Arch. Environ. Contam. Toxicol. 52, 418–430 (2007)
DOI: 10.1007/s00244-005-0225-2

ARCHIVES OF
Environmental
Contamination
and Toxicology

Trace Elements in Three Marine Birds Breeding on Reunion Island (Western Indian Ocean): Part 1—Factors Influencing Their Bioaccumulation

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Received: 19 September 2005/Accepted: 26 June 2006

Abstract. This work aimed to use seabirds as bioindicators of trace element levels in the tropical waters and food webs of the Western Indian Ocean. The accumulation patterns of selected toxic (Cd and Hg) and essential (Cu, Fe, Mn, Se, and Zn) elements were determined in liver, kidney, and pectoral muscle of 162 marine birds belonging to 3 species collected in Reunion Island between 2002 and 2004. These pelagic seabirds belong to the following species: Barau's Petrel (*Pterodroma baraui*), Audubon's Shearwater (*Puffinus lherminieri bailloni*), and White-Tailed Tropicbird (*Phaethon lepturus*). Hg levels were also measured in breast feathers. Highest mean kidney Cd and liver Hg levels (respectively, 27.79 ± 13.78 $\mu g.g^{-1}$ dry weight (dw) and 24.31 ± 14.13 $\mu g.g^{-1}$ dw) were found in the squid-eating Barau's Petrel. Barau's Petrel feather Hg levels fell in the range of 0.6 to 2.7 $\mu g.g^{-1}$ dw previously reported for other petrels and shearwaters. The values of the other elements were also in the same range as those previously reported in the published literature concerning related seabirds, although Se and Zn burdens in the Reunion birds were among the highest values. Levels of Zn, Fe, and, to a lesser extent, Cu appeared to be regulated in seabird tissues. Uptake and pathways of metabolism and storage seemed to be similar for the five essential elements. The reproductive status of the bird did not seem to affect element levels, which, moreover, were not significantly different between male and female birds. However, trace elements in sampled birds varied according to the tissue considered, the age of the animal, and its species. Diet was seemingly a major influencing factor. Health status also appeared to have an impact on element levels.

Contaminants, such as metals and metalloids, reach oceans through natural erosion, geochemical cycles, and human activity. Once in the marine environment, they can enter the food web where bioaccumulation and/or biomagnification can occur. Top predators, such as marine birds, are particularly exposed to high levels of these trace elements through their food (Bearhop *et al.* 2000). Because of their trophic position and bioaccumulation capacities, seabirds are often used as bioindicators of elemental levels in the marine environment (Walsh 1990; Furness *et al.* 1993; Monteiro and Furness 1995; Gray 2002).

Despite the great variety and number of seabirds present in the southern hemisphere, most studies have been carried out on species inhabiting the northern hemisphere. The few studies conducted on trace elements in the southern hemisphere focused on temperate and subantartic species, e.g., from Chili (Ochoa-Acuna *et al.* 2002), New Zealand (Fiona *et al.* 1999), Gough island (Muirhead and Furness 1988), the Kerguelen islands (Bocher *et al.* 2003), and the Antarctic Ocean (Szefer *et al.* 1993; Ancora *et al.* 2002; Gonzalez-Solis *et al.* 2002). The tropical zone of the Indian Ocean has, up to the present day, received little attention from researchers in reference to biocenosis trace element contamination. Overall, tropical waters are less monitored than marine environments from temperate and polar regions, more particularly southern tropical oceans, which are often considered less polluted than the northern ones (Fiona *et al.* 1999). However, the study of unpolluted sites can be of great interest by allowing estimation of "background" levels of contaminants and can thus be used as a reference in space and time.

This article presents novel data on elemental contamination in seabirds of the western part of the tropical Indian Ocean, which was gathered through the study of trace element levels in marine birds from Reunion Island. This work focused on three seabirds species chosen for their contrasting dietary ecology. Barau's Petrel (*Pterodroma baraui*) is a pelagic bird that feeds almost exclusively on cephalopods. Audubon's Shearwater (*Puffinus lherminieri bailloni*), forages closer to the coast and feeds as much on cephalopods as fish. Finally, White-Tailed Tropicbird (*Phaethon lepturus*) also forages over oceanic waters but at intermediate distances from the island compared with the other two species and is less dependent on squid than Barau's Petrel.

In organisms, elements are transported in the blood, deposited in various tissues, and excreted or stored. Understanding

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the global patterns of accumulation and excretion is useful in evaluating sources of contamination and potential risks to an individual species. In the first part of this article, we examined the levels of two nonessential elements (Cd and Hg) and five essential elements (Cu, Fe, Mn, Se, and Zu) in liver, kidney, and muscle, as well as Hg levels in feathers. We also studied factors influencing elemental burdens and correlations among levels of these trace elements.

