Ingested plastic and trace element concentrations in Short-tailed Shearwaters *(Ardenna tenuirostris)*

Peter S. Puskic[1], Jennifer L. Lavers[2, *], Louise R. Adams[1], Alexander L. Bond[2,3]

[1] Institute for Marine and Antarctic Studies, University of Tasmania, School Road, Newnham, Tasmania 7250, Australia
[2] Institute for Marine and Antarctic Studies, University of Tasmania, 20 Castray Esplanade, Battery Point, Tasmania 7004, Australia
[3] Bird Group, Department of Life Sciences, The Natural History Museum, Akeman Street, Tring, Hertfordshire, HP23 6AP, United Kingdom

*****Corresponding Author: Jennifer L. Lavers (Jennifer.lavers@utas.edu.au, +61 (03) 6324 3868), Institute for Marine and Antarctic Studies, University of Tasmania, 20 Castray Esplanade, Battery Point, Tasmania 7004, Australia

ABSTRACT

Pollution of marine environments is concerning for complex trophic systems. Two anthropogenic stresses associated with marine pollution are the introduction of marine plastic and their associated chemicals (e.g., trace elements) which, when ingested, may cause harm to wildlife. Here we explore the relationship between plastic ingestion and trace element burden in the breast muscle of Short-tailed Shearwaters (*Ardenna tenuirostris*). We found no relationship between the amount of plastic ingested and trace element concentration in the birds' tissues. Though the mass and number of plastic items ingested by birds during 1969 - 2017 did not change significantly, trace element concentrations of some elements (Cu, Zn, As, Rb, Sr and Cd), appeared to have increased in birds sampled in 2017 compared to limited data from prior studies. We encourage policy which considers the data gleaned from this sentinel species to monitor the anthropogenic alteration of the marine environment.

Keywords: Ardenna tenuirostris; ecological indicator; marine debris; muttonbird; plastic pollution; toxicology

Marine and terrestrial environments face ever-growing pressures in the human-induced Anthropocene epoch (Crutzen, 2002; Waters et al., 2016). In the case of the ocean environment, which fosters life and drives global climate (Hoegh-Guldberg and Bruno, 2010), there is growing concern following predictions of further biodiversity loss and increasing pollution levels (MacArthur et al., 2016; Worm, 2016). Understanding and mitigating pressures on marine ecosystems typically requires two pieces of important information: data which provide an indicator of historical (baseline) conditions, and follow-up (monitoring) data to identify any changes over time and guide future research (Pauly, 1995). To achieve this in the vast, dynamic marine environment, we can make use of sentinel (or indicator) species which can provide large volumes of data at relatively low cost while being specific to an area of interest (Caro, 2010; Durant et al., 2009; Van Franeker et al., 2011). As apex predators that faithfully return to breeding islands, seabirds are an ideal sentinel for the marine environment, reflecting changes in prey abundance and pollution, including a suite of chemicals (Mallory et al., 2010; Piatt et al., 2007).

Contamination of the marine environment comes from myriad sources, including influx from rivers, agricultural and urban runoff, and long-range atmospheric transport (Amunsen et al., 1992). Chemicals accumulate throughout marine waters (Lamborg et al., 2014; Lohmann et al., 2007) and the species that inhabit them (Eagles-Smith et al., 2009; Finger et al., 2015). Some elements are essential for metabolic and biological function, but in high concentrations are toxic (e.g., copper (Cu); Lemos et al., 2013). However, exposure to low concentrations of non-essential elements (e.g., mercury (Hg)) can contribute to poor health of individuals and populations. Elevated trace element concentrations in birds has direct, and indirect health impacts, such as liver and kidney damage (e.g. Cd; Hoffman et al., 2011; Nicholson and Osborn, 1983; Salamat et al., 2014), reduced survival (Goutte et al., 2015), and changes in feather pigmentation (Pacyna et al., 2018).

