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Seasonal variation and annual trends of metals and metalloids in the blood of the Little Penguin (*Eudyptula minor*)

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ABSTRACT

Little Penguins (*Eudyptula minor*) are high-trophic coastal feeders and are effective indicators of bioavailable pollutants in their foraging zones. Here, we present concentrations of metals and metalloids in blood of 157 Little Penguins, collected over three years and during three distinct seasons (breeding, moulting and non-breeding) at two locations: the urban St Kilda colony and the semi-rural colony at Phillip Island, Victoria, Australia. Penguin metal concentrations were foremostly influenced by location (St Kilda > Phillip Island for non-essential elements) and differed among years and seasons at both locations, reflecting differences in seasonal metal bioaccumulation or seasonal exposure through prey. Mean blood mercury concentrations showed an increasing annual trend and a negative correlation with flipper length at St Kilda. Notably, this study is the first to report on blood metal concentrations during the different stages of moult, showing the mechanism of non-essential metal mobilisation and detoxification.

1. Introduction

Studying annual and seasonal trends of contaminants in coastal waters is essential to understand impacts of anthropogenic activities on sensitive species and inshore marine ecosystems, which are under increasing pressure due to continuing human population growth. In Australia, the proportion of the country's population living near the coast in 2001 was 85% and is predicted to increase (ABS, 2011). Higher concentrated coastal populations are likely to increase coastal pollution levels. Another factor adding pressure to coastal ecosystems is climate change, which will raise sea surface temperatures and sea levels, change rainfall run-off patterns into marine areas, increase the frequency and intensity of severe weather events and increase the acidity of sea-water, all of which affect contaminant exposure and toxic effects (Sokolova and Lannig, 2008; Millero et al., 2009; Noyes et al., 2009). While point-source industrial output of contaminants has been more tightly regulated and has in some locations decreased over time (Fabris et al., 1999), historical discharges persist for decades in the sediments of bays and inlets near industrialised areas (Aly et al., 2013). Highly toxic pollutants can become bioavailable, if sediments are physically disturbed and re-suspended in the water column, e.g. due to dredging (Hedge et al., 2009; Edge et al., 2015; Fetters et al., 2015). Taken up by plankton, these elements can enter the food web and potentially accumulate with each increase in trophic position. Hence, high-level predators are often used to indicate the ecological risks of bioavailable coastal pollution (Becker, 2003).

An increasing number of studies use blood of seabirds to elucidate issues relating to marine contamination (Eagles-Smith et al., 2008; Carvalho et al., 2013; Tartu et al., 2015). Taking a small blood sample non-destructively from a randomly selected individual has the advantage of being more ethical than destructive (sacrificial) sampling, but also more representative than opportunistic collection of specimens that died of unknown causes (Becker, 2003). As blood concentrations reflect recent dietary exposure, these data can give insight into seasonal variations of exposure through prey, but also highlight differences in bioaccumulation due to varying seasonal needs in the species' life stages. It may even be possible to observe sampled individuals over time to gauge an effect of the contaminant load on their behaviour (Tartu et al., 2013). However, studies that document annual variations often report on metal content in feathers (e.g. Carravieri et al., 2014; Bond et al., 2015), while studies investigating variations in pollution load of resident high-trophic feeders between seasons within a given year are rare. The benefits of such studies include getting a more accurate gauge on small-scale temporal changes and providing insight into the physiological mechanisms of the study species. This line of research has the potential to influence scientific investigation more broadly and to provide more comprehensive information as to what factors, other than diet, affect metal load in top feeders.

Port Phillip Bay is adjacent to the City of Melbourne, Australia - a metropolitan city, which currently hosts a human population of 4 million

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(ABS, 2011). Port Phillip Bay is Australia's largest shipping port, is 1930 km^2 in area, 13.6 m deep on average and joined to the Bass Strait through a narrow 3 km-wide channel (Fig. 1). Semi-enclosed bays like Port Phillip Bay may contain contamination hotspots as reduced wave action hinders the transport of polluted particles into open waters (Fukushima et al., 1992; Aly et al., 2013). Not surprisingly, sediments in Hobsons Bay (Fig. 1) and the shipping channels historically contain high concentrations of arsenic, mercury and lead (Phillips et al., 1992; Fabris et al., 1999). These locations were recently directly impacted by dredging in 2008/09 when 23 million m^3 of rock, silt, clay and sand were removed from the mouth of the Yarra River and the shipping channels to increase vessel accessibility to the port (PoMC, 2010). Notably, the same areas have been identified as foraging hot spots for the all-year resident Little Penguin population, nesting at the breakwater in St Kilda (Preston et al., 2008; Kowalczyk et al., 2013). Currently, records of metal and metalloid contaminants in the biota of Port Phillip Bay are only sporadic and not consistent in their choice of study species (Walker, 1988; EPA, 2009, 2013; Finger et al., 2015). Given the recent scale of developments and disturbances, as well as general pressures on the area, the lack of long-term data on contamination levels in biota is of concern.

