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Trace Metal Concentrations in the Little Penguin (*Eudyptula minor*) from Southern Victoria, Australia

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Little Penguins (*Eudyptula minor*) are among the smallest of the warm-climate penguins (Stonehouse, 1975). Part of the family Spheniscidae, Little Penguins breed at several points along the southern coast of Australia and around Tasmania, mainly on offshore islands or parts of the coast where access by mammalian predators is reduced. In Victoria, the two most famous and accessible penguin colonies are on Philip Island in Westernport Bay, and Middle Island in southwest Victoria (Fig. 1). Middle Island lies just off-shore from the coastal city of Warrnambool, approximately 260 km from Melbourne, and fringes the Merri Marine Sanctuary. Although southwest Victoria is often considered to be a relatively uncontaminated region, because of its low scale industrial base and the distance from major sources of metal pollution, the island's local marine environment may be influenced by the nearby municipal waste water treatment plant outfall and contaminants in the Merri River, whose estuary fringes the northern shore of the island. Philip Island is approximately 140 km from Melbourne, on the southern, ocean side of Westernport Bay. Westernport Bay is one of Victoria's most important embayments and has many environmental values, from its important seagrass beds to the extensive mangrove and saltmarsh fringes. Some of the threats to the bay include urban and agricultural runoff, sedimentation, and turbidity from catchment, shipping and port activities (dredging of shipping channels and berths, and associated threat of oil spills and pollution), introduced marine pests, and removal of saltmarshes and mangroves for other land uses (Central Coastal Board, 2003).

There have been few studies of contaminants in Australian birds, and only one previous study of metals in Australian Little Penguins (Gibbs, 1995). Little Penguins are an ideal indicator of contaminant accumulation in the inshore coastal environment, because they are higher level predators, feeding primarily on fish and squid within defined feeding areas close to their breeding grounds, and contaminant concentrations can be compared with penguins of the higher, less polluted latitudes of the Southern Ocean and Antarctic. In this baseline survey, we investigated the concentrations of a range of metals, including As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Pb, and Zn, in the pectoral muscles and livers of male and female Little Penguins on Phillip Island and Middle Island. Herein we report our results, and examine any localized differences in metal contamination in the penguins.

Materials and Methods

Five adult male and female penguins were collected from Middle Island and Phillip Island in 2005. Permission to collect, receive and retain, dissect, and digest tissues was granted by the Department of Sustainability and Environment (DSE), under the provisions of the Wildlife Act 1975 (Permit Numbers 10002589; 10003245; 10003297). Little

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Fig. 1 Location of Middle Island, Warrnambool (MI) and Philip Island (PI) in Victoria, Australia

Penguins killed by foxes were utilized for this project to avoid the need to sacrifice a protected native animal. Collection of penguin carcases was undertaken within 4 hr to 24 hr of fox kills. Immediately after collection, penguins were given an identification number, bagged individually, and stored in a freezer until further processing.

All dissection equipment, glassware, and plasticware used during the processing and preparation of samples were soaked for at least 24 hr in a 5% solution of Extran 300, followed by three rinses with deionized water prior to use. Digestion equipment was soaked in 10% nitric acid solution (69.5% purity; Trace select for trace analysis, Sigma-Aldrich Pty. Ltd, Sydney, Australia) for a minimum of 24 hr. Before use, equipment was again rinsed three times with deionized water to remove excess nitric acid solution. Penguin samples were taken out of the freezer and left to thaw for two days. Thereafter, a straight line was cut down the middle of the penguin, and the skin was pulled back. A large section of the pectoral muscles and the entire liver were removed from the carcass and were placed in separate brown paper envelopes, labelled with information regarding tissue type, the origin of the sample, the sex of the sample, an identification number, and the flora and fauna permit number. These were then placed in individually labelled polyethylene bags and were frozen prior to further preparation and analysis. The samples were taken out of the freezer and left to thaw for a day. A thin layer of the outer part of the muscle samples was cut away and discarded. A smaller sample of muscle was then subsampled for digestion, ensuring that the extraneous matter did not contaminate the sample. The same process was employed for the liver samples.

