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Baseline

Trace element concentrations in feathers from three seabird species breeding in the Timor Sea

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ABSTRACT

Mobile marine predators, such as seabirds, are frequently used as broad samplers of contaminants that are widespread in the marine environment. The Timor Sea off remote Western Australia is a poorly studied, yet rapidly expanding area of offshore development. To provide much needed data on contamination in this region, we quantified trace element concentrations in breast feathers of three seabird species breeding on Bedout Island. While adult Masked Boobies *Sula dactylatra* exhibited some of the highest concentrations, values for all species were below toxicology thresholds for seabirds and were comparable to those reported in other closely related species. The low concentrations detected in the birds provide a valuable baseline and suggest that the local marine environment around Bedout is in relatively good condition. However, careful monitoring is warranted in light increasing anthropogenic activity in this region.

The introduction of pollution into the environment is a major feature of anthropogenic change to our planet (Khan, 2018). Some well-known pollutants, including some trace elements, also occur naturally as part of biogeochemical cycles and volcanism (Burger et al., 1994). However, the background concentrations of many trace elements are undergoing dramatic increases in the industrial age due in large part to fossil fuel extraction and combustion (Nriagu and Pacyna, 1988). For example, mercury (Hg) in the atmosphere and oceans has increased three-fold from pre-industrial times (Lamborg et al., 2014).

Trace elements are typically differentiated into two categories: essential elements required for important processes within organisms, and non-essential elements that are not required for any biological purpose and are therefore particularly toxic when they accumulate within organisms (Burger, 1993; Ceyca et al., 2016; Moura et al., 2018). Several trace elements are of particular concern due to the concentrations released into the environment, as well as the documented toxicological effects at relatively low concentrations. They include the essential elements copper (Cu), chromium (Cr), nickel (Ni), selenium (Se), tin (Sn) and zinc (Zn) along with non-essential elements including Hg, arsenic (As), cadmium (Cd) and lead (Pb; Burger, 1993; Moura et al., 2018).

In the marine environment, three elements (Hg, Pb, and Cd) have been extensively studied because they bioaccumulate and biomagnify in aquatic food webs (Burger, 1993). Exposure can harm species in a variety of ways, including impaired biological function of proteins, enzymes, and cell damage (Burger, 1993; Ceyca et al., 2016). These impacts are often demonstrated using marine birds as sentinels, or bioindicators, as apex predators can also provide information on broader ecosystem health (Borghesi et al., 2016; Durant et al., 2009). Seabirds are also widespread, and unlike fish or marine mammals, are more readily sampled given they spend substantial time out of the water (Burger, 1993). Seabirds also display relatively predictable overwintering and breeding behaviours, allowing repeat sampling of individuals over time (Burger and Gochfeld, 2004; Mallory et al., 2010).

Feathers are often the preferred sampling media in avian studies as collection is largely non-invasive compared to sampling blood or other tissues (Monteiro and Furness, 1997). Being keratinous structures, feathers are chemically and physically stable, resistant to heat and deterioration, and are therefore easily stored over time (Burger et al., 1994; de Assis Padilha et al., 2018; Monteiro and Furness, 1997). Many seabird species sequester trace elements within growing feathers and exhibit well defined relationships between feather concentration and prey items (Borghesi et al., 2016; de Assis Padilha et al., 2018; Dolci et al., 2017). Feathers can also be collected from museum specimens, providing long-term data sets to analyse changes in trace elements over time (Bond et al., 2015a; Monteiro and Furness, 1997; Vo et al., 2011).

