

# Ingested plastic as a route for trace metals in Laysan Albatross (*Phoebastria immutabilis*) and Bonin Petrel (*Pterodroma hypoleuca*) from Midway Atoll



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## ABSTRACT

Seabirds are declining faster than any other group of birds, with plastic ingestion and associated contaminants linked to negative impacts on marine wildlife, including >170 seabird species. To provide quantitative data on the effects of plastic pollution, we sampled feathers and stomach contents from Laysan Albatross (*Phoebastria immutabilis*) and Bonin Petrel (*Pterodroma hypoleuca*) on Midway Atoll, North Pacific Ocean, and assessed our ability to detect change over time by synthesizing previous studies. Between 25 and 100% of fledglings exceed international targets for plastic ingestion by seabirds. High levels of ingested plastic were correlated with increased concentrations of chlorine, iron, lead, manganese, and rubidium in feathers. The frequency of plastic ingestion by Laysan Albatross and concentration of some elements in both species is increasing, suggesting deterioration in the health of the marine environment. Variability in the frequency of plastic ingestion by Laysan Albatross may limit their utility as an indicator species.

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## 1. Introduction

Plastic debris is ubiquitous in the marine environment despite the implementation of international legislation (e.g., MARPOL Annex V) more than three decades ago that aimed specifically to reduce the amount of debris entering the oceans from marine-based sources. Increasing contamination of our oceans led to plastic pollution being identified by the United Nations Environment Programme (UNEP) as a global environmental issue (UNEP, 2014; Vegter et al., 2014). The list of species known to ingest plastic floating in the ocean is lengthy, increasing, and worrisome as it spans the entire marine food web, from copepods and polychaete worms, to mussels, squid, and an array of pelagic fish, as well as numerous apex predators including one of the most at-risk groups, the seabirds (Gall and Thompson, 2015; Laist, 1997; Wright et al., 2013). Effects of plastic debris range from the molecular to organismal level (Browne et al., 2015).

Ingested plastic debris negatively affects seabirds and other marine wildlife in a number of ways, including direct effects such as nutritional deprivation and physical damage to the digestive tract (Auman et al., 1998; Lavers et al., 2014; Pierce et al., 2004). Ingested plastic may also indirectly contribute to mortality or morbidity of wildlife through increasing exposure to metals and other contaminants such as

polychlorinated biphenyls (PCBs) and toxic trace elements that are absorbed from the surrounding sea water (Ashton et al., 2010; Endo et al., 2013; Engler, 2012; Rochman et al., 2013) and transferred to wildlife once ingested (Lavers et al., 2014; Tanaka et al., 2013; Teuten et al., 2009). For many wildlife populations already under pressure from anthropogenic alterations to the marine environment, ingested plastic and the associated contaminants is an additional stressor that has important implications for population sustainability (Lavers et al., 2014; McCauley and Bjørndal, 1999).

Of the 21 species of albatross and 39 species of *Pterodroma* petrels currently recognised (Brooke, 2004), plastic has been reported in the stomach of at least 12 (57%) and 17 (43.6%) species, respectively (Table 1). The most severely contaminated albatross species with regards to the frequency and mass of ingested plastic is the Laysan Albatross (*Phoebastria immutabilis*; Table 1). Consequently, this species has been the focus of numerous studies of plastic ingestion dating back to the 1960s (Auman et al., 1998; Fry et al., 1987; Gray et al., 2012; Hyrenbach et al., 2012; Kenyon and Kridler, 1969; Sievert and Sileo, 1993; Young et al., 2009) and has acted as a flagship species for marine debris awareness campaigns. In contrast, plastic and associated copollutant data are limited for most *Pterodroma* spp. (Table 1), including the Bonin Petrel (*Pterodroma hypoleuca*) which breed and forage sympatrically with Laysan Albatross (Kuroda, 1991).

Detailed plastic ingestion data from multiple years and locations are available for only a handful of seabird species (Table 1; Provencher et al.,

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**Table 1**A summary of plastic ingestion studies of albatrosses and *Pterodroma* petrels. FO: frequency of occurrence (%).