While toxicity thresholds for some non-essential trace elements are poorly understood, especially in birds, effect levels have been proposed for elements such as Hg (5 µg/g, though as high as 20 µg/g in some marine species), lead (Pb; 4 µg/g), and cadmium (Cd; 0.1 – 5 µg/g) determined from feathers samples of seabirds (Burger, 1993; Burger and Gochfeld, 2000b) however, these are highly species-specific (Bond et al., 2015a). Analysis of muscle tissue from birds provides information on trace element burden over a period of ~4 weeks (Lewis and Furness, 1991; Ramos and Gonzalez-Solis, 2012), and is a reliable indicator of chemicals associated with metabolic function (Fromant et al., 2016). Chemical pollutants in wildlife, including trace elements, are obtained from a variety of sources, including prey (Bustamante et al., 1998), with some species experiencing heightened exposure through the ingestion of plastic (Chua et al., 2014; Lavers et al., 2014; Sleight et al., 2017). The nature of plastics to concentrate environmentally prevalent chemicals (Ashton et al., 2010; Mato et al., 2001) and release them into an animal's tissues following ingestion (Tanaka et al., 2013; Turner, 2018) is worrying as plastic ingestion has been documented in a variety of species and across trophic levels, from invertebrate primary consumers (Dawson et al., 2018) to large marine mammals (Fossi et al., 2018). The ingestion of plastic by wildlife has also been linked to direct impacts, such as starvation (Pierce et al., 2004), as well as a range of lesser-known physiological effects, including altered behaviour (e.g., lowered predator avoidance; Mattsson et al., 2017; Seuront, 2018), tissue damage (Peda et al., 2016; Rochman et al., 2013) and changes in blood chemistry (Lavers et al., 2019).

In seabirds, adults often provision chicks with prey collected from well-defined foraging grounds adjacent to breeding colonies (Cleeland et al., 2014; Miller et al., 2018). Studies of Short-tailed Shearwater (*Ardenna tenuirostris*) therefore provide information on the health of the Southern Ocean, where parent birds forage during the chick rearing period (Cleeland et al., 2014), feeding on low trophic level prey such as Antarctic krill (*Euphausia superba*; Skira et al., 1996). While foraging for prey, adult birds mistakenly consume plastic and feed it to their chicks (Carey, 2011; Waller et al., 2017), with >80% of Short-tailed Shearwater fledglings containing plastic debris (Acampora et al., 2014; Cousin et al., 2015). It is unclear whether the chemicals detected in Short-tailed Shearwater tissues (Honda et al., 1990; Lavers and Bond, 2013) originated primarily from their prey, or ingested plastics as has been correlated in other seabird studies (Lavers and Bond, 2016a; Lavers et al., 2014).

In this study, we quantified plastic ingestion and trace element burdens of Short-tailed Shearwater fledglings and compare with findings from similar data from 2011 (Lavers and Bond (2013) to (a) examine the relationship between plastic ingestion, body condition, and trace element burden in these birds, (b) provided updated data on the trace element concentration of Tasmanian Sort-tailed Shearwaters, and (c) examine changes in plastic ingestion over time to perform a post hoc power analysis of our current ability to assess these trends. We purchased 38 whole frozen Short-tailed Shearwater fledglings from harvesters under recreational (individually held) collection permits on Great Dog Island in the Furneaux Group, Tasmania, Australia (40.247°S, 148.249°E) in late-April 2017. Birds were immediately frozen intact after collection and remained in frozen during transportation and when stored at the Institute for Marine and Antarctic Studies aquatic animal health laboratory. We recorded standard bird morphometric measurements (mass (± 10 g), wing chord (unflattened and straightened; ± 1 mm), culmen (± 0.1 mm mm), head + bill (± 0.1 mm)) for 38 fledglings (approx. 80-90 days old) and followed the recommended protocol for quantifying plastic in seabird necropsies (van Franeker, 2004). Ingested plastic items including micro-plastic (>1 µm; Barnes et al., 2009) and visible macro-plastic (> 5mm diameter; Provencher et al., 2017), from the proventriculus and gizzard were categorised by type and colour following Provencher et al. (2017), with a minor modification to the colour categories in order to be consistent with a long-term monitoring study of Australian Procellariiformes (Lavers and Bond, 2016b; Puskic et al., 2019). Plastic items were dried and weighed to the nearest 0.001 g using an electronic balance. The whole breast muscle (pectoralis major; approximately 4.5 g (dry weight) in total) was removed and stored individually in aluminium foil and sterile sealed bags at -20°C.

