anthropocene (Zalasiewicz et al., 2017), and the mass of human-made objects now exceeds global dry biomass (Elhacham et al., 2020). Research into its presence, abundance, and effects on marine birds dates back more than 50 years (Kenyon and Kridler, 1969; Carpenter et al., 1972; Roman et al., 2019; Roman et al., 2021). We have demonstrated that Flesh-footed Shearwater fledgling boluses can be a low-effort, high-statistical power monitoring tool that could quantify progress against domestic and international environmental policies using more than a decade of data. The incorporation of specific, measurable targets akin to the Ecological Quality Objectives in the North Sea is an urgent policy need in Australia and other jurisdictions.

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Table 1. Plastic ingestion summary for boluses recovered from Flesh-footed Shearwater colonies on Lord Howe Island during 2002-2020.

Year	n	Number of pieces				Mass (g)			
		Mean	SD	Min	Max	Mean	SD	Min	Max
2002	4	28.75	13.15	16	47	5.41	3.92	0.70	9.96
2005	115	23.78	15.19	3	112	4.37	2.38	0.34	13.19
2006	30	26.1	17.12	4	93	4.25	2.64	0.90	15.73
2010	3	12.33	8.08	5	21	4.72	1.47	3.04	5.71
2012	62	29.58	16.24	6	81	5.73	3.13	0.73	20.96
2013	13	9	5.07	2	18	1.91	1.77	0.58	5.79
2014	2	9	0	9	9	2.75	0.13	2.65	2.84
2015	15	5.13	4.05	1	12	0.61	0.42	0.01	1.44
2016	26	14.54	10.75	4	51	2.89	2.34	0.10	8.71
2017	13	17.31	9.89	5	41	3.48	1.77	0.76	6.34
2018	8	14.62	13.86	1	40	2.67	2.23	0.48	6.66
2019	19	16.95	12.64	1	53	3.08	2.24	0.83	8.81
2020	5	22.8	14.36	11	47	4.91	2.71	2.48	9.42
Total	315	21.82	15.51	1	112	4.04	2.75	0.00	20.96
SE		2.20				0.41			

Figure 1. Examples of Flesh-footed Shearwater boluses containing anthropogenic debris on Lord Howe Island. Panels A/B and C/D show the same bolus intact (top panel) and separated into components in the lab (bottom panel). Photo: S. Stuckenbrock.



Figure 2. Number of ingested plastic items in Flesh-footed Shearwater boluses on Lord Howe Island during 2002-2020. Letters represent similar groups determined using Tukey's Honest Significant Differences post hoc tests; years sharing the same letter are not different from each other.