Albatrosses		FO	Source <sup>a</sup>
Atlantic yellow-nosed	<i>Thalassarche chlororhynchos</i>	0.0–8.0	Colabuono et al. (2010), Furness (1985)
Black-browed	<i>Thalassarche melanophris</i>	3.1–12.0	Colabuono et al. (2009), Jiménez et al. (2015)
Black-footed	<i>Phoebastria nigripes</i>	45.0–100.0	Blight and Burger (1997), Robards (1993)
Gray-headed	<i>Thalassarche chrysotoma</i>	0.0–10.0	Nel and Nel (1999), Nel et al. (2000)
Laysan	<i>Phoebastria immutabilis</i>	74.0–100.0	Kenyon and Kridler (1969); This study
Northern royal	<i>Diomedea sanfordi</i>	38.9	Jiménez et al. (2015)
Short-tailed	<i>Phoebastria albatrus</i>	63.6	McDermond and Morgan (1993)
Shy	<i>Thalassarche cauta</i>	0.0–0.9	Hedd and Gales (2001)
Sooty	<i>Phoebetria fusca</i>	0.0–1.4	Furness (1985), Hedd and Gales (2001)
Southern royal	<i>Diomedea epomorphora</i>	≤5.0–33.0	Day et al. (1985), Jiménez et al. (2015)
Tristan	<i>Diomedea dabbenena</i>	0.0–33.0	Colabuono et al. (2010), Jiménez et al. (2015)
Wandering	<i>Diomedea exulans</i>	0.0–4.5	Jiménez et al. (2015), Ryan (1987)
White-capped	<i>Thalassarche steadi</i>	0.0	Jiménez et al. (2015)
<b>Petrels</b>			
Atlantic	<i>Pterodroma incerta</i>	7.7–10.0	Day et al. (1985), Ryan (1987)
Black-winged	<i>Pterodroma nigripennis</i>	2.7–4.5	Ainley et al. (1990), Spear et al. (1995)
Bonin	<i>Pterodroma hypoleuca</i>	75.0–82.0	Sileo et al. (1990b); This study
Collared	<i>Pterodroma brevipes</i>	66.7	Spear et al. (1995)
Cook's	<i>Pterodroma cookii</i>	10.0–44.4	Ainley et al. (1985), Day et al. (1985)
Dark-rumped	<i>Pterodroma phaeopygia</i>	50.0–100.0	Robards (1993), Sileo et al. (1990b)
Juan Fernandez	<i>Pterodroma externa</i>	0.5–1.0	Ainley et al. (1985)
Gould's	<i>Pterodroma leucoptera</i>	11.8–15.2	Sileo et al. (1990b), Spear et al. (1995)
Great-winged	<i>Pterodroma macroptera</i>	7.7–10.0	Day et al. (1985), Ryan (1987)
Kermadec	<i>Pterodroma neglecta</i>	0.0–7.0	Ainley et al. (1985), Imber et al. (1995)
Henderson	<i>Pterodroma atrata</i>	0.0	Imber et al. (1995)
Mottled	<i>Pterodroma inexpecta</i>	0.0–60.0	Ainley et al. (1985), Robards (1993)
Murphy's	<i>Pterodroma ultima</i>	0.0–16.0	Ainley et al. (1985), Imber et al. (1995)
Phoenix	<i>Pterodroma alba</i>	0.0	Ainley et al. (1985), Spear et al. (1995)
Providence	<i>Pterodroma solandri</i>	12.1–50.0	Bester (2003), Robards (1993)
Pycroft's	<i>Pterodroma pycrofti</i>	40.0–50.0	Ainley et al. (1985), Spear et al. (1995)
Soft-plumaged	<i>Pterodroma mollis</i>	5.6–20.7	Furness (1985), Ryan (1987)
Stejneger's	<i>Pterodroma longirostris</i>	52.0–100.0	Ainley et al. (1985), Blight and Burger (1997)
Trinidad	<i>Pterodroma arminjoniana</i>	0.0	Ainley et al. (1985), Spear et al. (1995)
White-necked	<i>Pterodroma cervicalis</i>	8.3–10.0	Sileo et al. (1990b), Spear et al. (1995)

<sup>a</sup> Sources provided for minimum and maximum frequency of occurrence.

2014), therefore the Laysan Albatross represents a particularly valuable contribution to the study of plastic pollution impacts on marine wildlife. Despite this, knowledge of the relationship between ingested plastic and exposure to contaminants in this species, and other seabird species which have not benefited from targeted studies, remains poorly understood. Our goal was therefore to assess long-term trends in the ingestion of plastic by two offshore, surface feeding species (Harrison et al., 1983), the Laysan Albatross and Bonin Petrel on Midway Atoll, North Pacific Ocean in order to determine whether a relationship between plastic load and trace element concentrations exists for fledglings of either species.

## 2. Methods

### 2.1. Sample collection

Freshly dead (<24 h) Laysan Albatross ( $n = 40$ ) and Bonin Petrel ( $n = 7$ ) fledglings were collected from the colony surface on Midway Atoll, Papahānaumokuākea Marine National Monument (28°12'N, 177°21'W) from 19 to 26 June 2012 when birds were approximately 140 and 80 days of age, respectively (Grant and Whittow, 1983; Rice and Kenyon, 1962). Their cause of death was unknown, but presumed to be dehydration as has been reported in prior years (Sileo et al., 1990a). While lead poisoning due to the accidental ingestion of paint chips by albatross has been identified as a source of mortality on Midway in previous years (Finkelstein et al., 2003; Work and Smith, 1996), a range of mitigation measures have reduced the risk of exposure. For this study, care was taken to collect birds away from areas where lead paint chips could be present.

Ingested plastic was collected from the proventriculus and gizzard by necropsy. Plastic items were dried, weighed to the nearest 0.001 g using an electronic balance, and sorted by colour and type following

Lavers and Bond (2016). Four breast feathers were collected from each fledgling. Fledgling feathers were the preferred tissue as they are grown in a relatively short window (Pettit et al., 1984), have been found to be the best indicator of whole-body metal burden (Furness et al., 1986), represent exogenous input, with a minimal body pool of most elements (Braune and Gaskin, 1987), and elemental concentrations are often correlated with those of internal tissues (Eagles-Smith et al., 2008; Finger et al., 2015). Feathers were stored in sterile polyethylene bags at  $-20^{\circ}\text{C}$  prior to analysis. For each individual, we pooled all four feathers to reduce within-individual variability (Bond and Diamond, 2008). We chose feathers because they enabled comparisons with other studies and were easily collected, stored, and transported.

