



Short communication

Influence of age, sex and breeding status on mercury accumulation patterns in the wandering albatross *Diomedea exulans*S. Tavares^{a,*}, J.C. Xavier^{b,c}, R.A. Phillips^b, M.E. Pereira^d, M.A. Pardal^a^a CFE (Centre for Functional Ecology), Department of Life Sciences, University of Coimbra, PO Box 3046, 3001-401 Coimbra, Portugal^b British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK^c IMAR (Institute of Marine Research), Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal^d CESAM (Centre for Environmental and Marine Studies), Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

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ABSTRACT

Although mercury bio-amplifies through the food chain and accumulates in top predators, mercury concentrations in tissues of the wandering albatross are greater than in any other vertebrate, including closely related species. In order to explore the alternative explanations for this pattern, we measured total mercury concentrations in feathers, plasma and blood cells of wandering albatrosses of known age, sex and breeding status sampled at South Georgia. Mercury concentrations were low in feathers and blood components of chicks, and higher in the feathers of young pre-breeders than in feathers or blood of older pre-breeders and breeding adults. There was no effect of sex on mercury concentrations in the feathers of pre-breeders or breeding adults, whereas levels were significantly higher in blood cells of breeding females than males. The high feather mercury concentrations of young pre-breeders compared with older birds suggest an increase in moult frequency as birds approach maturity.

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1. Introduction

Marine ecosystems provide crucial resources and services for humans, but have been greatly altered by the effects of fisheries, climate change and release of hazardous contaminants (Halpern et al., 2008). Given its toxicity and tendency to bioaccumulate, contamination by mercury (Hg) is a major concern for environmental agencies and policy makers. Mercury also biomagnifies through the food web, concentrating in the tissues of top predators, including marine and freshwater fish, which raises human health issues (EPA, 2001).

Marine top predators are widely regarded as effective monitors of ocean health because they integrate processes occurring at lower trophic levels (Monteiro and Furness, 1995). Also, given their large foraging ranges, levels of pollutants in their tissues reflect those of a wide area, including potentially remote regions that would otherwise be difficult to sample (Thompson et al., 1993; Stewart et al., 1999; Blévin et al., 2013). Moreover, their distributions change seasonally (Phillips et al., 2008), permitting a comparison between pollutant levels in breeding and nonbreeding areas (Ramos and González-Solís, 2012).

Antarctica is considered to be one of the most undisturbed areas of the world. However, mercury is widely distributed as a consequence of long-range atmospheric transport, and wet and dry deposition processes, and some of the highest organic mercury concentrations observed in the open ocean were recorded in Antarctic waters (Cossa et al., 2011). Mercury emissions are predicted to increase (Streets et al., 2009), raising concern about impact on these remote areas. Previous studies have highlighted an increase in mercury contamination of several seabird species from South Georgia and New Zealand over the last few decades (Thompson et al., 1993; Becker et al., 2002).

The wandering albatross, *Diomedea exulans*, is a wide-ranging top predator, and its mercury concentrations reflect contamination over a huge foraging area that encompasses Antarctic, subantarctic and subtropical waters. Mercury levels measured previously in this species were much higher than in related taxa with similar diets from the same localities (Thompson et al., 1993; Hindell et al., 1999; Stewart et al., 1999; Anderson et al., 2009). Even more surprising is that these values exceed those in seabirds in the Northern Hemisphere (e.g. Doi et al., 1984; Monteiro and Furness, 1995; Stewart et al., 1997; Bearhop et al., 2000).