The samples were digested using the EPA Method 200.3 for Total Recoverable Elements in Biological Samples (Environmental Protection Agency, 1991), with minor modification. The EPA method recommends 5 g (wet weight) of sample. This sample weight was halved in the current study, and all reagent volumes used were adjusted accordingly. In short, the samples were digested in batches in a temperature-controlled digestion block (AIM500 digestion block, A.I. Scientific). Field and quality control/quality assurance samples (e.g., spiked samples, blanks, certified reference material [CRM; DORM-2, National Research Council of Canada (NRCC) and BL1577b (National Institute of Standards and Technology, Gaithersburg, MD, USA)]) were randomly placed into separate glass tubes on the digestion block. The CRMs were dry powders, and therefore only 0.5 g of the material was used in the digestion. Concentrated nitric acid (5 mL) was added to each tube, and the stoppered tube was left for 24 hr at room temperature. A small number of antibumping granules was placed into each of the tubes, which were then heated to 40°C for 30 min, and were then allowed to cool to room temperature. Additional nitric acid (4.5 mL) was subsequently added to each tube, the solutions were heated to 90–95°C for 3 hr, and then again were allowed to cool to room temperature. After the solution had cooled, hydrogen peroxide (2 mL) was added, and the solution was heated to boiling for 30 min with the stoppers off. The step requiring hydrochloric acid (as suggested by the EPA) was not employed for the current study, as it is mainly applied to extract methyl-mercury from biological tissues. Samples were cooled to room temperature, transferred to 50 mL volumetric flasks, and made up to the mark with deionized water. The digests were then filtered through 0.45 μm mixed cellulose ester membrane filters (Advantec, MFS, USA) and stored in centrifuge tubes prior to despatch to the NATA (National Association of Testing Authorities) accredited Marine and Freshwater Research Laboratory at Murdoch University, Perth, Western Australia. Deionized water with a resistivity of at least 18M Ω cm was prepared by distilled water through a Milli-Q water purification system. Nitric acid (AnalaR grade), hydrogen peroxide (AnalaR grade), and Extran 300 detergent were obtained from BDH Chemicals. Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) was used to measure metal concentrations in the digests (MAFRL method ICP001: Determination of Elements in Waters and Other Appropriate Solutions by ICP-AES). The instrument used was a Vista AX CCD Axial View Simultaneous ICP-AES (Varian, Palo Alto, USA). The following analytical wavelengths were monitored: As, 189.0; Cd, 228.8; Cr, 205.6; Cu, 324.8; Fe, 239.6; Hg, 193.1; Mn, 257.6; Mo, 202.0; Pb, 220.4; and Zn, 206.2 nm, respectively. Analytical reporting limits (LOR) quoted by the laboratory were: Hg, 0.02 mg L^{-1}; As, Pb, 0.01 mg L^{-1}; Mo, 0.004 mg L^{-1}; Fe, Zn, 0.002 mg L^{-1}; Cr, Cu, 0.001 mg L^{-1}; Cd, 0.0006 mg L^{-1}; Mn, 0.0002 mg L^{-1}, respectively. Method detection limits (ML), that is, the minimum total metal concentration that could be quantified in 2.5 g raw muscle, were twenty times higher than these values.

Table 1 Average metal concentrations (mg kg^{-1} wet weight) in Little Penguins from Middle Island and Philip Island, Victoria, 2005