### 2.2. Trace element analysis

Feathers were digested in nitric acid and peroxide and trace element concentrations were measured in a PerkinElmer ELAN DRCII Inductively Coupled Plasma Mass Spectrometer (ICP-MS) using a protocol developed by Friel et al. (Friel et al., 1990) and detailed in Lavers et al. (Lavers et al., 2014). The reference materials used are mussel tissue NIST 2976 ( $n = 2$ ) and NIST-2977 ( $n = 2$ ). Reference materials were certified for concentrations of Mg, Al, P, S, Cl, Ca, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Ag, Cd, Sn, I, Cs, Ba, La, Ce, Hg, Tl, Pb, and U, and we restricted our statistical analysis to those elements that could be analysed reliably as assessed by the recovery of reference materials. We therefore included only Cl, Ca, Fe, Mn, Zn, As, Br, Rb, Sr, Ag, Cd, Hg, and Pb in our analysis. Recovery of the secondary reference material for these elements ranged from 86 to 110% among all runs. Values were corrected for background levels using procedural blanks. For each element, we used the keratin reference material with the same magnitude of concentration as the unknowns to correct for recovery.

Comprehensive quality assurance and quality control (QA/QC) details are provided in the supplementary material (Table S1).

2.3. Statistical methods

The relationship between ingested plastic mass and number of items on trace metal concentrations in Laysan Albatross and Bonin Petrel fledgling feathers was investigated using linear regression in RStudio (v 2.15.2, Boston, Massachusetts, USA). Outliers with a Cook's Distance >3 were excluded from the analysis (Kim and Storer, 1996; Rousseeuw and Leroy, 1987). Data were not transformed, as log-transforming values did not improve normality. We used published data to conduct a power analysis to assess the sample size needed to detect changes in Laysan Albatross plastic ingestion (frequency of occurrence, mass and number of ingested items) over time, as described in van Franeker and Meijboom (van Franeker and Meijboom, 2002). We estimated the sample size required to detect changes of 5%–100% (in 5% increments) with  $p < 0.05$ , and a power of 80% (Provencher et al., 2014; van Franeker and Meijboom, 2002). While post-hoc power analyses have several shortcomings (Hoening and Heisey, 2001), we used previously collected data to inform potential future monitoring schemes. Relationships were considered statistically significant when  $p < 0.05$  and values are reported as mean  $\pm$  SD, except regression parameters, which use standard error around parameter estimates.

Finally, we estimate the daily intake (mass of plastic;  $M_{adv}$ ) that may present a toxicologically important dose of elements using the formula:

$$M_{adv} = LOAEL/C_{BA}$$

where LOAEL represents the Lowest Observed Adverse Effect Level reported for each metal in piscivore birds (mg/kg body mass/day) (Sample et al., 1996) and  $C_{BA}$  is the estimated bioaccessible metal concentration for white plastic ( $\mu\text{g/g}$ ) (Holmes, 2013). Bird body mass was assumed to be 1.9 kg and 0.195 kg for Laysan Albatross and Bonin Petrels, respectively (Auman et al., 1998; Seto and O'Daniel, 1999).

3. Results

3.1. Ingested plastic

All Laysan Albatross ( $n = 40$ ) and 75% of Bonin Petrel ( $n = 8$ ) fledglings contained plastic in their digestive tract (Table 2). Prevalence of the avian pox virus (*Poxvirus avium*) can approach 100% in Laysan

Albatross on Oahu during high rainfall years, but has little or no effect on breeding success (Young and VanderWerf, 2008). We classified birds as having active pox if there were warty growths, open sores, or crusty scabs on the feet, legs, or face (Young and VanderWerf, 2008). A quarter ( $n = 10$ ) of Laysan Albatross fledglings in this study were infected with the avian pox virus on Midway Atoll in 2012. The amount of ingested plastic and concentration of trace elements in infected birds did not differ significantly from healthy birds ( $F_{1,39} = 1.72, p = 0.20$ ), so the data were pooled.

Overall, Laysan Albatross contained  $132.5 \pm 102.7$  pieces of plastic weighing  $52.0 \pm 43.6$  g and Bonin Petrel contained  $3.7 \pm 1.4$  pieces of plastic weighing  $0.05 \pm 0.07$  g (Table 2). Mean mass of the single largest ingested plastic item in each bird was  $3.5 \pm 3.6$  g ( $n = 40$ ) for Laysan Albatross and  $0.02 \pm 0.01$  g ( $n = 8$ ) for Bonin Petrel. These values are considered conservative as most Laysan Albatross expel a bolus just prior to fledging (Hyrenbach et al., 2012).

The majority of plastic ingested by Laysan Albatross and Bonin Petrels was white (albatross: 73.5%,  $n = 3882$ ; petrel: 63.6%,  $n = 14$ ; Table 3). We identified 5299 pieces of plastic ingested by Laysan Albatross, the majority of which were plastic fragments (88.1%) and industrial pellets (nurdles; 5.2%; Table 3). The types of plastic ingested by Bonin Petrel were similar, with fragments and pellets accounting 77.3% and 4.5% of the 22 items ingested, respectively (Table 3).