Materials and Methods

Study Site and Species

The seabirds used in this study originated from colonies on Reunion Island (21°7′S, 55°33′E), a French territory (2 512 km^2) located 700 *km* east of Madagascar in the Western Indian Ocean.

Barau's Petrel, described for the first time in 1963 (Jouanin 1963), is endemic to Reunion Island, where its population is estimated at 4000 to 6500 pairs (Le Corre and Safford 2000). This pelagic bird is present on the island from September (courtship and mating) to April (fledgling's first flight) (Bretagnolle and Attié 1991); the eggs are laid in November. During its reproductive season, Barau's Petrel scatters to feed in the tropical and subtropical waters as far as several hundred kilometers south of Reunion Island. Its diet consists of 98% cephalopods (*Stenoteuthis oualaniensis* and *Taonius sp*., given as a percentage of the total number of prey items) and 2% fish (Gigan & Lebon, 2001, personal communication). From March to September, Barau's Petrel leaves Reunion waters and migrates toward the north and the east of the Indian Ocean (Barre *et al.* 1996; Stahl and Bartle 1991).

Aububon's Shearwater is a pantropical seabird, but the subspecies *P. l. bailloni* is found only on the islands of Reunion and Europa (22°20′S and 40°E; 650 km from Reunion Island) (Barré *et al.* 1996; Le Corre 2000). Three thousand to 5000 pairs breed in 235 colonies located in the interior of Reunion Island at altitudes ranging from 50 to 1500 m as well as on the coastal cliffs (Bretagnolle and Attié 1991). This species breeds all year with peak laying occurring between August and November. During the breeding season, Aububon's shearwater does not seem to forage further than 50 km from the coast (Bailey 1967; Jaquemet *et al.* 2004). During that time, its diet consists of 50% *S. oualaniensis* and 50% fish (Gigan & Lebon, 2001, personal communication).

Two thousand to 5000 pairs of White-Tailed Tropicbirds breed in ravines or on the coastal cliffs of Reunion Island. They do not form true colonies (Le Corre, 1998, unpublished data). This indigenous species breeds on the island all year. Its diet consists of 80% *S. oualaniensis* and 20% fish, mainly of the Mollidae and Dactylopteridae families (Gigan & Lebon, 2001, personal communication).

Outside of their breeding season, individuals scatter in the tropical waters of the Indian Ocean (Barré *et al.* 1996). During that period, the dietary habits of the three species remain unknown.

Bird Sampling

Fifty-two Barau's Petrels, 61 Audubon's Shearwaters, and 49 White-Tailed Tropicbirds were sampled. All birds used in this study died accidentally. Petrels and Shearwaters are known to be attracted by urban lights. This is particularly common in Reunion (Le Corre *et al.* 2001). Of the hundreds of fledgling Petrels and Shearwaters found stranded annually, 90% are rescued successfully (Le Corre *et al.* 2001). The remaining 10% are usually found dead or fatally injured. The adult Procellariiformes died of various causes, including light attraction, collisions, and poaching. Poaching was also one of the main causes of the death of White-Tailed Tropicbirds. Because these birds were found before or shortly after their death, we are confident that the conditions under which the birds died had no effect on the element levels in their tissues.

Each bird was measured and weighed. Two age classes were determined (juvenile or adult) using characteristic features of the beak and feathers. For adults, the presence of an incubation patch was an indicator of the reproductive status of the individual. Because these birds do not show any sexual dimorphism, their sex was determined during dissection. Only adults were sexed. Gonads were not developed enough in fledglings to differentiate males from female birds. The liver, kidneys, and pectoral muscles were removed and refrozen for freeze drying. Breast feathers were also sampled on each bird. Emaciation was estimated by evaluating the muscular condition (MC), which was given a range from 1 to 3 in relation to the shape of the pectoral muscles: 1-well developed pectoral muscles; 2-slight muscle atrophy; and 3-severe pectoral muscle atrophy (Bolton *et al.* 1991). Body condition (*BC*) was also evaluated using a condition index proposed by Wenzel and Adelung (1996). This condition index is the ratio of liver mass to kidney mass. It is significantly negatively correlated to the degree of emaciation of the bird: the smaller the index, the more emaciated the bird (Debacker *et al.* 2001a).

Sample Preparation

Livers, kidneys, and muscles were blended, dried, and ground to a fine powder. Muscles were dried in an oven at 55°C to constant mass for 72 hours, whereas livers and kidneys were freeze dried. The moisture content was 66% to 72% in liver, 73% to 78% in kidney and 65% to 71% in muscle.