Here we investigate trace element concentrations within the breast feathers of three seabird species from Bedout Island, Western Australia

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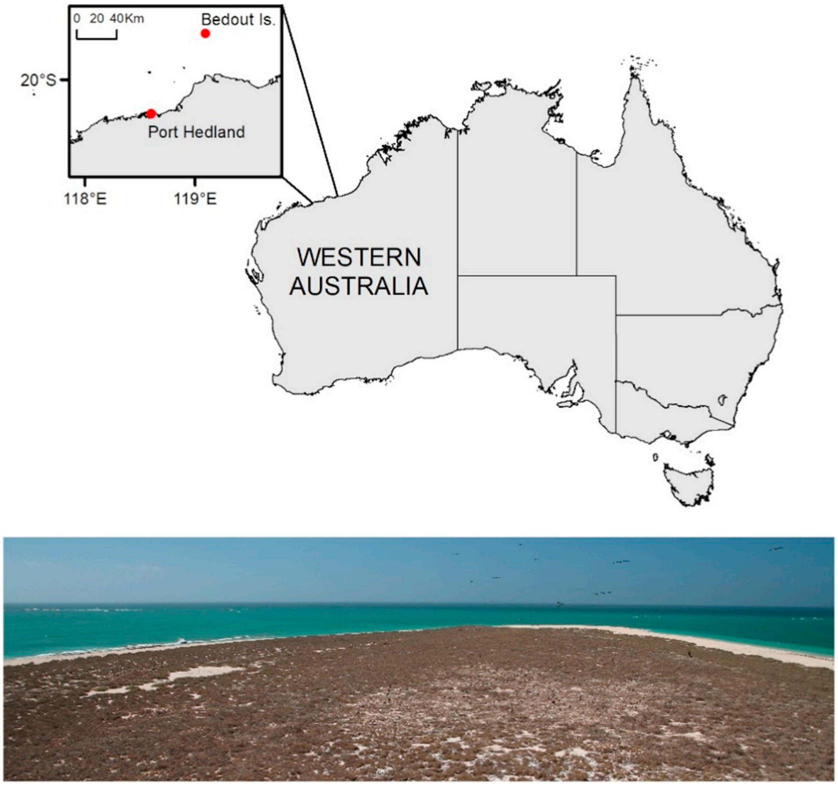

Fig. 1. Top panel: map showing the location of Bedout Island. Bottom panel: the Lesser Frigatebird colony (center of image; looking north) and the main Masked Booby colony (unvegetated area on left) on Bedout Island. Brown boobies breed along the shoreline, especially on the eastern side. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(19.578°S, 119.094°E; Fig. 1): Brown Boobies (*Sula leucogaster*), Masked Boobies (*S. dactylatra*) and Lesser Frigatebirds (*Fregata ariel*). Generally, low concentrations of dissolved trace elements have been detected in the coastal and estuarine waters off north west Australia, indicating the region is in near pristine condition (Mackey, 1984; Munksgaard and Parry, 2001). However the Bedout, Browse, Canning, and northern Carnarvon Basins adjacent to Bedout Island hold economical deposits of oil and gas and are all undergoing varying degrees of exploration and extraction (DMIRS, 2014; Lavers et al., 2014; Thompson et al., 2018). Therefore, there is an urgent need to assess pollution levels in the otherwise poorly-documented seabirds breeding in this rapidly developing region. Thus, the aims of this study were to 1) establish baseline trace element concentrations in three seabird species breeding in the region, and 2) assess whether current trace element concentrations may adversely affect the health of these species.

Adult boobies were captured by hand from their nest at night using a net, while juvenile frigatebirds (nearly ready to fledge) were captured by hand from their nest pedestals during daylight from 22 to 24 November 2016 and 8–10 November 2017 on Bedout Island. Bedout Island is a 31 ha uninhabited island with low vegetation located in a remote, sparsely populated region of the north west coast of Australia (Johnson et al., 2013; Munksgaard and Parry, 2001), and is listed as an Important Bird and Biodiversity Area (BirdLife International, 2019). Four breast feathers were obtained from each bird and stored in sterile polyethylene bags at −20 °C until analysis. Breast feathers were used as they are thought to be more representative of body metal burdens compared to flight feathers (Furness et al., 1986).

Feather preparation and analysis was completed at the Natural History Museum Core Research Laboratories using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) across a range of trace elements. Two feathers from each individual were placed using ethanol cleaned plastic tweezers in separate clean 12 ml plastic vials and washed by submersing feathers in Suprapur® 0.25 M NaOH solution for 15 min, followed by two rinses with deionised water, and then dried at 60 °C for 48 h. We used two feathers per individual to account for within-individual variability (Bond and Diamond, 2008), which gave sample weights between 9 mg and 27 mg.