Based on the current sample sizes available for Laysan Albatross (Table 1), and the estimated coefficients of variation for frequency of occurrence, mean number ingested items, and mass of ingested plastic (CV = 0.257, 1.256, and 1.249, respectively), detecting a 5% change in the mean number of ingested items and mean mass of ingested plastic with a power of 80% would require annual sampling of >14,000 birds (Fig. 1b, c). Approximately 600 birds would be required to detect a 5% change in the frequency of occurrence of ingested plastic over time. The mean sample size over these studies ( $49 \pm 70$  birds; Table 2) would detect only a 455% change in the number of pieces of plastic, and a 20% change in the frequency of occurrence of ingested plastic (Fig. 1a).

3.2. Ecological Quality Objective (EcoQO)

Long-term data on plastic ingestion rates in Northern Fulmars (*Fulmarus glacialis*) in the North Sea led to the development of the Ecological Quality Objective (EcoQO), with an established target of no >10% of seabirds with 0.1 g of plastic (van Franeker et al., 2005). If the EcoQO was applied to Bonin Petrel fledglings, adjusting the value to 0.03 g of

Table 2

A review of published and unpublished studies reporting plastic ingestion by Laysan Albatross and Bonin Petrel. FO: frequency of occurrence (%), N/R: plastic recorded as being present, but values not reported.

Year	Age	Location	n	FO	Mean pieces	Max pieces	Mean mass (g)	Max mass (g)	Source
Laysan Albatross									
2012	Chick	Midway Atoll	40	1.000	132.5	450	52.02	161.00	This study
2009	Adult	Japan	1	1.000	8.00		0.46		Kuramochi et al. (2011)
2006–2008	Adult	North Pacific	18	0.833			0.99		Gray et al. (2012)
2005	Chick <sup>a</sup>	Oahu	8	1.000	70.60		4.37		Young et al. (2009)
2005	Chick <sup>a</sup>	Kure Atoll	15	1.000	17.46		38.03		Young et al. (2009)
1994–1995	Chick	Midway Atoll	168	0.979			20.95	136.30	Auman et al. (1998)
1986–1987	Chick	Hawaiian islands	233	0.906					Sileo et al. (1990b)
1986–1987	Adult	Hawaiian islands	31	0.350					Sileo et al. (1990b)
1982–1983	Chick	Midway Atoll & Oahu	50	0.875			43.51	175.00	Fry et al. (1987)
1982–1983	Adult	Midway Atoll & Oahu	4	0.500					Fry et al. (1987)
1978–1981	Chick	Hawaiian islands	12	N/R					Harrison and Seki (1987)
1979–1980	Chick	Midway Atoll	4	1.000					Pettit et al. (1981)
1979–1980	Chick <sup>a</sup>	French Frigate shoals	5	1.000			96.60		Pettit et al. (1981)
1966	Chick	Pearl & Hermes reef	100	0.740	2.43	8	1.84		Kenyon and Kridler (1969)
Bonin Petrel									
2012	Chick	Midway Atoll	8	0.750	3.7	8	0.05	0.19	This study
1986–1987	Both	Hawaiian islands	99	0.820					Sileo et al. (1990b)
1978–1981	Chick	Laysan & Lisianski Is.	144	N/R					Harrison and Seki (1987)

<sup>a</sup> Boluses (regurgitated pellets) from albatross fledglings.

**Table 3**

Colour and type of plastic ingested by Laysan Albatross and Bonin Petrel fledglings on Midway Atoll, June 2012. Frequency of occurrence (%), sample size (n).

	White		Blue		Green		Red/pink		Yellow		Orange		Black/brown		Purple	
	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n
Laysan Albatross	73.5	3882	6.2	328	6.1	323	3.9	207	0.8	40	0.7	36	8.7	458	0.2	8
Bonin Petrel	63.6	14	0.4	1	22.7	5					0.9	2				