From a subset of fledglings (n = 20), the whole breast muscle sample was freeze-dried for 72 h to a constant weight, to determine dry matter and moisture content. Approximately 0.2000 g of dried sample was homogenised to a fine powder using a mortar and pestle, placed in 100 ml glass vials, and digested in 2 ml of concentrated nitric acid (HNO_3) (supplier details) with an antibumping granule at 90°C for 2 h. The solution was analysed for 19 elements (Na, Mg, Al, S, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd and Pb) against the certified reference material dogfish muscle (DORM-2; certified for Hg, and arsenic (As) from the National Research Council of Canada) using a Sector Field Inductively Coupled Plasma Mass Spectrometer (ICP-MS Element 2, Thermo Fisher, Bremen, Germany). International certifications for the remaining elements were sourced from the Geological and Environmental Reference Materials database (GeoReM: georem.mpch-mainz.gwdg.de; Jochum et al., 2005). To monitor analytical recovery and limit erroneous data due to element rich matrices, a known quantity (10 ng/g) of a random sample digest was spiked with a multi-element on top of the matrix. A total of 5 sample blanks were run for quality control. The mean recovery across all elements in the spiked samples tested was ~80 - 130%, indicating the sample digest matrix had no detrimental effect on the results (Table S1).

Samples with a concentration below the detection limit of the ICP-MS were excluded (136/380 total cases, 36%), and the remaining analytical duplicates averaged. Analysis of the resulting dataset was completed using R (version 3.4.3; R Core Team, 2018) in RStudio (v.3.4.0, Boston, Massachusetts, USA). The relationship between trace element concentrations and the mass and number of ingested plastic items was examined using linear regression. We examined outliers by calculating Cooks Distance, and as none had a value >3, all data were retained (Cook, 1977, 1979). Relationships were considered significant when $p < 0.05$ and final results are reported as µg/g and expressed as mean ± standard deviation (range). We extracted data from existing publications on the frequency of occurrence, number and mass of plastic ingested by Short-tailed Shearwaters (n = 17 papers). We separated these data by age class (adult and fledgling) and excluded papers that did not report bird age (2 papers) or had sample sizes < 10 (5 papers; Table 1). We conducted a power analysis (van Franeker and Meijboom, 2002) on this data following Provencher et al. (2015), to estimate the sample size required to determine percent changes in the frequency of occurrence, number of plastic pieces, and plastic mass in adult and fledgling samples at 5, 10, 15, 25, 50, 75 and 100 %.

Plastic ingestion in Short-tailed Shearwaters appears to have remained consistent over time, with a frequency of occurrence (FO) of >80% across most studies (Fig. 1, Table 1), though the inconsistent monitoring of this species over time, age classes, and locations make this data difficult to compare, and the collection methods (i.e., beach-washed, by-catch and road-killed birds) of each of these studies vary and may influence our overall understanding of the interactions of these birds and plastic (Rodríguez et al., 2018). Of the total fledglings necropsied (n = 38), 89.5% contained plastic in their proventriculus (0.76 ± 1.34 items, range: 0-5; 19.5% of all plastic items) and/or gizzard (3.24 ± 3.89 items, range: 0-13; 80.5% of all plastic items). Overall, the mean number of plastic pieces recorded in individuals was 3.78 ± 3.79 items weighing 0.06 ± 0.06 g. The dominant types of ingested plastic were hard fragments (n = 103 pieces, 75.7%) and pre-production pellets (‘nurdles’; 30 items, 22.1%), followed by thread, sheet and foam (1 item (0.7%) per type). The most common colour of ingested plastic was white (87 items, 63.9%), followed by green (n = 24, 17.6%), black (n = 20, 14.7%), blue (n = 3, 2.2%), red and yellow (n = 1,0.7%) each (Table S2).. Short-tailed Shearwater fledgling body mass varied greatly (583.5 ± 93.4 g; range 300-730 g), similar to birds randomly collected as roadkill (522.64 ± 105.47 g; range 254-784 g; Rodríguez et al., 2018), suggesting recreational harvesters selected birds at random. Fledgling morphometric measurements were not related to plastic mass (bird mass: $F_{1,36} = 0.01$, $p = 0.91$, wing chord: $F_{1,36} = 2.22$, $p = 0.15$, culmen: $F_{1,36} = 0.46$, $p = 0.50$, head + bill length: $F_{1,36} = 2.35$, $p = 0.13$,) or number of pieces (bird mass: $F_{1,36} = 0.13$, $p = 0.73$, wing chord: $F_{1,36} = 1.22$, $p = 0.28$, culmen: $F_{1,36} = 1.68$, $p = 0.20$, head + bill length: $F_{1,36} = 4.08$, $p = 0.05$). Plastic in high quantities can impact fledgling development in some seabird species (Lavers et al., 2014), and has the capacity to introduce trace elements when ingested (Browne et al., 2013; Lavers and Bond, 2016a; Luís et al., 2015). However, we found minimal evidence of ingested plastic having a significant impact on Short-tailed Shearwater body condition, in line with previous studies (Acampora et al., 2014; Carey, 2011; Cousin et al., 2015; Rodríguez et al., 2018), and no evidence of a relationship between plastic and elevated trace element concentrations in these birds. Short-tailed Shearwaters ingest relatively small amounts of plastic compared to other Procellariforms, such as Flesh-footed Shearwaters (*Ardenna carneipes*; Lavers et al., 2014) and Laysan Albatross (*Phoebastria immutabilis*; Young et al., 2009), which may explain our observation. Additionally, our ability to detect changes may be influenced by our small sample size (n = 20 shearwaters analysed for trace elements).