Although several studies have measured mercury levels in albatrosses (Thompson et al., 1993; Hindell et al., 1999; Stewart et al., 1999; Becker et al., 2002; Anderson et al., 2009; Blévin et al., 2013), none included data from birds ranging in age from chicks to adults,

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despite the potential for elucidating the key factors contributing to lifetime mercury accumulation. In this study, we measured total mercury concentration in feathers of chicks, young and old pre-breeders, and in feathers, blood cells and plasma of breeding adults, all of known-age, in the wandering albatross at South Georgia. Mercury in feathers is considered to be a reliable measure of total body burden at the time of feather formation (Monteiro and Furness, 2001). This is because mercury is sequestered in the sulfhydryl groups of keratin, so concentrations reflect the uptake and storage of mercury between moults (Ochoa-acuña et al., 2002). Mercury bound in the plumage can account for up to 93% of the accumulated body burden (Braune and Gaskin, 1987). In adult wandering albatrosses, feather replacement takes place exclusively during the nonbreeding period (Weimerskirch, 1991). In chicks, two generations of feathers are grown post-hatching, an initial down and later pennaceous (or 'true') feathers. Down sampled early in development may include a residual signal of the maternal mercury burden via the egg, but this is likely to be diluted quickly, as the rate of plumage growth in procellariiform chicks is rapid (Phillips and Hamer, 2000). The utility of analysing mercury concentrations in blood is that these largely reflect accumulation from the time of the previous moult, less any mercury that has been demethylated from its more toxic methyl, to less toxic inorganic form and potentially sequestered in internal tissues (Thompson and Furness, 1989) or, in females, was deposited in the egg (Lewis et al., 1993). Hence our sampling programme allowed us to determine mercury dynamics in relation to age, sex and breeding status, and to determine the key factors underpinning long-term mercury accumulation.

2. Methodology

Fieldwork was undertaken on Bird Island, South Georgia (54° 00' S, 38° 03' W). Between January 2005 and February 2010, 6–8 body feathers (selected at random) were obtained from 20 returning pre-breeders visiting the colony in the early-midsummer; 7 were young pre-breeders (4–6 years) and 13 were old pre-breeders (9–15 years). In May–October 2009, blood samples (1 ml blood from the tarsal vein) and body feathers were collected from 6 breeding adults, also of known age, each month. Down and blood were also sampled in 4 chicks in May and September 2009. Feathers were stored dried, and blood was separated into plasma and cells using a centrifuge (15 min at 3000 rpm) and stored frozen within 2 h of collection. No bird was sampled more than once, nor a sample taken from both members of any pair. Birds were sexed using plumage and morphology (Tickell, 1968), and all had been ringed as chicks and so were of known age.

Feathers were cleaned with a chloroform and diethyl ether solution (2:1) and dried at 50 °C prior to analysis. Repeatability in mercury measurement was assessed using paired feather samples from the same individuals. Samples of both plasma and blood cells were subsequently freeze-dried and homogenized, and total mercury determinations of samples of 0.20–7.62 mg was performed by thermal decomposition atomic absorption spectrometry with gold amalgamation, using a LECO AMA-254. Accuracy and precision were assured by the daily analysis of a certified reference material (CRM) of similar matrix to the samples (TORT-2), obtained from the National Research Council of Canada. The results for the CRM were always within the certified value ($0.27 \pm 0.06 \text{ mg kg}^{-1}$) with a recovery efficiency of $102.8 \pm 7.85\%$ ($n = 49$). The results were corrected for the daily recovery percentage of the CRM analyses.

After checking for normality, data were analysed using parametric procedures after logarithmic transformation of mercury concentrations. ANOVAs were used to evaluate the effects of age, sex and breeding status on tissue Hg concentrations, followed by unequal N Tukey HSD post-hoc test (given the unequal group sizes). Pearson correlations or ANCOVA were used to assess relationships between mercury concentrations, age and sex. Differences in mercury levels between chicks and breeding adults were examined using unpaired *t*-tests. Consistency in mercury levels measured in two different feathers sampled from the same individual was examined using intraclass correlation. Significant levels were set at $p < 0.05$.

Table 1
Review of the published data on the Hg levels in a number of albatrosses species (mean \pm SD in mg kg^{-1} dry wt).