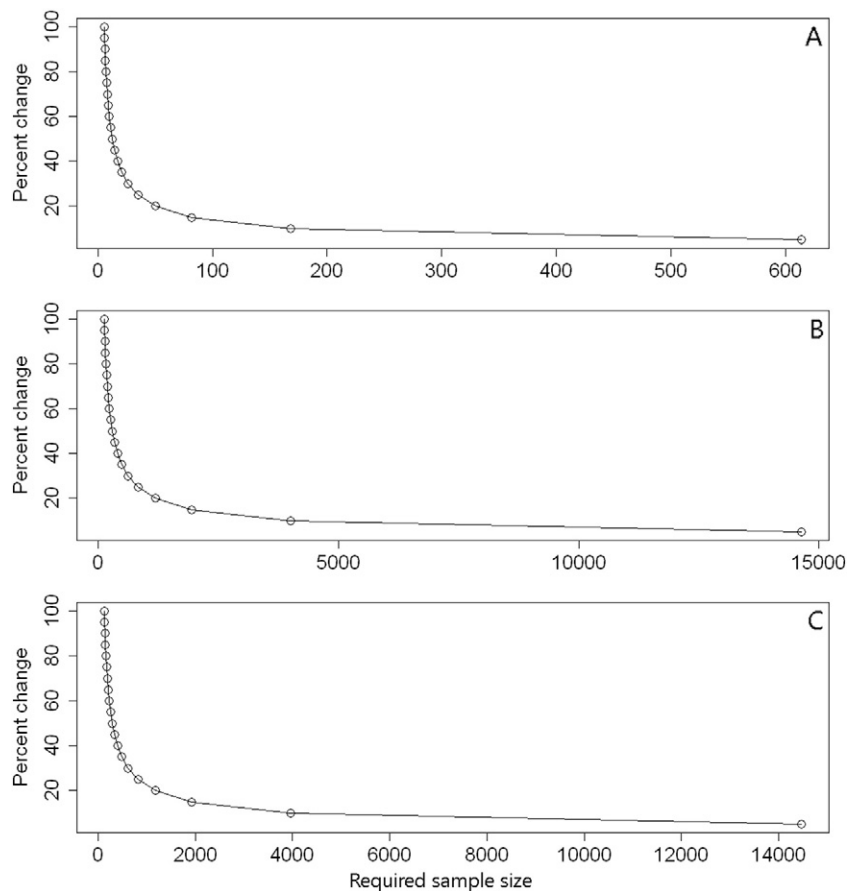
  

	Fragment		Pellet		Foam		Cap		Tubing		Line		Rope		Lighter		Bag		Strapping		Pen lid	
	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n
Laysan Albatross	88.1	4668	5.2	275	2.5	132	1.3	69	1.2	64	0.7	37	0.5	26	0.1	5	0.1	5	0.1	5	0.1	5
Bonin Petrel	77.3	17	4.5	1							18.2	4										

plastic following Bond and Lavers (Bond and Lavers, 2013) and assuming a 0.65 kg fulmar and 0.20 kg petrel, then 2/8 birds in our study (25%) fall above this threshold (ingested plastic  $0.05 \pm 0.07$  g). In contrast, 100% of Laysan Albatross exceeded the EcoQO threshold for this species [0.30 g, assuming a mean fledgling mass of 1.9 kg; 8] having ingested  $52.0 \pm 43.6$  g of plastic. On average, the albatross assessed in this study exceed the EcoQO by a minimum of six times (2.2 g of ingested plastic) with one bird ingesting 450 pieces of plastic totalling 161.0 g. This mass of plastic exceeds the relative EcoQO by  $5366\times$  (more than three orders of magnitude) and accounted for approximately 8.4% of the bird's body mass. Assuming albatross chicks are fed on average every 7 days for 140 days (Hyrenbach et al., 2002), and that ingested plastic is retained for this period (Ryan, 2015; Ryan and Jackson, 1987), 33 (83%) of the Laysan Albatross fledglings in this study exceed the species-adjusted EcoQO after a single feeding (range: 0.32–8.05 g of plastic per feeding).

### 3.3. Trace elements

We detected measurable concentrations of 13 trace elements in breast feathers from Laysan Albatross and Bonin Petrel fledglings (Table 3). The mass of ingested plastic was positively related to the concentrations of Fe ( $\beta = 3637 \pm 819 \mu\text{g/g plastic}^{-1}$ ,  $r^2 = 0.75$ ,  $p < 0.01$ ), Mn ( $\beta = 71.35 \pm 5.97 \mu\text{g/g plastic}^{-1}$ ,  $r^2 = 0.95$ ,  $p < 0.01$ ), Rb ( $\beta = 0.768 \pm 0.165 \mu\text{g/g plastic}^{-1}$ ,  $r^2 = 0.77$ ,  $p < 0.01$ ), Sr ( $\beta = 177.1 \pm 63.3 \mu\text{g/g plastic}^{-1}$ ,  $r^2 = 0.53$ ,  $p = 0.04$ ), and Pb ( $\beta = 16.44 \pm 5.21 \mu\text{g/g plastic}^{-1}$ ,  $r^2 = 0.60$ ,  $p = 0.03$ ) in Bonin Petrel, and Cl ( $\beta = 29.67 \pm 13.79 \mu\text{g/g plastic}^{-1}$ ,  $r^2 = 0.09$ ,  $p = 0.04$ ) in Laysan Albatross. The relationship approached significance for Cl in Bonin Petrel ( $\beta = -248.8 \pm 770.0 \mu\text{g/g plastic}^{-1}$ ,  $r^2 = 0.35$ ,  $p = 0.09$ ). The number of plastic items ingested was also positively related to the concentration of Cl in Laysan Albatross ( $\beta = 11.43 \pm 4.45 \mu\text{g/g pieces of plastic}^{-1}$ ,  $r^2 = 0.13$ ,  $p = 0.01$ ).



**Fig. 1.** Power analysis graphs for ingested plastic by Laysan Albatross Showing the number of individuals required to detect changes in plastic ingestion with 80% power and  $p < 0.05$  (a) frequency of occurrence (%), (b) number of plastic items ingested, and (c), mass of ingested plastic.