The analysis of Cd, Cu, Fe, Mn, Se, and Zn called for an extra step in the preparation protocol. Two aliquots of 50 to 400 mg, of each sample were digested with 3.5 ml 15 N nitric acid at 60°C for 48 hours before dilution in 10 ml deionized water. Accuracy and reproducibility of the preparation were tested by preparing 28 replicates of lobster hepatopancreas (TORT-2), dogfish liver (DOLT-2), and dogfish muscle (DORM-2) reference standards (National Research Council, Canada) and 11 blanks along with each set of samples. Glass and plastic utensils were washed with detergent, plunged in a bath of mixed nitric acid (35 ml.l^{-1}) and hydrochloric acid (50 ml.l^{-1}) for a minimum of 24 hours, rinsed 3 times in deionized (Milli-Q quality) water, and dried in an oven at 50°C before use.

Pectoral feathers were washed vigorously in triple baths of 0.25 N sodium hydroxide solution alternated with triple baths of deionized water to remove adherent external contamination and airborne contamination (Walsh 1990; Schreiber and Burger 2001), which could have altered the results of the analysis of Hg sequestered in the feathers. Feathers were then dried in an oven for 24 hours at 50°C and analyzed for Hg content.

Metal Analysis

Cd, Cu, Fe, Mn, Se, and Zn were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES Varian Vista Pro CCD, La Rochelle, France). Total Hg analyzes were carried out with an advanced mercury analyzer (ALTEC AMA 254) on aliquots ranging from 5 to 20 mg dried sample weighed to the nearest 0.01 mg.

Detection limits and recovery rates were, respectively, equal to 0.322 μg.g^{-1} and 98% for Cd, 0.32 μg.g^{-1} and 94% for Cu, 0.64 μg.g^{-1} and 89% for Fe, 0.0025 μg.g^{-1} 94% for Hg, 0.32 μg.g^{-1} and 90% for Mn, 6.45 μg.g^{-1} and 85% for Se, and 1.29 μg.g^{-1} and 101% for Zn. Element levels are expressed in μg.g^{-1} dry weight (dw).

Table 1. Levels (mean ± SD, $\mu g.g^{-1}$ *dw*) and interspecific comparison results for seven trace elements in the tissues of juvenile and adult seabirds from Reunion Island