Species		Location	Feathers	Blood	Liver	<i>n</i>	Source
<i>Diomedea exulans</i>	Wandering albatross	South Georgia	20.1 \pm 7.6	9.6 \pm 4.3		31	<i>Present study</i>
			19.6 \pm 10.1			66	Thompson et al. (1993)
			27.4 \pm 8.1			14	Anderson et al. (2009)
			24.8 \pm 12.4			29	Thompson et al. (1993)
		Marion Island			360.0 \pm 183.0	9	Stewart et al. (1999)
		Southern Pacific and Indian Oceans			482.3 \pm 120.7	22	Hindell et al. (1999)
<i>Diomedea dabbenena</i>	Tristan albatross	Gough Island	28.0 \pm 14.3			27	Thompson et al. (1993)
					1343 \pm U	2	Thompson and Furness (1989)
<i>Diomedea epomophora</i>	Royal albatross	New Zealand	11.5 \pm 13.9			22	Thompson et al. (1993)
						4	Stewart et al. (1999)
						9	Hindell et al. (1999)
		Southern Pacific and Indian Oceans			449.3 \pm 490.1	4	Stewart et al. (1999)
					108.6 \pm 35.8	9	Hindell et al. (1999)
<i>Thalassarche melanophris</i>	Black-browed albatross	South Georgia	4.6 \pm 1.9	4.4 \pm 1.1		20	Thompson et al. (1993)
			5.4 \pm 2.0			16	Becker et al. (2002)
			8.3 \pm 2.6			16	Anderson et al. (2009)
		Chilean coast	2.7 \pm 0.8			4	Ochoa-acuña et al. (2002)
		Falkland Islands	2.7 \pm 1.2			30	Thompson et al. (1993)
<i>Thalassarche impavida</i>	Campbell albatross	New Zealand	10.1 \pm 4.4			35	Thompson et al. (1993)
<i>Thalassarche chrysostoma</i>	Grey-headed albatross	New Zealand	6.9 \pm 2.4	6.6 \pm 1.1		6	Stewart et al. (1999)
			4.2 \pm 2.3			36	Thompson et al. (1993)
			8.9 \pm 2.9			34	Thompson et al. (1993)
			9.5 \pm 2.8			19	Becker et al. (2002)
			10.9 \pm 4.6			15	Anderson et al. (2009)
<i>Thalassarche steadi</i>	White-capped albatross	New Zealand	10.9 \pm 4.6			20	Thompson et al. (1993)
					35.0 \pm 17.6	42	Stewart et al. (1999)
		Southern Pacific and Indian Oceans			39.6 \pm 4.6	29	Hindell et al. (1999)
<i>Phoebastria immutabilis</i>	Laysan albatross	Midway Atoll, Hawaii	3.5 \pm 0.4			13	Burger and Gochfeld (2000)
<i>Phoebastria nigripes</i>	Black-footed albatross	British Columbia, Canada			11.9 \pm U ^a	11	Elliott (2005)
		Midway Atoll, Hawaii	19.6 \pm 1.8			17	Burger and Gochfeld (2000)
		British Columbia, Canada			121 \pm U ^a	12	Elliott (2005)

^a U – unknown.

3. Results and discussion

The observed levels in feathers and blood confirm that mercury concentrations in wandering albatrosses are much higher than those of several other albatrosses, including those from South Georgia (Table 1). Such high values have been attributed to the high rate of mercury intake from their upper trophic level diet combined with a slow moult cycle, biennial breeding and a physiological capability to demethylate mercury and sequester the inorganic form with selenium (Thompson et al., 1993; Stewart et al., 1999; Xavier et al., 2004). The low moult frequency is a contributing factor, but not the complete explanation as other albatrosses moult almost as infrequently (Weimerskirch, 1991; Prince et al., 1993). At South Georgia, wandering albatrosses tend to maintain a consistent feeding preference for fish and squid, whereas other albatrosses eat more lower trophic level prey such as Antarctic krill *Euphausia superba* during the austral summer (Xavier et al., 2003, 2004; Phillips et al., 2009, 2011).