LOAEL and  $C_{BA}$  data were available for Pb (11.3 mg/kg/d, 0.079 mg/g) and Cd (20.3 mg/kg/d, 0.025 mg/g). The mass of ingested plastic thought to cause adverse effects ( $M_{adv}$ ) to Bonin Petrels and Laysan Albatross through contamination with Pb = is >27.7 g and >270.0 g, respectively,

and that for Cd is 153.2 g and 1494.2 g respectively (Table 4) and would not have been exceeded by any of the individuals we examined.

**Table 4**

Trace element concentrations ( $\mu\text{g/g}$ ; mean  $\pm$  S.D.) in feathers from juvenile Laysan Albatross (LAAL) and Bonin Petrel (BOPE) on Midway Atoll.

Element	$\mu\text{g/g} \pm$ S.D.	Year	n	Source
<b>Mercury (Hg)</b>				
LAAL	1.74 $\pm$ 0.66	2012	40	This study
LAAL	2.15 $\pm$ 0.12	1997	25	Burger and Gochfeld (2000c)
BOPE	2.04 $\pm$ 0.91	2012	8	This study
BOPE	3.87 $\pm$ 0.31	1997	20	Gochfeld et al. (1999)
<b>Lead (Pb)</b>				
LAAL	1.61 $\pm$ 3.39	2012	40	This study
LAAL	0.73 $\pm$ 0.10	1997	25	[68]
BOPE	1.19 $\pm$ 0.92	2012	8	This study
BOPE	0.80 $\pm$ 0.16	1997	20	[69]
<b>Cadmium (Cd)</b>				
LAAL	0.08 $\pm$ 0.09	2012	40	This study
LAAL	0.49 $\pm$ 0.19	1997	25	[68]
BOPE	0.05 $\pm$ 0.04	2012	8	This study
BOPE	0.10 $\pm$ 0.01	1997	20	[69]
<b>Arsenic (As)</b>				
LAAL	0.06 $\pm$ 0.07	2012	40	This study
LAAL	0.18 $\pm$ 0.04	1997	25	[68]
BOPE	0.14 $\pm$ 0.13	2012	8	This study
BOPE	0.19 $\pm$ 0.03	1997	20	[69]
<b>Manganese (Mn)</b>				
LAAL	0.72 $\pm$ 0.86	2012	40	This study
LAAL	1.63 $\pm$ 0.21	1997	25	[68]
BOPE	1.95 $\pm$ 3.31	2012	8	This study
BOPE	1.14 $\pm$ 0.16	1997	20	[69]
<b>Iron (Fe)</b>				
LAAL	48.34 $\pm$ 85.32	2012	40	This study
BOPE	230.26 $\pm$ 185.77	2012	8	This study
<b>Zinc (Zn)</b>				
LAAL	66.16 $\pm$ 19.01	2012	40	This study
BOPE	26.88 $\pm$ 10.06	2012	8	This study
<b>Bromine (Br)</b>				
LAAL	40.21 $\pm$ 10.83	2012	40	This study
BOPE	47.69 $\pm$ 32.40	2012	8	This study
<b>Rubidium (Rb)</b>				
LAAL	0.09 $\pm$ 0.07	2012	40	This study
BOPE	0.05 $\pm$ 0.04	2012	8	This study
<b>Strontium (Sr)</b>				
LAAL	5.68 $\pm$ 3.72	2012	40	This study
BOPE	16.76 $\pm$ 10.34	2012	8	This study
<b>Silver (Ag)</b>				
LAAL	0.04 $\pm$ 0.17	2012	40	This study
BOPE	0.03 $\pm$ 0.05	2012	8	This study
<b>Calcium (Ca)</b>				
LAAL	682.02 $\pm$ 386.04	2012	40	This study
BOPE	1824.67 $\pm$ 882.38	2012	8	This study
<b>Chlorine (Cl)</b>				
LAAL	7913.90 $\pm$ 3017.13	2012	40	This study
BOPE	4930.71 $\pm$ 3458.75	2012	8	This study
<b>Chromium (Cr)</b>				
LAAL	1.94 $\pm$ 0.27	1997	25	[68]
BOPE	2.11 $\pm$ 0.13	1997	20	[69]
<b>Selenium (Se)</b>				
LAAL	1.70 $\pm$ 0.18	1997	25	[68]
BOPE	4.86 $\pm$ 0.22	1997	20	[69]
<b>Tin (Sn)</b>				
LAAL	3.42 $\pm$ 0.64	1997	25	[68]

## 4. Discussion

### 4.1. Ingested plastic

The results of our study indicate the proportion of Laysan Albatross fledglings contaminated by the ingestion of plastic debris has increased steadily over the past fifty years from 74% in 1966 (Kenyon and Kridler, 1969) to 100% in 2012 (Table 1, Fig. 2;  $r^2 = 0.90$ ,  $\beta = +0.06 \pm 0.04\%$  year $^{-1}$ ,  $p = 0.01$ ). The sample size required to detect this 25% increase in the frequency of occurrence of ingested plastic with 80% power is 35 (Fig. 1a). The frequency of plastic ingestion by seabirds has been identified as a potential indicator of the quality of the marine environment as well as the risk to seabird health (Lavers et al., 2014; van Franeker et al., 2011).