	Barau's Petrel		Audubon's Shearwater		White-tailled Tropicbird		Hypotheses tests results	
	Juveniles $n = 32$	Adults $n = 20$	Juveniles $n = 38$	Adults $n = 23$	Juveniles $n = 17$	Adults $n = 32$		
	Mean ± SD CV (%)	Mean ± SD CV (%)	Mean ± SD CV (%)	Mean ± SD CV (%)	Mean ± SD CV (%)	Mean ± SD) CV (%)	Juveniles	Adults
Cd								
Liver	**6.30 ± 4.27**^{a} 68	**66.8 ± 31.8** 48	**3.29 ± 2.57**^{b} 78	**53.0 ± 38.0** 72	**4.53 ± 3.06**^{ab} 67	**47.0 ± 28.0** 59	ANOVA***	ANOVA:NS
Muscle	**0.12 ± 0.05**^{a} 48	**9.28 ± 9.70** 104	**0.07 ± 0.09**^{b} 134	**4.55 ±3.93** 86	**0.37 ± 0.32**^{c} 87	**3.67 ± 3.23** 88	ANOVA***	ANOVA:NS
Kidney	**27.8 ± 13.8**^{a} 50	**145 ± 70** 48	**12.6 ± 11.5**^{b} 92	**147 ± 55** 37	**19.5 ± 23.5**^{b} 121	**117 ± 58** 50	ANOVA:***	KW:NS
Cu								
Liver	**29.3 ± 45.8**^{a} 156	**20.2 ± 23.5**^{x} 116	**11.1 ± 3.2**^{a} 29	**16.5 ± 5.9**^{x} 36	**31.5 ± 13.5**^{b} 43	**29.3 ± 22.6**^{y} 77	ANOVA:***	ANOVA:***
Muscle	**143 ± 2.9**^{ab} 20	**27.7 ± 10.2** 37	**12.7 ± 1.5**^{a} 12	**21.0 ± 9.0** 41	**18.3 ± 7.2**^{b} 39	**28.1 ± 18.5** 66	ANOVA:***	ANOVA:NS
Kidney	**11.7 ± 3.1**^{a} 27	**19.5 ± 5.1**^{x} 26	**8.55 ± 4.72**^{b} 55	**15.4 ± 5.0**^{y} 32	**17.0 ± 6.0**^{c} 37	**24.2 ± 28.5**^{x} 118	ANOVA:***	ANOVA:*
Fe								
Liver	**1240 ± 571**^{a} 45	**2620 ± 1670**^{x} 64	**1350 ± 550**^{a} 40	**1540 ± 940**^{y} 61	**4050 ± 2890**^{c} 71	**2120 ± 1880**^{xy} 88	ANOVA:***	ANOVA:*
Muscle	**201 ± 37**^{a} 18	**404 ± 186** 46	**206 ± 28**^{a} 13	**365 ± 146** 40	**337 ± 162**^{b} 48	**367 ± 171** 47	ANOVA:***	ANOVA:NS
Kidney	**327 ± 81**^{a} 25	**526 ± 141** 27	**538 ± 461**^{b} 86	**499 ± 187** 37	**524 ± 162**^{b} 31	**539 ± 163** 30	ANOVA:***	KW:NS
Hg								
Liver	**1.10 ± 0.33**^{a} 30	**24.3 ± 14.1**^{x} 58	**0.26 ± 0.07**^{b} 27	**1.72 ± 1.32**^{y} 77	**1.26 ± 2.47**^{c} 196	**1.89 ± 1.23**^{y} 65	ANOVA:***	ANOVA:***
Muscle	**0.13 ± 0.06**^{a} 46	**2.84 ± 2.09**^{x} 74	**0.06 ± 0.01**^{b} 23	**0.38 ± 0.19**^{y} 49	**0.30 ± 0.50**^{a} 167	**0.75 ± 0.47**^{z} 63	ANOVA:***	ANOVA:***
Kidney	**0.55 ± 0.28**^{a} 50	**24.2 ± 18.5**^{x} 76	**0.17 ± 0.06**^{b} 35	**1.16 ± 0.51**^{y} 44	**0.86 ± 1.29**^{a} 150	**1.88 ± 1.12**^{z} 59	ANOVA:***	ANOVA:***
Feathers	**0.30 ± 0.07**^{a} 58	**0.96 ± 0.31**^{x} 69	**0.07 ± 0.01**^{b} 87	**0.25 ± 0.04**^{y} 47	**0.29 ± 0.02**^{a} 8	**0.84 ± 0.10**^{x} 12	ANOVA:***	ANOVA:***
Mn								
Liver	**7.37 ± 2.52**^{a} 34	**11.1 ± 3.3**^{x} 30	**11.1 ± 2.3**^{b} 21	**13.4 ± 3.9**^{x} 29	**27.9 ± 10.8**^{c} 39	**17.9 ± 7.7**^{y} 43	ANOVA:***	KW:***
Muscle	**1.38 ± 0.25**^{a} 18	**1.65 ± 0.34**^{x} 20	**1.46 ± 0.23**^{ab} 16	**1.83 ± 0.32**^{x} 17	**1.60 ± 0.50**^{b} 30	**2.33 ± 0.49**^{y} 21	KW:*	KW:***
Kidney	**6.26 ± 3.03** 48	**8.33 ± 2.26**^{x} 27	**6.24 ± 5.00** 80	**8.86 ± 2.65**^{x} 30	**7.72 ± 4.09** 53	**733 ± 5.48**^{y} 75	ANOVA: NS	KW:***
Se								
Liver	**36.1 ± 7.7**^{a} 21	**81.7 ± 29.9**^{x} 37	**48.5 ± 13.7**^{b} 28	**57.3 ± 18.9**^{y} 33	**43.7 ± 18.4**^{ab} 42	**68.5 ± 23.2**^{xy} 34	KW:***	KW:**
Muscle	**15.8 ± 3.6**^{a} 23	**37.9 ± 16.7**^{x} 44	**16.5 ± 4.5**^{a} 28	**25.7 ± 9.1**^{y} 35	**11.2 ± 5.7**^{b} 51	**23.3 ± 9**^{y} 38	ANOVA:***	ANOVA:***
Kidney	**50.9 ± 19.1**^{a} 38	**148 ± 62** 42	**89.9 ± 79.9**^{b} 89	**145 ± 65** 45	**97.7 ± 55.3**^{b} 57	**160 ± 64** 40	ANOVA:***	KW:NS
Zn								
Liver	**119 ± 46**^{a} 39	**316 ± 172** 54	**209 ± 76**^{b} 36	**288 ± 129** 45	**528 ± 198**^{c} 37	**305 ± 190** 62	ANOVA:***	ANOVA:NS
Muscle	**73.1 ± 12.1**^{a} 16	**101 ± 50** 50	**55.6 ± 7.8**^{b} 14	**73.0 ± 27.0** 37	**101 ± 43**^{a} 42	**86.7 ± 56.9** 66	ANOVA:***	ANOVA:NS
Kidney	**137 ± 31**^{a} 22	**235 ± 61** 26	**110 ± 56**^{b} 51	**224 ± 58** 26	**193 ± 50**^{c} 26	**241 ± 101** 42	ANOVA:***	ANOVA:NS

For each metal and each tissue, the significance of the level differences among juvenile birds (respectively, adults) of the three species is given in the last but one (respectively last) column. NS: $p > 0.05$.