Bioaccumulation of mercury with size has been documented in many species of fish (McArthur et al., 2003) and squid (Bustamante et al., 2006, 2008; Pierce et al., 2008; Pereira et al., 2009), which are the major components of albatross diets (Xavier et al., 2003). An estimated 86% of the mass of cephalopods consumed by wandering albatrosses is scavenged, and includes large species (Xavier and Croxall, 2007), and they also consume offal and discards from commercial fishing, which frequently consist of long-lived, large-bodied demersal species (Xavier et al., 2004). Hence, wandering albatrosses are probably more likely than other albatross species to consume a greater proportion of large prey. Indeed, previous work has highlighted that variation in mercury burdens in seabirds often reflects differences in feeding strategies, including the relative importance of mesopelagic prey (Monteiro et al., 1998; Stewart et al., 1999; Becker et al., 2002; Anderson et al., 2009).

Mercury levels in feathers from wandering albatrosses ranging in age from chicks to mature adults showed no evidence of an overall linear trend ($r = 0.143$, $p = 0.297$; Fig. 1). This is consistent with a number of previous studies that tested in a similar way for correlations between mercury levels in tissues and bird age (Thompson et al., 1991, 1993; Becker et al., 2002). However, having the opportunity to sample young pre-breeders, we detected a surprisingly steep increase in mercury burden from the time of fledging to first return at the age of 4–6 years. This was followed by a decline to a lower level by 9 years which was maintained

Table 2

Mercury concentration in feathers and chick down of the wandering albatross according to breeding status and sex (mean \pm SD and range, mg kg^{-1} dry wt).

	<i>n</i>	Mean value	SD	Range
Chicks	8	6.14	1.91	4.25–9.91
Young pre-breeders				
Males	2	45.81	4.28	42.78–48.84
Females	4	49.69	8.16	41.25–57.18
Pooled data	7	48.13	6.34	41.25–57.18
Old pre-breeders				
Males	7	23.93	5.64	16.77–33.20
Females	3	20.22	8.10	10.89–25.46
Pooled data	13	21.20	6.32	10.89–33.20
Adults				
Males	16	18.90	7.41	8.09–36.58
Females	16	21.43	8.18	13.18–39.82
Pooled data	34	20.14	7.64	8.09–39.82

thereafter in breeding adults. There were also significant differences in mercury concentrations in feathers of chicks (<1 year), young pre-breeders (4–6 years old), old pre-breeders (9–15 years old) and breeding adults (11–33 years old) ($F = 51.62$, $p < 0.001$). Levels were lowest in chicks, intermediate in old pre-breeders and breeding adults, and greatest in young pre-breeders (Table 2). Repeatability in mercury concentration between the two feather samples taken from each individual ($n = 55$) was high, and significant ($F = 5.08$, $p < 0.0001$, $r_i = 0.804$).

The higher mercury levels measured in the feathers of the young pre-breeders are probably linked to differences in moulting strategies. In breeding adults, as in most seabirds, moulting and breeding tend to be temporally segregated because both are energetically highly demanding (Bridge, 2006). Hence, albatrosses moult almost exclusively in the nonbreeding period, and must balance the extent of moult necessary to maintain flight efficiency and the demands of reproduction to the extent that in several species, birds may ultimately be forced to defer breeding in order to replace worn plumage (Langston and Rohwer, 1996). In wandering albatrosses, immature birds and individuals breeding for the first time possess fewer new feathers than experienced birds, which seems likely to reflect their greater difficulties in replacing feathers because of energy allocation constraints associated with their lower foraging skills (Weimerskirch, 1991). Since moult is a crucial mechanism for mercury excretion, this reduces the opportunities for pre-breeders and, it would appear from our results, particularly