While the EcoQO has broad appeal as a target for policy makers, its success hinges on the identification of reliable indicator species. And while the EcoQO was established for a particular species and system as an indicator of plastic in the environment (rather than its effects on birds), by using a mass-adjusted value, this could be applied to systems elsewhere. The Northern Fulmar and Common Murre (*Uria aalge*) exhibit low levels of inter-annual variation in the amount of plastic ingested, and as a consequence, have been proposed as indicators of plastic pollution in the North Atlantic because the number of birds required to reliably detect changes over time is manageable (Provencher et al., 2014; van Franeker et al., 2004). In contrast, the mean mass of ingested plastic and number of plastic items ingested by Laysan Albatross fledglings is highly variable from year to year (Table 2), a pattern which has been highlighted in prior studies (Fry et al., 1987; Sileo et al., 1990a). Though Laysan Albatrosses are often used as a flagship species for outreach regarding the impacts of plastic pollution in the North Pacific because of their charismatic nature, the number of individuals required for annual monitoring is prohibitively high (600–14,000 per year) due to the inherent variability in their ingestion of plastic. Bonin Petrel and other candidate species such as the Black-footed Albatross (*Phoebastria nigripes*) are less variable in their plastic ingestion (Table 1), but the data required to fully assess their potential as reliable indicators is lacking (Blight and Burger, 1997; Gray et al., 2012; Robards, 1993).

The ingestion of large, sub-lethal volumes of plastic in around 27% of Laysan Albatross fledglings has been linked to a significant reduction in fledging weights and chick survival to fledging of up to 5.7% (Auman et al., 1998; Sievert and Sileo, 1993). Seabirds reared under unfavourable conditions (as inferred by reduced growth rate and fledging mass) are known to exhibit low survivorship (Morrison et al., 2009; Sarau et al., 2011) and low return rates have been linked to population declines (Chastel et al., 1993; Croxall et al., 1988; Kitaysky et al., 2006). Despite this, numerous studies suggest the overall impact of ingested plastic on Laysan Albatross is either unclear or negligible (Auman et al., 1998; Fry et al., 1987; Sievert and Sileo, 1993). These conclusions were based largely on evidence of an increase in Laysan Albatross populations (Arata and Sievert, 2009; Naughton et al., 2007). However, recent surveys suggest the Laysan Albatross population on Midway is no longer increasing (USFWS unpublished data) and the less visible, but potentially equally damaging consequences of exposure to plastic-associated toxins has been largely ignored despite the issue being raised for Laysan Albatross in the 1960s (Kenyon and Kridler, 1969).

### 4.2. Trace elements

While the relative contributions of plastic- and trophic-derived contaminants (e.g., from the ingestion of contaminated prey) is difficult to quantify (Bakir et al., 2014; Holmes, 2013) the potential for ingested plastic to transfer toxins to marine wildlife has been highlighted by a

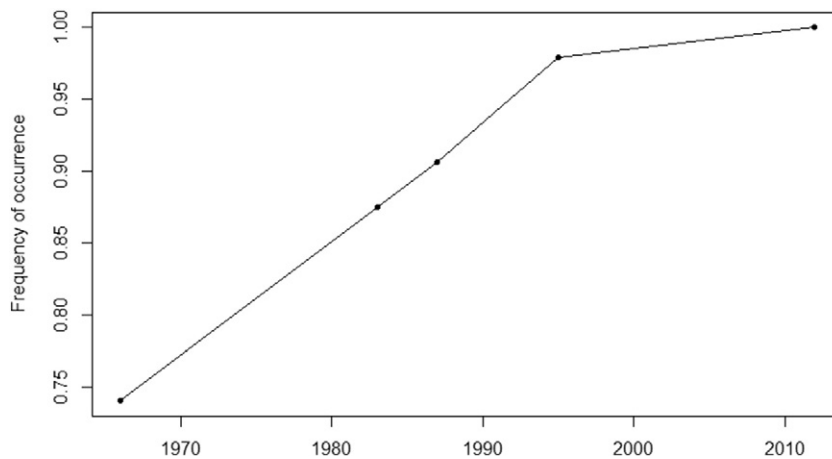


Fig. 2. Frequency of occurrence of ingested plastic in Laysan Albatross fledglings from Hawaii increased significantly during 1966–2012 (adapted from Table 2).

handful of recent studies showing plastic-associated contaminants, including metals and metalloids, in the tissues of animals that had ingested plastic (Browne et al., 2013; Tanaka et al., 2013), with higher concentrations of toxins linked to the mass of plastic consumed (Lavers et al., 2014). In Laysan Albatross, high concentrations of Cl ( $7913 \pm 3017 \mu\text{g/g}$ ; Table 4) were positively related with the mass and number of plastic items ingested ( $p < 0.05$ ) while plastic mass was positively related to concentrations of Fe, Mn, Rb, Sr, and Pb in Bonin Petrel ( $p < 0.05$ ). In Flesh-footed Shearwaters (*Ardenna carneipes*), ingested plastic has been linked to increased concentrations of chromium and silver in feathers (Lavers et al., 2014) suggesting the transfer of bioavailable contaminants is species and/or site specific. Critically, this highlights the implication that broad generalizations about the transfer of trace elements from ingested plastics are not yet possible, and the mechanisms that underpin this process remain unknown. The degree to which contaminants adhere to plastic is influenced by a number of factors, including pH and duration of exposure (Bryan, 1971; Holmes, 2013; Sharom and Solomon, 1981), and may obscure the relationship between the amount of plastic ingested and the concentration of trace elements in animal tissues. The type of plastic polymer can also influence concentrations (Ashton et al., 2010; Holmes et al., 2014; Nakashima et al., 2012); Laysan Albatrosses on nearby Kure Atoll ingest a variety of plastic types (Nilsen et al., 2014), but these have not been related to associated concentrations of trace elements.