*$p < 0.05$.

**$p < 0.01$

***$p < 0.001$. In case of significant difference, the letters a, b, c (respectively, x, y, z) indicate which subgroups differ; sub-groups sharing the same letter do not differ significantly.

Statistical Analysis

Statistical analyzes were performed using the GNU R statistical system (R Development Core Team 2005). All statistical samples submitted to tests were first checked for normality by means of Shapiro-Wilk test. In the case of nondeparture from normality, parametric tests were used in the subsequent analyzes; otherwise, nonparametric analogues were used.

The validity of the replicates of the level measures was tested by means of Student *t*-test for paired samples. The significance of differences of trace element levels among tissues was tested by one-way repeated measures analysis of variance (ANOVA) or Friedman test and followed by Tukey's Honest Significant Difference (HSD) test. The influences of species, age, sex, MC, and reproductive status on element levels were tested by means of ANOVA or Kruskal-Wallis test, followed, when necessary, by Tukey's HSD test. Before using ANOVA for independent samples, besides normality, homogeneity of the variances of the tested samples was checked by means of Bartlett test. In the case of departure from normality or non homogeneity of the variances, Kruskal-Wallis test was applied instead.

For each species, associations among element levels in different tissues were studied by means of Pearson's linear correlation coefficient. The resulting correlation matrices were represented by dendrograms built using the average linkage as aggregation criterion.

Levels of significance of the null hypotheses associated with these tests was divided into classes of *p*-values represented by the following codes: NS ≥ 0.05; * < 0.05; ** < 0.01; *** < 0.001. SD stands for standard deviation, and CV stands for coefficient of variation.

Results

Levels of Cd, Cu, Fe, Hg, Mn, Se, and Zn in liver, muscle, kidney, and Hg in feathers of each species and age subgroup, are listed in Table 1 and their dispersion illustrated in Figs. 1 through 3.

With the exception of Cu, which presented particularly high CV values in Barau's Petrel liver (115%) and in White-Tailed Tropicbird kidney (up to 117%), essential elements showed little variability in seabird tissues. In contrast, Cd and Hg levels showed high variability in all three species. For Cd, CV values ranged from 38% to 134%. For Hg, the highest fluctuations were found in the soft tissues of juvenile White-Tailed Tropicbirds (196%) and the lowest in their feathers (8%). In soft tissues, CV values were higher in juvenile than in adult tropicbirds (up to 3 times higher), whereas they were higher in adult than in juvenile Procellariiformes.

Elemental levels varied from one tissue to an other, except for Cu, for which differences were not significant between liver and muscle (Table 1). The general pattern in all 3 species showed that liver (followed by kidney, then muscle) accumulated the highest levels of Fe, Hg, and Mn. This was also the case for Zn, except in juvenile petrels, in which the highest levels were found in kidney. Cd and Se accumulated mostly in kidney. Furthermore, muscle and feather Hg levels were equivalent in juvenile shearwaters and tropicbirds. In petrels, juvenile birds showed higher Hg levels in feather than in muscle, whereas opposite results were found for adult birds.

Fig. 1. Comparison of element levels (μg.g^{-1} *dw*) in liver of adult (A) and juvenile (J) seabirds. Outliers are represented as individual points. For each species, the significances of the level differences between juvenile and adult birds are indicated below the boxplots. PB - *P.baraui*; PLB - *P. l. balloni*; PQ - *P. lepturus*; *t*: *t*-test; *W*: Wilcoxon test.

Factors Influencing Trace Element Levels

Influence of age classes, sex, and reproductive status. Clear morphologic traits differentiate fledgling from adult birds, therefore, elemental burden differences were tested between these two age classes. The type of test and level of significance are given in Figs. 1 through 3. Significantly higher elemental levels were revealed in adult than in juvenile Petrels, except for hepatic Cu. In the same way, levels were significantly higher in adult than in juvenile shearwaters, except for Fe in liver and kidney. In tropicbirds, differences between juvenile and adult birds were less systematic with, in some cases (*i.e.*, Fe, Mn, and Zn), higher levels in juvenile than in adult birds. In all three species, significant differences between juvenile and adult birds were observed in the three tissues for Hg and Se, for Cd in muscle and kidney, for Mn in muscle and liver, and for Fe and Zn in liver.