We used data from 12 existing studies from 1969 – 2017 (Table 1) to conduct a power analysis. Fledgling birds required lower sample sizes to detect changes in the frequency of occurrence of plastic (to detect a 5% change in fledglings; n = 41, adults; n = 658). However, to detect changes in the mass and number of plastic items ingested by these birds would require sample sizes much larger than any study has reported to date (Table 2). Results from our power analysis suggests changes within the frequency of occurrence of plastic ingested by Short-tailed Shearwaters can be monitored with lower samples sizes in fledgling birds (5% change when n = 41) compared to studies on adult birds (5% change when n = 798; Table 2). Fledgling seabirds are regarded as reliable sentinels as they are provisioned by adults from well-defined foraging regions and bird development is directly indicative of local conditions over a defined time-frame (Burger et al., 2001; Provencher et al., 2019). In contrast, studies of adult birds can be used to explore the biomagnification of contaminates over their life span. Mass and number of plastic pieces ingested appears much harder to monitor (Table 2) due to the higher variability over time.

In a subset of Short-tailed Shearwater fledglings (n = 20), we detected measurable concentrations of 10 trace elements in the dry breast muscle tissue (moisture content 62.5% ± 21.6; range: 8.25-92.94 %), the remaining nine elements were below the level of detection (LoD; Table S1). Mean concentration of six non-essential trace elements (Cu, Zn, As, Rb, Sr and Cd), had increased relative to the only comparable study on breast muscle samples of Tasmanian fledglings in 2011 (Lavers and Bond, 2013; Table 3). A lack of long-term fledgling health or recruitment monitoring and incidentally sparse datasets for trace element levels means we cannot draw trends or predict what this data means for the overall health of these populations. Continued monitoring of fledgling birds provides the best understanding of the anthropogenic impacts of the Southern Ocean, where parent birds forage to feed young (Cleeland et al., 2014). As seabirds tend to bioaccumulate trace elements over time monitoring of birds at a variety of life stages can also indicate the extent of this pollution and what levels are frequent (Burger and Gochfeld, 2004; Mallory et al., 2010). As the impacts of trace elements in seabirds appear to be highly species specific (Bond et al., 2015a), and in some cases show little impact on individuals (Carravieri et al., 2018), future research on

Short-tailed Shearwaters should aim to determine effect levels of various trace elements. Many trace elements, in high concentrations are associated with adverse health implications (Burger and Gochfeld, 1985, 2000a). In Flesh-footed Shearwaters, high plastic ingestion is thought to be directly related to mortality and the associated chemicals believed to incur morbidity (Fossi et al., 2018; Lavers, 2015; Lavers et al., 2014). Trace element concentrations were unrelated to the mass or number of plastic items ingested by fledgling shearwaters in our study and showed few significant relationships with bird morphology (Table 4). Plastic production is expected to increase (PlasticsEurope, 2018), therefore the number of species documented to be impacted by its ingestion is also growing (Barnes et al., 2009; Gall and Thompson, 2015) and the indirect impacts of plastic ingestion, such as the introduction of non-essential trace elements require urgent attention (Holmes, 2013).