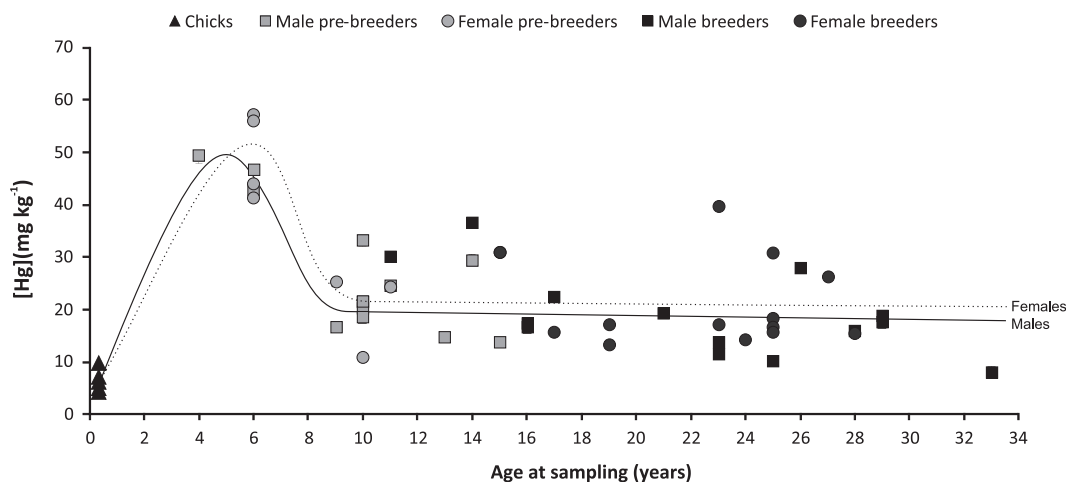


Fig. 1. Total mercury concentrations in body feathers of wandering albatrosses of known age and breeding status (mg kg^{-1}). The dashed line refers to the long-term pattern of mercury accumulation in males and females.

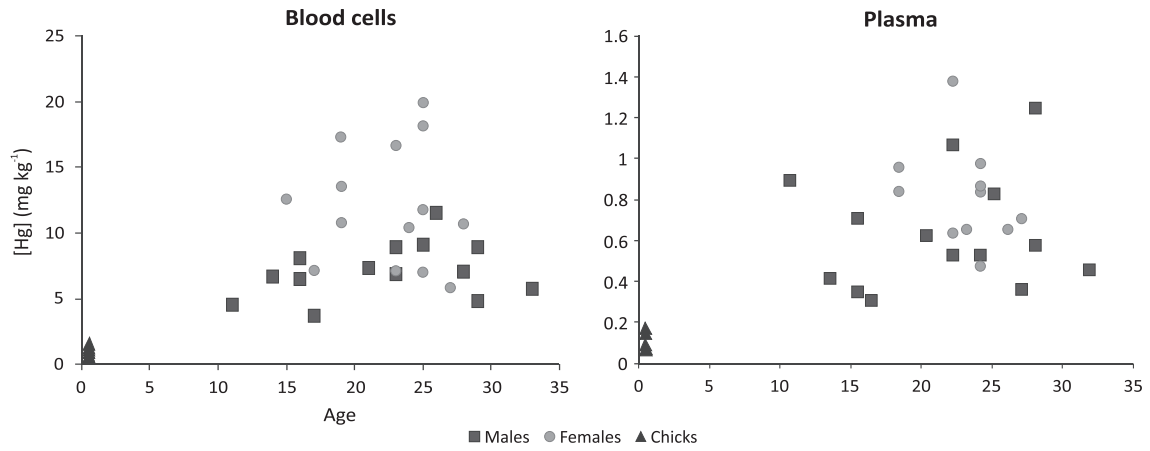


Fig. 2. Mercury levels in blood samples of albatrosses of known age (mg kg^{-1}): (a) blood cells, (b) plasma.

the very youngest birds, to reduce their body pool of mercury by excretion into feathers.