Following decontamination, the feathers were digested in 15 ml PFA vials which had been pre-cleaned using 2–3 repeated treatments of 8 M HNO_3 at 120 °C for a minimum of 48 h. Approximately 1 ml of Romil® 8 M HNO_3 was added to each sample vial which was then placed on a hotplate at 70 °C. An additional 1.5 ml of 8 M HNO_3 was added to each vial after 60 min. After 48 h, the hotplate was cooled from 70 °C to 50 °C. The sample vial caps were then removed and 1 ml of Suprapur® 9.7 M H_2O_2 was added to each vial. Following any reaction, each vial was recapped and left on the hotplate for a further 12 h at 70 °C. Each solution was then transferred to a dedicated clean, sealed container, where deionised water was added to dilute the sample to a total volume of 15 ml. For inductively coupled plasma mass spectrometry (ICP-MS) analysis, a 2 ml aliquot of each sample solution was added into clean 12 ml tubes, with a further 2 ml of deionised water.

The digests were analysed using ICP-MS to quantify trace metal concentrations (Table S1). ICP-MS analysis was conducted on an Agilent 7700x using a power of 1550 W, 1.07 ml/min nebuliser gas flow, and He mode (5 ml/min, He 99.9995% purity) for all elements to reduce molecular interferences. Total procedural blanks were analysed and gave no detectable concentrations above the limit of quantification and certified reference materials were run every 10–15 samples using two reference materials from the Institut National de Santé Publique du Québec (INSPQ QM-H-Q1818, and QM-H-Q1827) and one from the Institute for Reference Material and Measurements, Joint Research Centre, European Commission (ERM-DB001; Table S1) certified for concentrations of As, Cd, Cu, Hg, Pb, Tl and V.

To place our results in a broader context, we collated information on feather trace element concentrations in other sulids and frigatebirds from the literature (Table 1). In cases where summary statistics were not displayed in tables or the text, they were estimated from the plots provided in each paper using WebPlotDigitizer (Rohatgi, 2019).

Some samples were below the level of quantification (< LOQ), and therefore left-censored (Table 2; Helsel, 2012). Summary statistics were calculated using maximum likelihood estimation in cases where < 10 samples were below the level of quantification (V, Hg), and regression

Table 1
Trace element concentrations (μg/g) in breast feathers of adult Sulidae and juvenile Fregatidae, reported as mean ± SD. N/A = information not available.

Species	Site	Year	Sample size (n)	Hg	Cd	Pb	Se
Sula dactylatra	Bedout Is., Timor Sea	2016–2017	20	3.20 ± 0.62	0.03 ± 0.03	0.48 ± 0.36	
Sula leucogaster	Bedout Is., Timor Sea	2016–2017	16	1.55 ± 0.89	0.04 ± 0.07	0.59 ± 0.36	
	North Pacific	1990	12	1.90 ± 0.33	0.14 ± 0.02	2.33 ± 0.30	3.68 ± 0.48
	Ryukyu Is., Japan	1992	11	2.9 ± 1.0			
Sula sula	North Pacific	1990	12	3.57 ± 0.28	0.13 ± 0.03	2.08 ± 0.32	2.28 ± 0.34
	North Pacific	N/A	12	3.85 ± 0.09	0.05 ± 0.01	0.975 ± 0.04	2.34 ± 0.05
Morus serrator	Pakiri Beach, New Zealand	N/A	11	4.47 ± 0.18	0.09 ± 0.01	0.82 ± 0.09	1.03 ± 0.06
	Muriwai Beach, New Zealand	N/A	12	3.89 ± 0.21	0.22 ± 0.02	3.13 ± 0.28	1.85 ± 0.09
	Ninety Beach, New Zealand	N/A	21	3.8 ± 0.10	0.20 ± 0.01	1.13 ± 0.08	2.04 ± 0.04
	N/A	N/A	1	4.5		0.9	
Fregata ariel	Bedout Is., Timor Sea	2016–2017	23	0.22 ± 0.17	0.03 ± 0.02	0.82 ± 1.26	
	Ashmore Reef, Timor Sea	2013–2014	38	4.41[a]			
Fregata minor	Ashmore Reef, Timor Sea	2013–2014	6	2.6[a]			
Fregata magnificens	Cispata Bay, Colombia	2010–2011	7	2.10 ± 1.36	0.34 ± 0.32	0.19 ± 0.09	