Location, sex, tissue	Element								
	As	Cd	Cr	Cu	Fe	Hg	Mn	Mo	Zn
MI, F, m	1.42 (123)	0.98 (169)	0.77 (188)	2.9 (16)	120 (22)	0.60 (58)	0.40 (35)	0.95 (219)	8.5 (22)
	[0.40–4.49]	[0.06–3.87]	[0.13–3.36]	[2.1–3.2]	[76–140]	[<ML–0.60]	[0.26–0.54]	[<ML 4.30]	[5.5–10.4]
MI, F, l	0.94 (49)	1.0 (61)	0.12 (57)	6.2 (26)	230 (40)	0.55 (54)	1.9 (13)	0.70 (48)	37.0 (18)
	[0.60–1.70]	[0.1–1.8]	[0.06–0.23]	[3.9–8.5]	[120–360]	[<ML – 0.80]	[1.6–2.2]	[<ML - 0.90]	[32.0–46.0]
MI, M, m	1.46 (114)	0.99 (173)	0.81 (204)	3.0 (10)	130 (12)	0.50 (155)	1.16 (155)	0.92 (322)	11.1 (13)
	[0.40–4.39]	[0.07–4.04]	[0.05–3.77]	[2.6–3.4]	[110–160]	[<ML – 0.60]	[0.29–4.36]	[<ML - 4.40]	[9.2–12.8]
MI, M, l	0.92 (42)	2.4 (84)	0.28 (111)	5.8 (23)	190 (49)	0.82 (48)	2.00 (26)	0.62 (46)	35.7 (11)
	[0.60–1.60]	[0.8–6.0]	[0.04–0.82]	[5.0–8.2]	[93–350]	[<ML – 1.00]	[1.5–2.8]	[<ML - 0.70]	[31.9–40.9]
PI, F, m	0.90 (67)	0.08 (31)	0.05 (19)	2.8 (4)	190 (34)	<ML	0.31 (13)	0.05 (125)	9.8 (24)
	[0.59–1.90]	[0.04–0.09]	[0.04–0.06]	[2.6–2.8]	[120–260]		[0.24–0.34]	<ML - 0.15]	[6.2–12.3]
PI, F, l	1.7 (73)	2.0 (49)	0.08 (26)	6.1 (46)	670 (87)	0.76 (51)	2.6 (35)	0.66 (16)	41.5 (35)
	[0.1–3.5]	[1.0–3.6]	[0.07–0.12]	[3.9–10.7]	[110–1300]	[0.40–1.40]	[1.9–4.2]	[0.55–0.78]	30.0–66.9]
PI, M, m	0.92 (60)	0.07 (47)	0.04 (0)	2.6 (29)	250 (18)	0.12 (250)	0.34 (10)	0.07 (154)	10.7 (13)
	[0.40–1.70]	[0.04–0.13]		[1.5–3.5]	[200–300]	[<ML – 0.60]	[0.30–0.39]	[<ML - 0.21]	[8.9–12.5]
PI, M, l	1.3 (62)	1.3 (51)	0.05 (51)	5.7 (19)	1100 (39)	2.0 (60)	3.4 (27)	0.72 (17)	43.5 (30)
	[0.6–2.5]	[0.6–2.2]	[0.02–0.09]	[4.3–7.2]	[410–1600]	[0.6–3.3]	[2.6–4.5]	[0.66–0.75]	[30.0–63.9]

MI, Middle Island, Warrnambool; PI, Philip Island, Westernport Bay; F, female; M, male; m, muscle, l, liver. Figures in parentheses (x) represent the coefficient of variation of data (%); figures in square brackets [a-b] represent the range of concentrations determined; ML, method detection limit. For Pb, all data < ML except for Middle Island female muscle, where mean Pb concentration was 0.58 mg kg^{-1} wet weight (CV, 249%; range, <ML – 2.89 mg kg^{-1} wet weight)

Statistical Analysis

The concentrations of all 10 elements measured in the digests were certified in one or other of the CRMs, for example, all except Mo in DORM–2, and all except Cr in BL1577b. By using the two CRMs, we were able to estimate procedural recovery even when digest concentrations of an element were < LOR in one CRM (e.g., Cd and Pb in the muscle CRM (DORM-2), and As, Hg, and Pb in the liver CRM (BL1577b)). All elements have therefore been reported and included in the statistical analysis. Chemical residue data did not comply with prerequisites of homogeneity of variance and normality of data required for parametric tests, therefore the nonparametric Kruscal-Wallis Median Test was used to assess the overall differences in metal concentrations in female and male liver and muscle tissue at both sites. If differences were detected, post-hoc analysis was performed using the nonparametric Mann-Whitney U test. For pooled comparisons using site only as the independent measure, the Mann-Whitney test was also used. SPSS version 11.5 (SPSS Inc, Chicago) was used to test for differences in analyte concentrations between sites, and was used to test for gender within and between sites.

Results and Discussion

To check analytical accuracy and precision, analysis of two CRMs (DORM-2 and 11577b) was undertaken. For the most part, metal concentrations were found to be within 25% of expected values (80%–109% recovery). The recovery of Hg and Mn in DORM-2 was low (69% and 60% expected value, respectively) but was accepted, because there was little variation between CRM digests (12% and 5%, respectively). In discussing elemental concentrations, data has not been corrected for recovery.

Some caution should be taken when assessing statistical variability in the biophysical or chemical data, since sample numbers were small, and the samples were not collected from the field to a rigorous ecological protocol, but were collected following fox kills. No statistically significant differences in body weight were observed between male and female animals at either site (Middle Island females, 1130 ± 140 g; males, 1210 ± 110 g; Philip Island females, 884 ± 200 g; males, 926 ± 150 g). No statistically significant differences in liver weight were observed between male and female animals at either site (Middle Island females, 36.6 ± 8.8 g; males, 36.5 ± 3.8 g; Philip Island females, 34.4 ± 11.9 g; males, 29.3 ± 9.2 g). Statistically significant differences ($p < 0.05$) were observed

Table 2 Average metal concentrations (mg/kg wet weight) in penguins reported in other studies (post-1990 publications only)