Another factor contributing to differences in mercury concentrations in feathers of young pre-breeders compared with older wandering albatrosses could be variation in at-sea distribution or diet. The plumage of juvenile and immature wandering albatrosses is easily distinguished, and at sea observations in combination with

recent tracking data indicate that young birds are more likely than adults to feed in subtropical or subantarctic waters, and that a significant proportion cross the Indian Ocean to wintering grounds around the southern and eastern coast of Australia (Weimerskirch et al., 2006).

Mercury concentrations in chick down were significantly lower than in feathers of pre-breeders or breeding adults probably

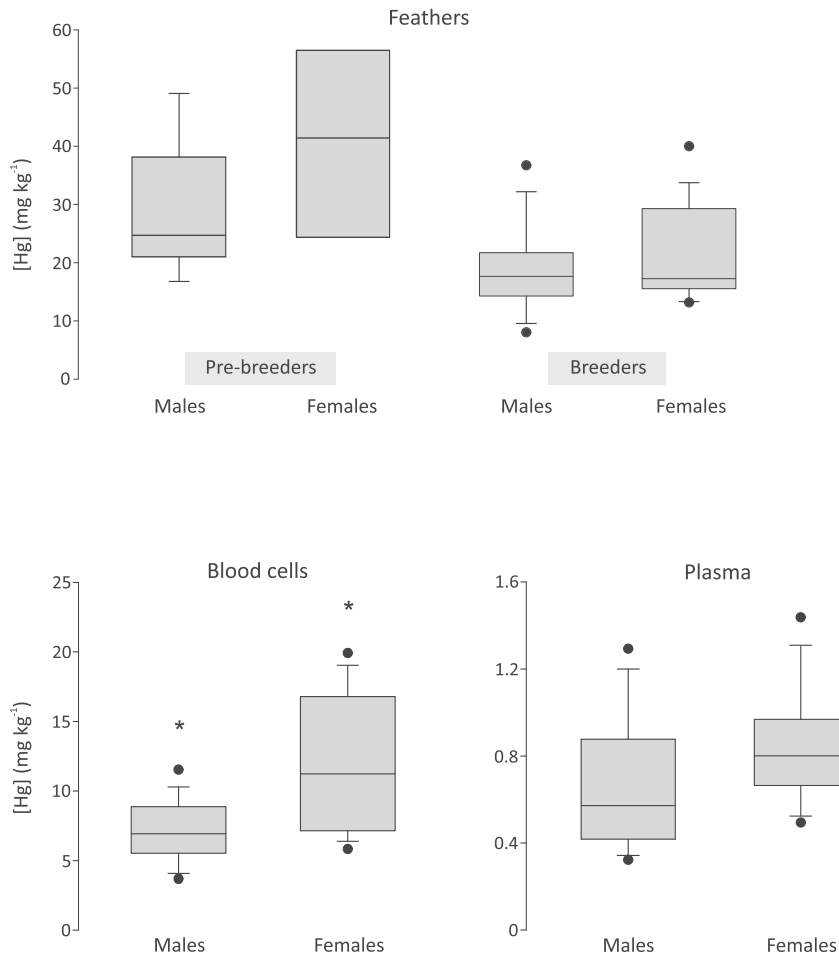


Fig. 3. Mercury levels in feathers, blood cells and plasma of male and female wandering albatrosses (mg kg^{-1}). Boxes represent interquartile range, and bars \pm range. * Indicates a significant difference.

because their exposure periods were short (chicks were only 1–6 months old when sampled), and also they were rapidly growing a complete set of plumage. The latter alone would tend to greatly dilute the levels of mercury excreted into new feathers, certainly by comparison with an older bird that might replace only half its plumage once every two years between breeding attempts.