Lead concentrations in Bonin Petrel and Laysan Albatross feathers from Midway Atoll are similar to values reported for other Hawaiian seabirds ( $1.19 \pm 0.92 \mu\text{g/g}$  and  $1.61 \pm 3.39 \mu\text{g/g}$ , respectively; Table 4) (Burger and Gochfeld, 2000c), but appear to have increased since the mid-1990s (Gochfeld et al., 1999). Lead concentrations exceeding  $4 \mu\text{g/g}$  in feathers are associated with negative effects on seabird behaviour and physiology, including lowered chick survival (reviewed in Burger and Gochfeld, 2000a). Three (7.5%) of the 40 Laysan Albatross fledglings in our study had lead concentrations more than twice this limit ( $12.71 \pm 4.34 \mu\text{g/g}$ ). Mercury concentrations in Bonin Petrel collected on Midway Atoll in 1997 were reported to be higher than levels known to cause adverse reproductive and behavioural effects (Burger and Gochfeld, 2000b), but appear to have decreased in recent years with only one bird approaching the hypothesized effect level of  $5 \mu\text{g/g}$  in feathers on Midway in 2012 ( $3.86 \mu\text{g/g}$ ; Table 4) (Burger, 1993). The Cd concentrations observed in Laysan Albatross and Bonin Petrel in this study (Table 4) fall within the known range of Cd concentrations from bird feathers, however one (11%) petrel and 10 (25%) albatross were above the hypothesized effect level of  $2.00 \mu\text{g/g}$  (Burger, 1993). Increased levels of Pb, Hg, and Cd may contribute to reduced body condition, and perhaps juvenile survival, in birds found to exceed safe levels. Using the LOAEL (Sample et al., 1996), the daily intake of plastic necessary to present a toxicologically important dose of Pb and Cd in Laysan

Albatross and Bonin Petrel is significantly larger than that reported in the birds included in this study (Table 5), suggesting some plastic associated pollutants may not pose an immediate threat to these species.

The apportionment of contaminants to either plastic- or prey-derived sources is very challenging, and currently only possible with compounds with multiple congeners (e.g., polybrominated diphenylethers; Tanaka et al., 2013). Tools such as stable isotopes of metals and metalloids may prove fruitful in this regard (Kidd et al., 2012; Tütken et al., 2011). The risk posed to seabirds from ingested plastic is significant and has been increasing for many decades despite growing awareness of the issue and implementation of targeted legislation. This upwards trend is likely to continue in line with growing demand for plastic products burdened with a diverse range of contaminants. Consequently, mortality and morbidity due to plastic and its associated contaminants are likely to play an increasingly important role in driving seabird population trends. Our results for Laysan Albatross and Bonin Petrel lend further support to correlative evidence from studies in which animals exposed to plastic exhibited signs of morbidity (Browne et al., 2013; Ryan, 1988; Teuten et al., 2009) and contained higher concentrations of contaminants (Lavers et al., 2014). Relatively small increases in the amount of plastic consumed (e.g.,  $100 \text{ cm}^3$  vs.  $200 \text{ cm}^3$  in Laysan Albatross) have been linked to a 12% reduction chick survival to fledging (Sievert and Sileo, 1993) and a proposed 11% reduction in the survival of shearwater fledglings to recruitment (Lavers et al., 2014). The ingestion of plastic has been recorded in all oceans with at least 174 species, or approximately 56% of the world's seabird species, known to be negatively affected (Gall and Thompson, 2015; Laist, 1997). Plastic, therefore, has potential broad implications for seabird populations and marine communities in general. Additional studies investigating species and regional variation in the burden of the plastic-derived chemicals to wildlife, their transfer to organisms, and potential adverse effects are urgently needed (Browne et al., 2015).

Table 5

Toxicity effect levels, lowest observed adverse effects levels (LOAEL), bioaccessible concentrations ( $C_{BA}$ ), and mass of plastic required on a daily basis for adverse effects to be possible in terms of metal toxicity ( $M_{adv}$ ) reported for selected trace metals.

Element	Effect level ( $\mu\text{g/g}$ ) <sup>a</sup>	LOAEL (mg/kg body mass/day) <sup>b</sup>	$C_{BA}$ (mg/g) <sup>c</sup>	$M_{adv}$ (g plastic) <sup>c</sup>	
				Bonin Petrel	Laysan Albatross
Cadmium	0.10–2.00	20.03	0.025	153.17	1492.4
Lead	4.00	11.3	0.079	27.7	270.0
Mercury	5.00	0.9	N/A	N/A	N/A

<sup>a</sup> Based on values reported by Burger (Burger, 1993).

<sup>b</sup> Based on values reported by Sample et al. (1996).

<sup>c</sup> Based on formulae provided by Holmes (Holmes, 2013).

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