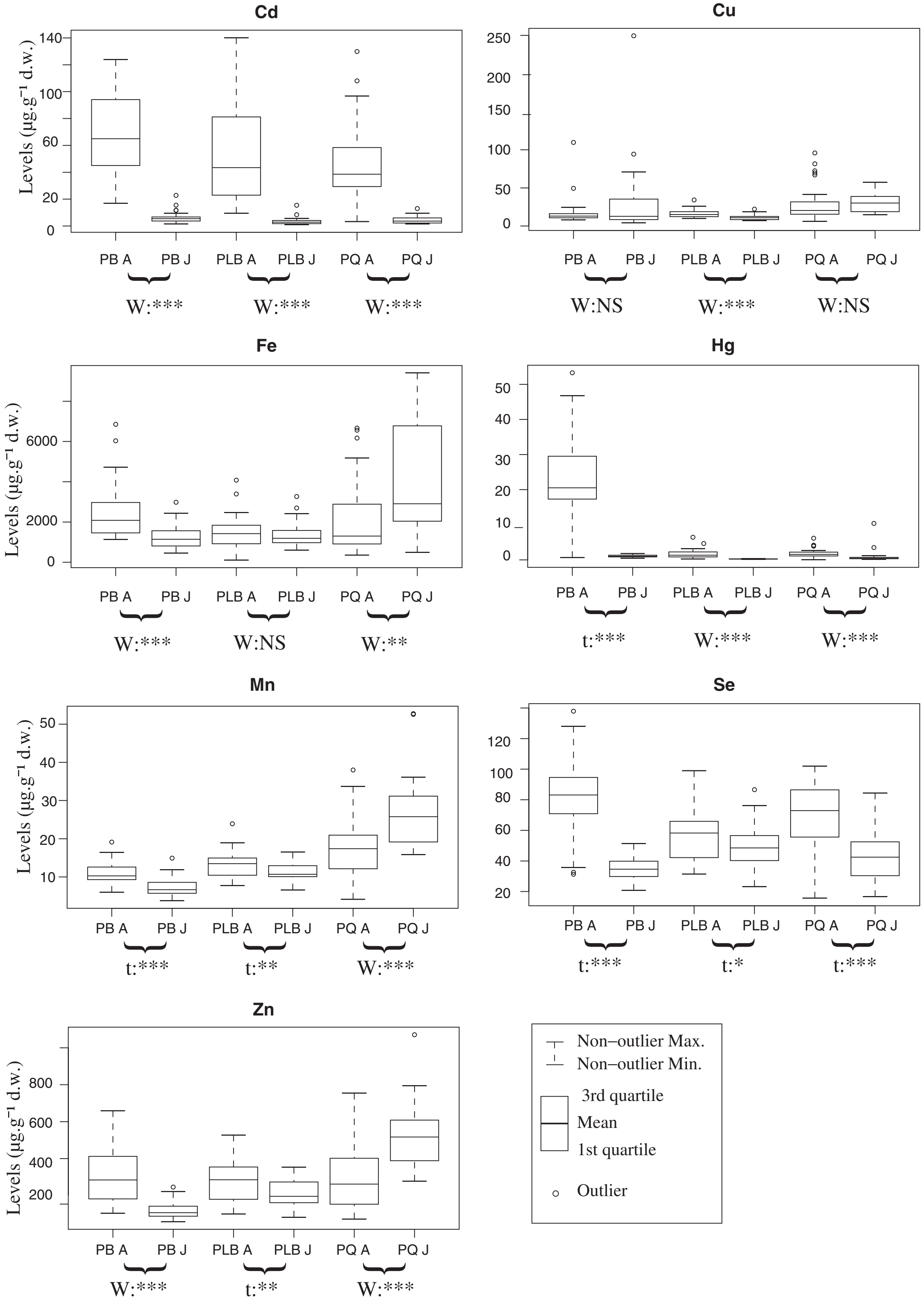
The influence of sex on element levels was tested on adult birds. Sex did not seem to influence element levels among adult birds of each species with the exception of hepatic Se, which was higher in male than in female tropicbirds (P_{Wilcoxon}: ***). This latter result suggests that eggs may constitute an excretion path for Se in female White-Tailed Tropicbirds. Reproductive status, defined by the presence or absence of an incubation patch, did not significantly influence trace element levels among adult birds for all species.