Mercury concentrations in blood cells and plasma were significantly higher in breeding adults than chicks ($t = 13.26$, $p < 0.001$ and $t = 10.05$, $p < 0.001$, respectively). There was no significant linear correlation between mercury concentrations in either blood cells or plasma, and age in breeding adults ($r = 0.107$, $p = 0.589$ and $r = 0.109$, $p = 0.597$, respectively; Fig. 2). However, the pattern of mercury accumulation in the blood cells with age differed between sexes, with a higher rate of mercury accumulation in females than males (ANCOVA, age $F = 0.445$, $p = 0.511$; sex $F = 13.54$, $p = 0.001$).

There was no effect of sex on mercury levels in feathers of young pre-breeders, old pre-breeders or breeding adults (Two-way ANOVA, effect of status $F = 16.29$, $p < 0.001$, effect of sex $F < 0.001$, $p = 0.985$, interaction $F = 0.836$, $p = 0.441$; Fig. 3). Nor was there an effect of sex on mercury levels in plasma of breeding adults ($t = 1.893$, $p = 0.185$; Table 3). In contrast, mercury levels in blood cells were significantly higher in female than male breeders ($t = 3.741$, $p < 0.001$; Table 3; Fig. 3). Blood cells presumably reflect dietary mercury intake since the end of the moult, prior to the onset of the current breeding attempt. Those concentrations remain higher in females than males, suggesting they do not excrete sufficient quantities of mercury into the egg for this to have a substantial long-term effect. Instead, the difference suggests some sexual segregation in foraging areas or diet of breeding birds in the incubation or early to mid chick-rearing period, prior to sample collection. Male and female wandering albatrosses do show preferences for different water masses during breeding; males favour cold, Antarctic waters whereas females mostly use subarctic and subtropical waters (Weimerskirch et al., 1993; Xavier et al., 2004; Xavier and Croxall, 2005). Moreover, in two years with differing environmental conditions, males consumed mainly fish (74% by mass) whereas females consumed mainly cephalopods (67%) (Xavier et al., 2004). However, differences in distribution or diet may be less pronounced during the nonbreeding season, given the lack of a sex effect on mercury levels in feathers. This accords with previous studies of stable isotope ratios; $\delta^{13}\text{C}$ in feathers was higher in females than males, but there was no difference in $\delta^{15}\text{N}$, indicating that females had a more northerly foraging habitat but did not feed at a higher trophic level during the nonbreeding period (Phillips et al., 2009; Ceia et al., 2012).

The high level of mercury contamination in the wandering albatross may constitute an additional stress in individuals within a species that is already facing conservation problems, mainly as a consequence of unsustainable incidental mortality associated with long-line fishing (Croxall et al., 1998; Nel et al., 2002; Xavier et al., 2004). Some degree of health surveillance, including contaminant monitoring, is advisable, as exposure to mercury may lead to deleterious effects (organ toxicity and reproductive or

neurobehavioral impairment) (Scheuhammer, 1987). Mercury concentrations of over 0.5 mg kg^{-1} in eggs and of over $9\text{--}20 \text{ mg kg}^{-1}$ in feathers have been correlated with decreased reproductive success in some piscivorous birds (Scheuhammer, 1987; Burger and Gochfeld, 1997). Concentrations reported in this and other studies of wandering albatross are considerably higher, and so although marine birds are expected to have higher toxicity thresholds than terrestrial birds (Blévin et al., 2013), we cannot dismiss the possibility of adverse effects in this already threatened species.

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Table 3

Mercury concentration in blood cells and plasma of the wandering albatross according to breeding status and sex (mean \pm SD and range, mg kg^{-1} dry wt).

	Blood cells				Plasma			
	n	Mean	SD	Range	n	Mean	SD	Range
Chicks	7	0.84	0.36	0.42–1.40	5	0.11	0.05	0.06–0.17
Adults								
Males	14	7.10	2.10	3.69–11.53	14	0.66	0.29	0.32–1.29
Females	14	12.04	4.55	5.83–19.91	12	0.83	0.25	0.49–1.43
Pooled data	28	9.57	4.29	3.69–19.91	26	0.74	0.28	0.32–1.43

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