The birds used in this study were destined for human consumption. Short-tailed Shearwaters or Yolla are harvested in Tasmania annually and are of cultural significance to some aboriginal Tasmanian communities (Hill et al., 1981). The harvesting of seabirds for their meat and fat is documented in historical and archaeological records across the globe (Anderson, 1996; West and Sim, 1995). While there is some concern regarding the consumption of high end consumers which have high trace element concentrations (Bond et al., 2015b; Hightower and Moore, 2003; Johansen et al., 2004; Johansen et al., 2006), globally, there is a lack of contaminant monitoring in these wild, free-living and traditional food sources. In the Arctic, marine mammals destined for First Nations consumption are assessed periodically (AFN, 2007; Moses et al., 2009). Dialogue between the communities which consume unmonitored wildlife, the researchers who monitor these populations and regulatory bodies to ensure traditional foods can be consumed safely while safeguarding traditional practices and culture.

In the Northern hemisphere similar species such as the Northern Fulmer (*Fulmarus glacialis*), are used in such a way that Ecological Quality Objectives (EcoQO) are put in place to monitor plastic impacts in the local marine environment. EcoQO targets developed for Northern Fulmars aim to see no more than 0.1 g of plastic in 10% of a population within a five year period (OSPAR, 2008; van Franeker and Kühn, 2018). We lack similar international policies and targets for pollution in Southern Hemisphere despite increasing data to develop these (Table 1). Due to the number of studies on plastic ingestion in this species, the Short-tailed Shearwater can be presented as one of the most studied Southern Ocean seabirds. The use of the Short-tailed Shearwater as sentinel species may aid in the preservation of marine environments and conservation of cultural heritage.

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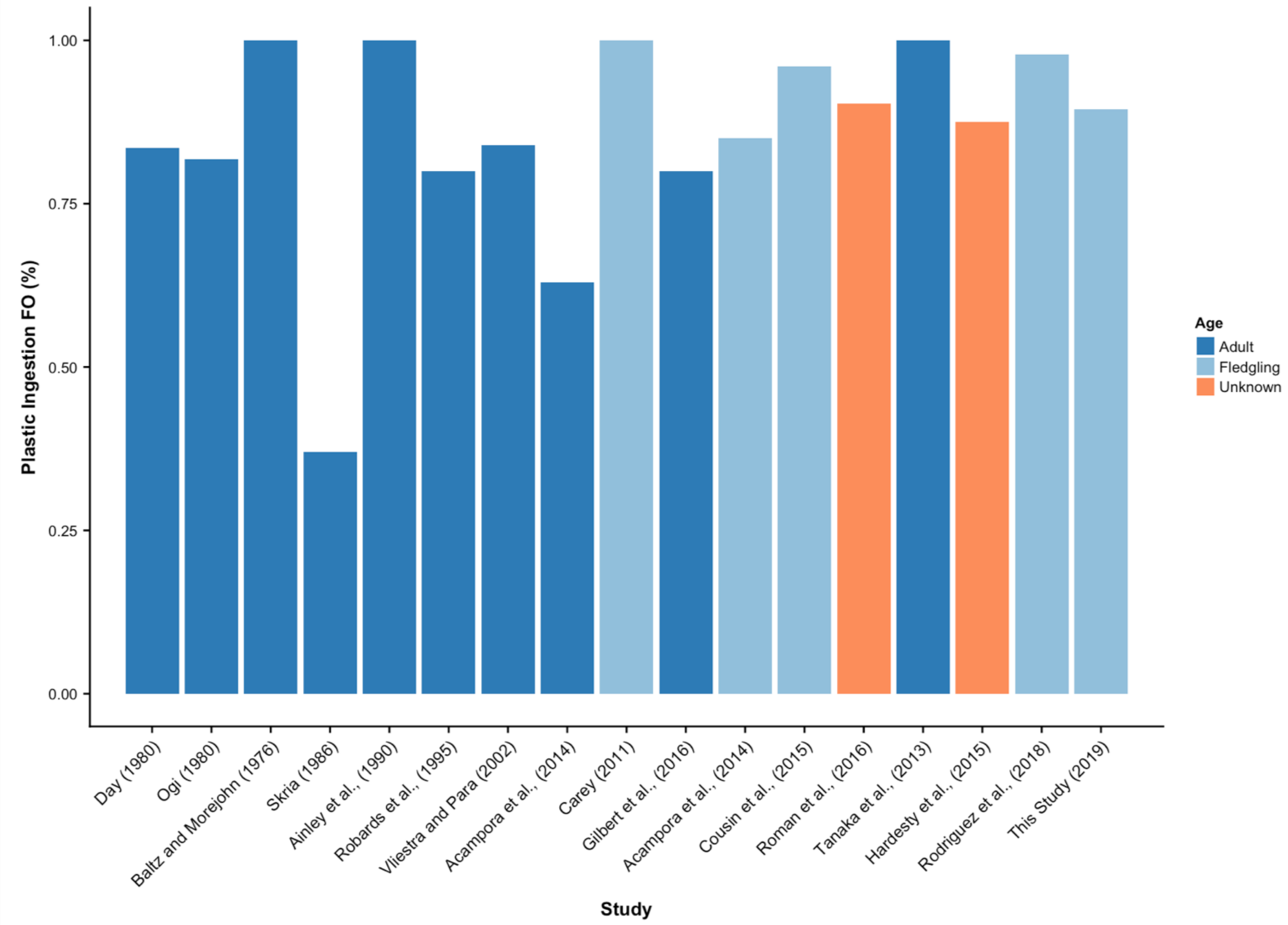