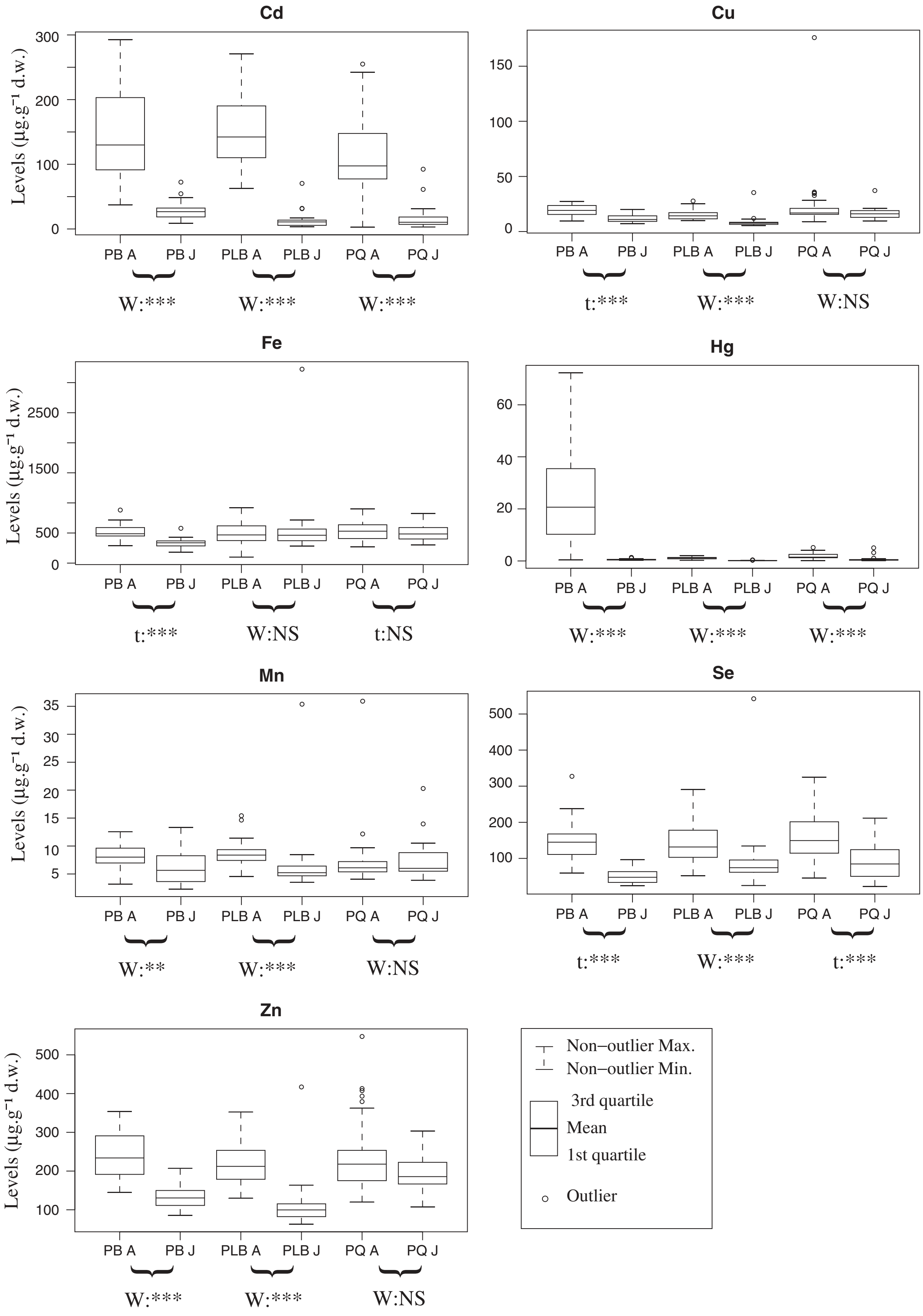
Interspecific differences. Element levels also differed from one species to another (Table 1). In juvenile birds, significant differences were found for every element in every tissue of the three species except for renal Mn. Hg levels were significantly higher in Barau's Petrels and White-Tailed Tropicbirds than in Audubon's Shearwaters. The highest levels of Cd were found in the petrels. Essential metal (Cu, Fe, Mn, and Zn) levels were highest in the tropicbirds.