Penguin species and location	Mean Metal Concentration (mg/kg wet weight)										Reference
	As	Cd	Cr	Cu	Fe	Hg	Mn	Mo	Pb	Zn	
LIVER											
Gentoo (*Pygoscelis papua*), ANT		< 0.1		0.5		< 0.1				23	de Moreno et al., 1997
Gentoo (*Pygoscelis papua*), ANT		0.6	< 0.03	5.3	260		1.8		< 0.01	20	Szefer et al., 1993
Adelie (*Pygoscelis adeliae*), ANT		0.2		1.5		<0.1				28	de Moreno et al., 1997
Adelie (*Pygoscelis adeliae*), ANT		0.1		17.6		0.2					Smichowski et al., 2006
Adelie (*Pygoscelis adeliae*), ANT		1.4	< 0.03	2.4	530		1.7		< 0.01	28	Szefer et al., 1993
Chinstrap (*Pygoscelis antartica*), ANT		2.1	< 0.03	2.5	380		1.5		< 0.01	25	Szefer et al., 1993
Rockhopper (*Eudyptes chrysocome*), NZ		18.5		4.3		1.5				52	Lock et al., 1992
Fiordland crested (*Eudyptes pachyrhynchus*), NZ		9.8		2.4		0.5				31	Lock et al., 1992
Erect-crested (*Eudyptes sclateri*), NZ		21.8		13.2		2.4				69	Lock et al., 1992
Little (*Eudyptula minor*), NZ		2.9		6.4		1.4				58	Lock et al., 1992
Little (*Eudyptula minor*), AUS	1.8	1.3	0.4	7.0		1.3				38	Gibbs, 1995
Little (*Eudyptula minor*), AUS	1.2	1.7	0.13	5.9	554	1.0	2.5	0.7		39	This study
MUSCLE											
Gentoo (*Pygoscelis papua*), ANT	< 0.01	< 0.01	< 0.03	1.6	120		0.1		< 0.01	7.1	Szefer et al., 1993
Adelie (*Pygoscelis adeliae*), ANT	< 0.01	0.09	< 0.03	1.6	140		0.1		< 0.01	9.3	Szefer et al., 1993
Chinstrap (*Pygoscelis antartica*), ANT	< 0.01	0.11	< 0.03	1.9	160		0.2		< 0.01	7.4	Szefer et al., 1993
Little (*Eudyptula minor*), AUS	1.2	0.53	0.42	2.8	170	0.3	0.6	0.5		10	This study

Authors' dry weight data has been converted to wet weight by dividing data by 5; ANT, Antarctic; AUS, Australia; NZ, New Zealand

between sites for Cr, Cu, and Fe concentrations in penguin muscle, and Cr, Fe, and Mn concentrations in the liver, although there was no consistent pattern to the differences. For instance, in the muscle, Cr and Cu concentrations are higher in penguins from Middle Island, whereas Fe concentrations are higher in penguins from Philip Island (Table 1). In the liver, Cr concentrations are higher in penguins from Middle Island, whereas Fe and Mn are higher in penguins from Philip Island. Some statistically significant differences ($p < 0.05$) were observed in the concentration of Cr and Fe in muscle between gender across sites. For instance, Middle Island male penguin muscle has higher concentrations of Cr than Port Phillip Island male muscle, whereas Middle Island female penguin muscle has higher concentrations of Cr than both Phillip Island male and female penguins. Conversely, Phillip Island male penguin muscle has higher concentrations of Fe than Middle Island male and female penguins. There were also statistically significant differences ($p < 0.05$) in the concentrations of Fe and Mn in the liver between gender across sites. For instance, Phillip Island male penguin liver has higher concentrations of these elements than Middle Island males and females. These observations are different from those of Gibbs (1995), who found no differences in metal concentrations in the livers of penguins that were hundreds of kilometers apart (on an island near Sydney on the eastern coast of Australia and Philip Island). The concentrations of metals determined in this study are similar to those found by Gibbs (1995), but lower than those reported by Lock et al. (1992) in New Zealand. The concentrations are, however, within in the range reported in the few recent studies on metals in the tissues of other penguins internationally (Table 2), adding weight to the suggestion that some elements, for example, Cu and Zn, are tightly regulated in seabirds (Gibbs, 1995). Although the concentrations of some elements, notably Cd, are higher than for penguins in the Antarctic, observed concentrations are well below those considered to cause physiological harm in vertebrates (Lock et al., 1992). Thus, although this study provides essential baseline data for risk assessment modelling of the Victorian inshore coastal environment, it appears unlikely that the penguins are suffering any harm from their metal burdens.

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