Fig. 1. The frequency of occurrence (FO (%)) of plastic ingested by Short-tailed Shearwaters (*Ardenna tenuirostris*) over 30 years of global studies of birds at various life stages; adult (dark blue), fledgling (light blue) and unknown (orange) where studies did not define age. A total of 17 studies are described, the details of each study are provided in Table 1. Only studies that provided FO% are included in this plot (n = 16 studies). Papers that provided data of both adults and fledglings were split (n = 1; Acampora et al., 2014).

Table 1. Review of published studies reporting plastic ingestion in Short-tailed Shearwaters (*Ardenna tenuirostris*). This data compliments that presented in Figure 1. FO: frequency of occurrence (%).

Year	Age	Location	n	FO (%)	Mean mass (g)	Mass range (g)	Mean pieces	Range pieces	Source
1969 - 1977	Adult	Sub-Antarctic, Pacific Ocean	200	83.5	0.10 ± 0.11	0 – 0.59	5.39 ± 5.72	0 – 32	(Day, 1980)
1970 - 1987	Adult	Sub-Antarctic, Pacific Ocean	324	81.8	0.14		8.79		(Ogi, 1990)
1974 - 1975	Adult	North Pacific Ocean	6	100					(Baltz and Morejohn, 1976)
1978	Adult	Tasmania, Australia	396	37			0-8		(Skira et al., 1996)
1984 - 1984	Adult	Pacific Ocean	1	100					(Ainley et al., 1990)
1988 - 1990	Adult	North Pacific Ocean	5	80					(Robards et al., 1995)
1997 - 2001	Adult	Bering Sea	330	84	0.114 ± 7.8		5.8 ± 0.4		(Vlietstra and Parga, 2002)
2003 - 2005	Adult	North Pacific Ocean	99		0.23 ± 0.18	0 – 0.9	15.1 ± 13.2		(Yamashita et al., 2011)
2010	Adult	Queensland, Australia	102	63			4.5		(Acampora et al., 2014)
2011	Fledgling	Victoria, Australia	67	100	0.113		7.6 ± 5.4		(Carey, 2011)
2011 - 2012	Adult	New South Wales, Australia	5	80		0.48 – 2.9		3 – 12	(Gilbert et al., 2016)
2012	Fledgling	Queensland, Australia	27	85			7.14		(Acampora et al., 2014)
2012	Fledgling	Tasmania, Australia	171	96	1.48		6.03 ± 0.3	0 – 30	(Cousin et al., 2015)
2013	Unknown	Eastern Australia	31	90.32			7.65		(Roman et al., 2016)
2013	Adult	North Pacific Ocean	12	100	0.24	0.04 – 0.59	27	1 – 9	(Tanaka et al., 2013)
2014	Unknown	Victoria, Australia	8	87.5			2.63	0 – 8	(Hardesty et al., 2015)
2015 - 2016	Fledgling	Victoria, Australia	140	97.85	0.15	0 – 1.04	6.46	0 – 26	(Rodríguez et al., 2018)
2017	Fledgling	Tasmania, Australia	38	89.5	0.06 ± 0.06	0 – 0.27	3.78 ± 3.79	0 – 15	This study