Species	Cu	V	Zn	As	Mn	Sn	Cr	Source
Sula dactylatra	2.92 ± 0.81	0.02 ± 0.01		0.01 ± 0.01				This study
Sula leucogaster	2.86 ± 0.92	0.04 ± 0.03		0.01 ± 0.01				This study
								Burger et al. (1992)
								Kim et al. (1996)
Sula sula								Burger et al. (1992)
				0.12 ± 0.01	1.46 ± 0.14	2.28 ± 0.12	2.53 ± 0.26	Burger and Gochfeld (2000)
Morus serrator					1.08 ± 0.05		1.78 ± 0.05	Burger et al. (1994)
					2.65 ± 0.18		3.43 ± 0.19	
					3.98 ± 0.13		6.25 ± 0.25	
	19.9		102.0					Lock et al. (1992)
Fregata ariel	1.32 ± 0.32	0.01 ± 0.03		< 0.01 ± 0.01				This study
								Mott et al. (2017)
Fregata minor								Mott et al. (2017)
Fregata magnificens	3.65 ± 4.99		61.64 ± 42.39					Burgos-Núñez et al. (2017)

[a] Median values were extracted from plots provided in Mott et al. (2017).

Table 2
Trace element concentrations (µg/g) in seabird breast feathers from Bedout Island.

Element	# < LOQ	Min	Median	Max	Mean (SD)
Brown booby (*Sula leucogaster*) – adults					
2016 (n = 11)					
V	0	0.03	0.04	0.09	0.06 (0.03)
Cu	0	2.27	2.56	5.94	2.93 (1.03)
As	2 (18%)	< 0.03	NA	0.10	NA
Cd	0	0.02	0.04	0.30	0.06 (0.08)
Hg	1 (9%)	< 0.10	NA	1.67	NA
Tl	11 (100%)	NA	NA	NA	NA
Pb	0	0.23	0.54	3.28	0.76 (0.88)
2017 (n = 5)					
V	0	0.06	0.07	0.18	0.09 (0.05)
Cu	0	2.50	2.87	3.26	2.88 (0.33)
As	0	0.04	NA	0.07	NA
Cd	0	0.02	0.03	0.04	0.03 (0.01)
Hg	0	0.84	NA	2.15	NA
Tl	5 (100%)	NA	NA	NA	NA
Pb	0	0.12	0.15	0.17	0.15 (0.02)
Masked booby (*Sula dactylatra*) – adults					
2016 (n = 16)					
V	0	0.01	0.04	0.07	0.04 (0.01)
Cu	0	2.35	2.77	5.52	3.08 (0.80)
As	4 (25%)	< 0.03	NA	0.13	NA
Cd	0	0.01	0.03	0.13	0.03 (0.03)
Hg	0	1.69	2.02	2.83	2.18 (0.38)
Tl	16 (100%)	NA	NA	NA	NA
Pb	0	0.32	0.54	5.04	0.85 (1.14)
2017 (n = 4)					
V	0	0.30	0.05	0.07	0.05 (0.02)
Cu	0	2.24	2.50	3.03	2.57 (0.34)
As	1 (25%)	< 0.03	NA	0.10	NA
Cd	0	0.02	0.03	0.09	0.04 (0.03)
Hg	0	1.58	1.84	2.66	1.98 (0.47)
Tl	4 (100%)	NA	NA	NA	NA
Pb	0	0.15	0.68	1.74	0.81 (0.73)
Lesser frigatebird (*Fregata ariel*) - juveniles					
2016 (n = 14)					
V	0	0.01	0.02	0.04	0.02 (0.01)
Cu	0	1.09	1.50	2.17	1.50 (0.27)
As	8 (57%)	< 0.03	NA	0.06	NA
Cd	0	0.01	0.03	0.07	0.03 (0.01)
Hg	0	0.14	0.27	0.37	NA
Tl	14 (100%)	NA	NA	NA	NA
Pb	0	0.23	0.56	1.48	0.66 (0.38)
2017 (n = 9)					
V	0	0.02	0.03	0.18	0.05 (0.05)
Cu	0	1.15	1.36	2.27	1.45 (0.34)
As	8 (89%)	< 0.03	NA	0.05	NA
Cd	0	0.02	0.04	0.10	0.06 (0.03)
Hg	6 (67%)	< 0.10	NA	0.39	NA
Tl	9 (100%)	NA	NA	NA	NA
Pb	0	0.15	0.33	0.60	0.33 (0.14)