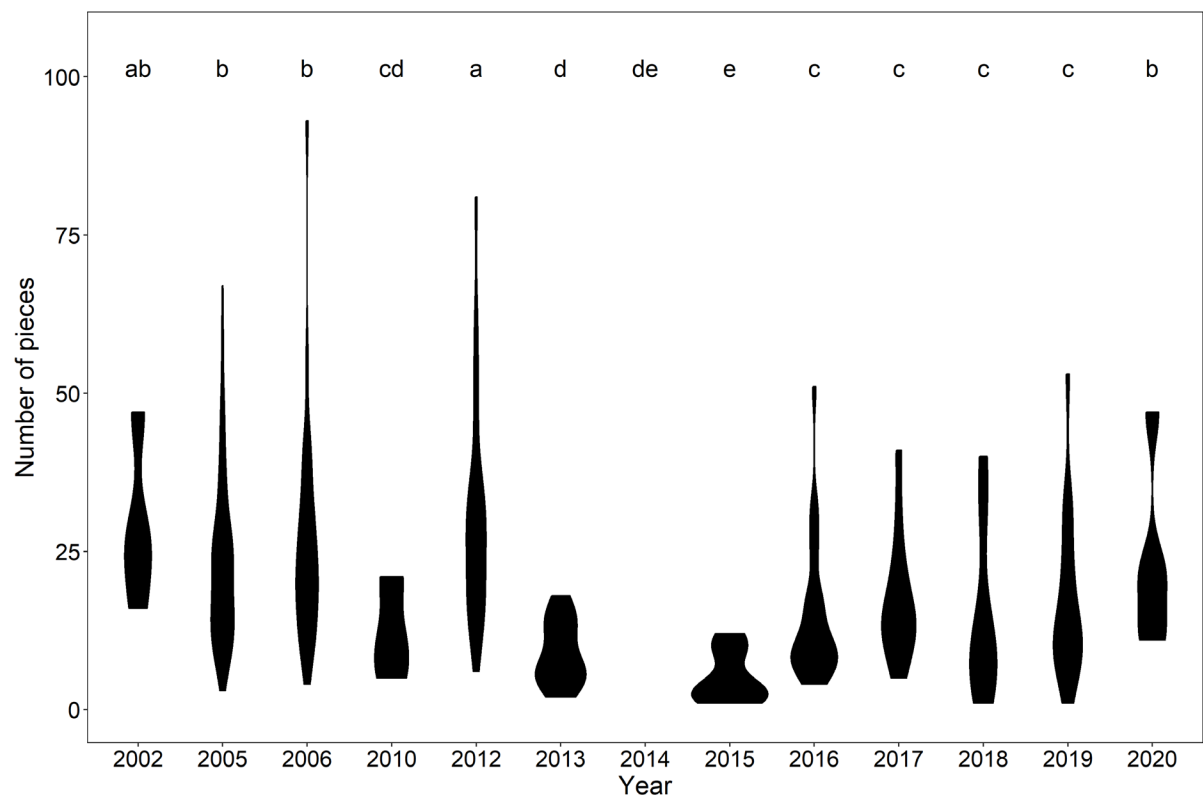


Figure 3. Mass (g) of ingested plastic items in Flesh-footed Shearwater boluses on Lord Howe Island during 2002-2020. Letters represent similar groups determined using Tukey's Honest Significant Differences post hoc tests; years sharing the same letter are not different from each other.

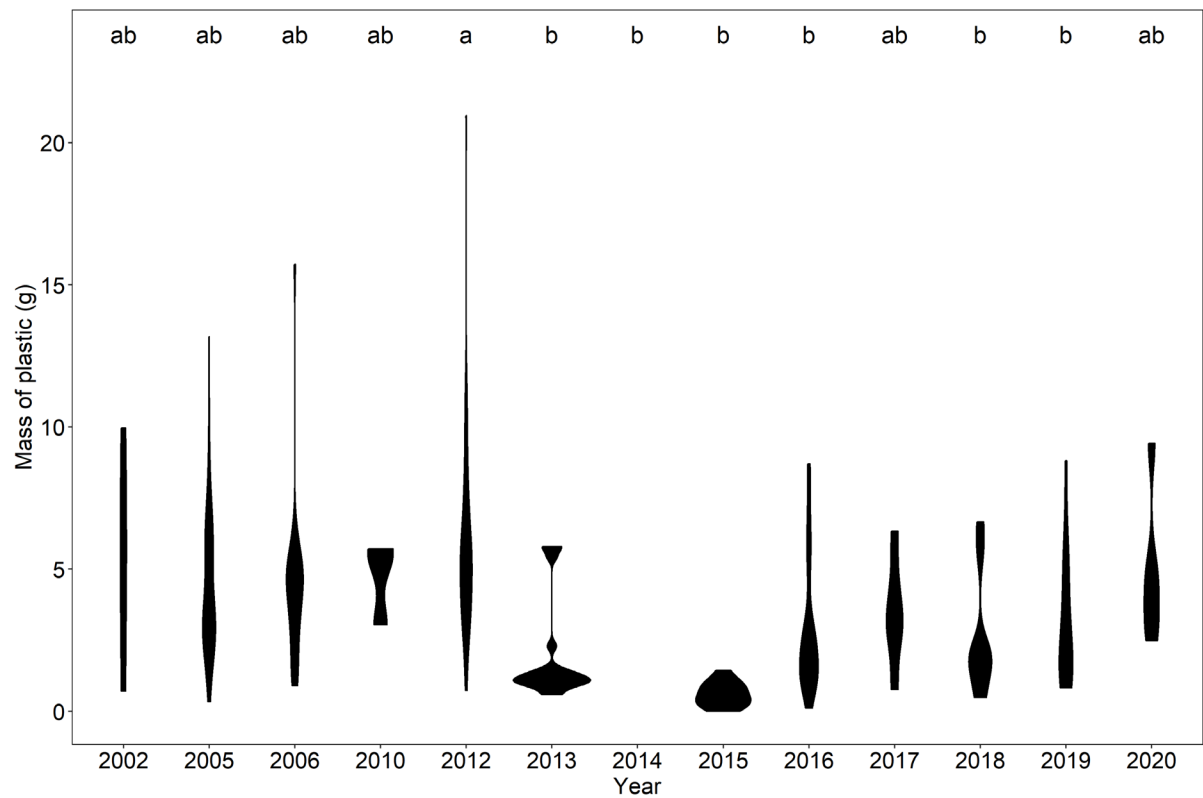


Figure 4. Power analysis graphs for plastic in Flesh-footed Shearwater boluses on Lord Howe Island during 2002-2020. Showing the number of individuals required to detect changes in plastic ingestion by number of plastic items ingested and mass (g) of ingested plastic.