Our previous study of metals and metalloids in Little Penguins, sampled at one point in time at three different locations, each with varying degrees of industrialisation, established the Little Penguin as a reliable bioindicator for local metal and metalloid contamination and showed Phillip Island to be a viable reference site for the more industrialised St Kilda (Finger et al., 2015). In this study, we extend this work and present the analysis of a comprehensive multi-year data set to investigate annual, seasonal and within-moult variation of blood metal and metalloid concentrations in this high trophic feeder.

2. Materials and methods

2.1. Study sites

Blood was collected from adult Little Penguins at two locations: St Kilda (n = 101) from March 2011 to December 2014 and Phillip Island (n = 56) from November 2011 to May 2013 (Fig. 1, Table 1). The St Kilda colony (37°51′S, 144°57′E) is located 5 km from the central business district of Melbourne, Australia. Approximately 1,000 Little Penguins nest between the rocks of a 650 m long man-made breakwater structure (Z. Hogg, unpublished data). St Kilda's Little Penguins adjust their diet depending on prey availability (Kowalczyk et al., 2015c), but mostly feed on clupeoids, such as Australian anchovy (*Engraulis australis*), southern garfish (*Hyporhamphus melanochir*), and luminous bay squid (*Loliolus noctiluca*) (Preston et al., 2008). The Phillip Island colony (38°30′S, 145°10′E) is located 140 km southeast of Melbourne, Australia, and has approximately 32,000 penguins nesting on the Summerland Peninsula (Sutherland and Dann, 2012). These penguins feed on Australian anchovy, Pilchard (*Sardinops sagax*), Barracouta (*Thyrsites atun*), Red Cod (*Pseudophysis bachus*), various other juvenile fish and Arrow squid (*Nototodarus gouldi*) (Chiaradia et al., 2010).

2.2. Sample collection

We collected all samples (n = 157) during three distinct life history stages, namely 'breeding', 'moult' and 'non-breeding'; henceforth called 'seasons'. Breeding starts with egg-laying and concludes with the fledging of the chicks, lasting between three and five months (Reilly and Cullen, 1981). During moult, which follows the breeding season, penguins stay inside or near their burrows and fast while the entire plumage is replaced (~22 days, Reilly and Cullen, 1983). Non-breeding is defined as the time between moult and breeding, where penguins forage without the restraints of caring for young. We captured penguins by hand on their way to or from their burrows. We restrained the animal in a light cotton bag and aspirated up to 2 mL of blood from the medial metatarsal (caudal tibial) vein using a 25-gauge butterfly needle with a 3 mL syringe. This was transferred into 6 mL Vacutainers® (BD Diagnostics, trace element tube plus K_2EDTA, product number 368381), which we placed in a cooler with ice packs, transferred to a freezer within 12 hours of sampling and kept frozen at −20 °C until analysis. We weighed penguins to the nearest 10 g using a hand-held spring balance. Animals that weighed less than 900 g were released

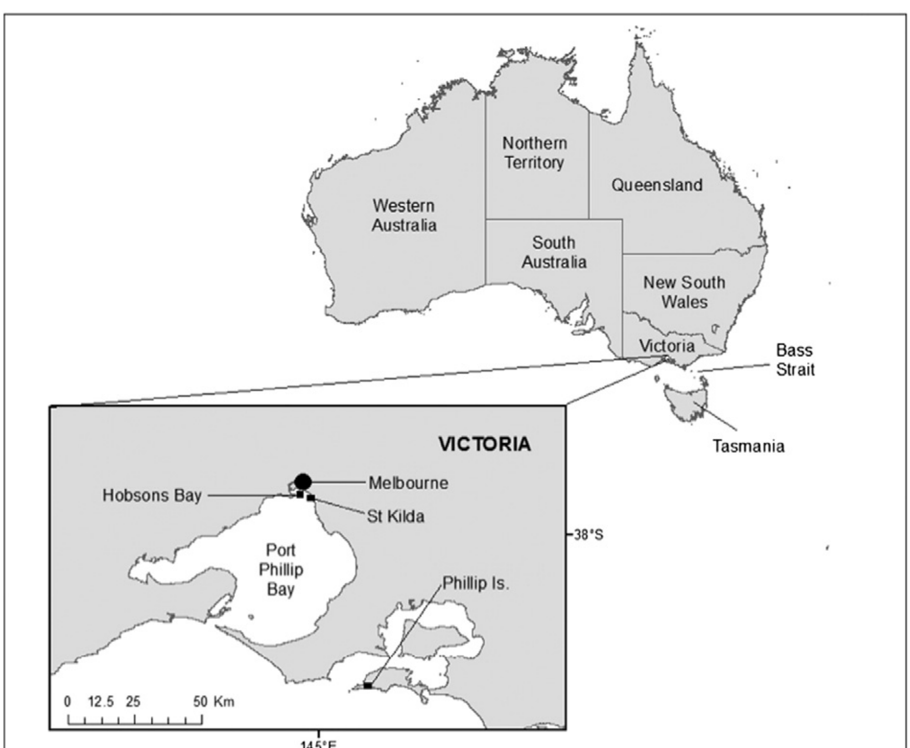

Fig. 1. Little Penguins were sampled at St Kilda and Phillip Island from 2011 to 2013.