on order statistics in the case of As, where 23/59 samples were below the level of quantification (Bond et al., 2015b; Helsel, 2012). This was done in the R package *NADA* (Lee, 2017). All analyses were conducted using *R* (R Core Team, 2019).

We compared feather elemental concentrations in adult Brown and Masked Boobies using general linear models, and log-transformed data when it did not meet the assumptions of normality (Levene, 1960). Differences were considered significant when $p < 0.05$.

A summary of elemental concentrations is provided for each of the three seabird species in Table 2. There was a significant difference in concentrations of V, Cu, As, and Hg among species, and in all cases, Lesser Frigatebirds had the lowest concentrations; Brown Boobies had higher V concentrations in feathers than Masked Boobies (Table 3). There was no difference in trace element concentrations among years except for V, where feathers from 2017 had significantly higher concentrations.

Table 3
Summary of linear models testing the effects of species and year on feather trace element concentrations.

Trace element	Factor	F[a]	p-Value	Post-hoc test	Log-transformed
V	Species	15.18	< 0.001	BB > MB > LF	Y
	Year	12.84	< 0.001	2017 > 2016	Y
	Species × Year	0.64	0.53	NA	Y
Cu	Species	79.65	< 0.001	MB = BB > LF	Y
	Year	0.92	0.34	NA	Y
	Species × Year	0.61	0.55	NA	Y
As	Species	14.11	< 0.001	BB = MB > LF	N
	Year	0.07	0.80	NA	N
	Species × Year	0.80	0.46	NA	N
Cd	Species	1.17	0.32	NA	Y
	Year	1.61	0.21	NA	Y
	Species × Year	1.43	0.25	NA	Y
Hg	Species	149.64	< 0.001	BB = MB > LF	N
	Year	0.23	0.63	NA	N
	Species × Year	4.20	0.020	NA	N
Pb	Species	0.41	0.67	NA	Y
	Year	0.01	0.99	NA	Y
	Species × Year	2.53	0.09	NA	Y

[a] Degrees of freedom are 2 & 53 for “Species” and “Species × Year”, and 1, 53 for “Year”.

Only one individual, an adult Masked Booby (5.04 µg/g), exceeded the lowest observed adverse effect level (LOAEL) for Pb (4.00 µg/g; Burger, 1993; Burger and Gochfeld, 2000), however an adult Brown Booby (3.28 µg/g) also approached this threshold. None of the birds tested approached the hypothesized LOAEL of 5.00 µg/g and 2.00 µg/g for Hg and Cd in seabird feathers, respectively (Burger, 1993; Burger and Gochfeld, 2000).