Table 2. Results of a power analysis for ingested plastic by Short-tailed Shearwaters (*Ardenna tenuirostris*) showing the number of individuals required to detect changes in the frequency of occurrence (FO (%)), number and mass of ingested plastic.

	Sample size required		
Age class & rate of change detected	**FO (%)**	**Number of plastic pieces**	**Mass of plastic**
Fledglings			
5%	41	530	2154
10%	11	145	5911
15%	5	71	2872
25%	<5	30	1221
50%	<5	11	440
75%	<5	7	266
100%	<5	5	195
Adults			
5%	798	5698	1476
10%	219	1563	405
15%	106	759	197
25%	45	323	84
50%	16	116	30
75%	10	70	18
100%	7	52	13

Table 3. Review of trace element concentrations in the breast muscle of Short-tailed Shearwaters (*Ardenna tenuirostris*) reported as mean ± standard deviation in µg/g from juvenile birds harvested in Tasmania (Lavers and Bond, 2013; This Study) and adult birds collected from the North Pacific (Honda et al., 1990).

Element	**(Honda et al., 1990)**	**(Lavers and Bond, 2013)**	**This study**
Ag	No Data	0.39 ± 0.20	<LoD
As	No Data	0.15 ± 0.14	1.04 ± 0.77
Br	No Data	1.27 ± 0.91	No Data
Cd	0.13 ± 0.07	0.01 ± 0.01	0.03 ± 0.03
Ce	No Data	0.02 ± 0.02	No Data
Co	No Data	0.01 ± 0.01	No Data
Cu	5.27 ± 0.64	4.00 ± 1.71	11.5 ± 2.69
Fe	63.1 ± 13.1	129.23 ± 38.69	No Data
Hg	0.04 ± 0.02	0.03 ± 0.00	No Data
Mg	No Data	326.54 ± 21.40	No Data
Mn	0.46 ± 0.11	0.53 ± 0.14	No Data
Pb	No Data	0.07 ± 0.13	No Data
Rb	No Data	1.39 ± 0.25	3.69 ± 0.45
Sb	No Data	<LoD (0.03)	No Data
Se	No Data	No Data	2.06 ± 0.31
Sr	No Data	0.13 ± 0.11	0.39 ± 0.47
Tl	No Data	<LoD (0.02)	No Data
V	No Data	<LoD (0.18)	No Data
Zn	16.9 ± 2.83	14.25 ± 6.11	62.31 ± 58.39

Table 4. P-values from linear models examining the relationships between elemental concentrations, morphometrics and plastic burden in Short-tailed Shearwaters.

	Bird Mass	**Wing Chord Length**	**Culmen Length**	**Head + Bill Length**	**Plastic Number**	**Plastic Mass**
As	0.35	**< 0.001**	0.68	0.61	0.27	0.50
Cd	**0.027**	**0.008**	0.44	0.20	0.87	0.78
Cu	0.11	**0.03**	0.30	**0.009**	0.49	**0.024**
Mg	**0.01**	0.06	0.26	**0.003**	0.31	0.16
Na	0.5	**0.004**	0.48	0.25	0.22	0.32
Rb	0.48	0.73	0.57	0.98	0.73	0.98
S	**0.034**	**0.041**	0.58	**0.006**	0.07	0.07
Se	0.77	0.07	0.90	0.05	0.06	0.26
Sr	0.66	0.40	0.59	0.97	0.48	0.19
Zn	0.70	0.67	0.78	0.97	0.54	0.28