The seabird community on Bedout Island provides a valuable opportunity to comprehensively sample the marine environment as the three species included in this study exhibit distinct foraging ecologies or seasonality while breeding at the same location. While adult Brown Boobies on Bedout Island exhibited some of the highest trace element concentrations of the three species considered in this study (Table 1), values were below toxicology thresholds for seabirds (Burger, 1993), and were comparable to those reported in other closely related species (Table 1). The data for juvenile Lesser Frigatebirds suggests spatial or temporal differences could be important, with notable differences in Hg concentrations across two locations in the Timor region (Bedout Island (mean): 0.22 ± 0.17 µg/g; Ashmore Reef (median): 4.50 µg/g; Table 1). Juvenile birds' feathers also represent a shorter period over which Hg can accumulate (Monteiro and Furness, 2001), so their low concentrations compared to the two boobies is expected. Overall, the low concentration of elements in all three seabird species sampled (Tables 1 and 2) suggests the local marine environment around Bedout Island is in relatively good condition, particularly compared to other areas with more offshore development (Boersma, 1986; Fraser, 2014).

For tropical sulids, numerous populations have undergone periods of rapid decline (Feare, 1978; Schreiber, 2000). For example, only a few sulid colonies remain in Indonesia, the Philippines, the South China Sea, and Papua New Guinea (Cao et al., 2005; de Korte and Meltofte, 1997; de Korte and Silvius, 1994; Jensen, 2007). The Masked Booby is nationally extinct in the Philippines and likely Indonesia (Jensen, 2007) and Australian colonies have also experienced historical declines or extirpations (Nelson, 2005; Serventy, 1952). These declines have been primarily attributed to anthropogenic disturbance, but chemical and physical pollution is increasingly identified as an issue of concern (e.g., Gilmour et al., 2019; Grant et al., 2018). Increasingly, seabirds are facing multiple pressures which, alone, may have been tolerable, but together may have additive or multiplicative effects, further affecting populations (Dias et al., 2019; Wiese et al., 2004). On Bedout Island, low numbers of Masked Boobies recorded in recent years (< 100 breeding pairs; Kingsley et al., 2019) and reductions in other seabirds in Australia and globally (Croxall et al., 2012; Dias et al., 2019; Gorta et al., 2019) suggests ongoing monitoring of this region is warranted. Furthermore, genetic analysis of Bedout's tiny Masked Booby population (likely subspecies *S. d. bedoutti*) indicates it rarely exchanges genes with other colonies in the region, meaning it has limited capacity to cope with pressures and recover from perturbations through immigration (Kingsley et al., 2019).

Along the north coast of Western Australia, seabirds increasingly overlap with offshore development (Lavers et al., 2014) as this is one of the most rapidly expanding regions of petroleum extraction (AERA, 2018; USGS, 2012). Drilling commenced in the Bedout Sub-basin, immediately adjacent to the island, in 2018 (Thompson et al., 2018). Risk of contamination or other harm to seabirds as a result of petrochemical activities is not insignificant, with Western Australia experiencing at least 13 offshore spillage events during 1988–2009 (May, 1992; Watson et al., 2009). When exposed to petroleum, seabirds exhibit elevated concentrations of trace elements and other pollutants in their tissues (Moreno et al., 2011; Pérez et al., 2008). Species that are resident year-round in areas of high development are at increased risk of exposure to spillage events, including members of the Sulidae which typically remain within 250–500 km of the nest site throughout the year (Huyvaert and Anderson, 2004; Kohno and Yoda, 2011; Weimerskirch et al., 2008).

The results of this study provide much-needed baseline data on trace element contamination for seabirds breeding on Bedout Island. While this is an important outcome, these data unfortunately resemble the majority of ecological studies, which focus on single stressors due to time or funding limitations (O'Brien et al., 2019). In reality, > 70% of seabird species face multiple threats that often occur simultaneously at-sea and on their breeding islands (Dias et al., 2019). Thus, it's increasingly important to consider cumulative impacts on seabird populations and their habitats if we are to ensure research outcomes have meaningful application to the real world.

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CRediT authorship contribution statement

Jennifer L. Lavers: Conceptualization, Funding acquisition, Writing - review & editing. **Emma Humphreys-Williams:** Methodology. **Nicholas J. Crameri:** Writing - original draft. **Alexander L. Bond:** Methodology, Formal analysis, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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