As far as adult birds were concerned, there were fewer differences among species. Hg was the only element for which there were differences between adult birds of all species. The highest Hg levels were found in Barau's Petrel and the lowest in Audubon's Shearwater. Adult Barau's Petrels also had the highest Fe and Se levels. The highest levels of Cu and Mn were found in White-Tailed Tropicbird. There were no significant differences between Cd and Zn levels in the three species.

Relationships Between Trace Element Levels

The correlation matrices among element levels in the various tissues of each species are not presented in this article.


Cd
Levels (µg.g⁻¹ d.w.)
140
100
60
20
0
PB A
PB J
PLB A
PLB J
PQ A
PQ J
W:***
W:***
W:***
Cu
250
200
150
100
50
0
PB A
PB J
PLB A
PLB J
PQ A
PQ J
W:NS
W:***
W:NS
Fe
Levels (µg.g⁻¹ d.w.)
6000
2000
0
PB A
PB J
PLB A
PLB J
PQ A
PQ J
W:***
W:NS
W:**
Hg
50
40
30
20
10
0
PB A
PB J
PLB A
PLB J
PQ A
PQ J
t:***
W:***
W:***
Mn
Levels (µg.g⁻¹ d.w.)
50
40
30
20
10
PB A
PB J
PLB A
PLB J
PQ A
PQ J
t:***
t:**
W:***
Se
120
100
80
60
40
20
PB A
PB J
PLB A
PLB J
PQ A
PQ J
t:***
t:*
t:***
Zn
Levels (µg.g⁻¹ d.w.)
800
600
400
200
PB A
PB J
PLB A
PLB J
PQ A
PQ J
W:***
t:**
W:***
Non–outlier Max.
Non–outlier Min.
3rd quartile
Mean
1st quartile
Outlier


Cd
Levels (µg.g^{-1} d.w.)
0
100
200
300
PB A
PB J
PLB A
PLB J
PQ A
PQ J
W:***
W:***
W:***
Cu
0
50
100
150
PB A
PB J
PLB A
PLB J
PQ A
PQ J
t:***
W:***
W:NS
Fe
Levels (µg.g^{-1} d.w.)
0
500
1500
2500
PB A
PB J
PLB A
PLB J
PQ A
PQ J
t:***
W:NS
t:NS
Hg
0
20
40
60
PB A
PB J
PLB A
PLB J
PQ A
PQ J
W:***
W:***
W:***
Mn
Levels (µg.g^{-1} d.w.)
5
10
15
20
25
30
35
PB A
PB J
PLB A
PLB J
PQ A
PQ J
W:**
W:***
W:NS
Se
100
200
300
400
500
PB A
PB J
PLB A
PLB J
PQ A
PQ J
t:***
W:***
t:***
Zn
Levels (µg.g^{-1} d.w.)
100
200
300
400
500
PB A
PB J
PLB A
PLB J
PQ A
PQ J
W:***
W:***
W:NS
Non−outlier Max.
Non−outlier Min.
3rd quartile
Mean
1st quartile
Outlier

◀

Fig. 2. Comparison of elemental levels ($\mu g.g^{-1}$ *dw*) in kidneys of adult (A) and juvenile, (J) seabirds. Outliers are represented as individual points. For each species, the significances of the level differences between juvenile and adult birds are indicated below the boxplots. PB - *P.baraui*; PLB - *P. l. balloni*; PQ - *P. lepturus*; *t*: *t*-test; *W*: Wilcoxon test.

However, two types of linear correlations were obvious: (1) correlations between different elements in the same tissue and (2) correlations of levels of one element in various tissues. Regarding (1), Hg and Cd stood out because of their strong tissue-to-tissue correlations in all species. Se and Mn were also well correlated in liver, kidney, and muscle of Barau's Petrel. Regarding (2), the following significant correlations between elements were common to the three species: Cu, Fe, and Zn in muscle and Fe and Zn in liver. Other relationships were more species-specific, such as correlations between muscle Mn and Se and kidney Cu and Mn in the tropicbird, and among renal Cu, Fe, Mn, Se, and Zn in the shearwater.

Discussion

Bioaccumulation in Barau's Petrel, Audubon's Shearwater, and White-tailed Tropicbird

Tissue distribution. As it has often been shown, trace elements are not all identically distributed in the different tissues of an organism. The preferential accumulation of Fe, Hg, Mn, and Zn in liver of Reunion birds reflects the usual trend, as does the preferential accumulation of Cd in kidney (Furness and Rainbow 1990; Furness *et al.* 1993; Thompson *et al.* 1996). Indeed, liver and kidney are considered long-term storage tissues for Hg and Cd in seabirds (Walsh 1990), implicating that large birds with long life spans accumulate high burdens of these elements (Fiona *et al.* 1999). This is obvious in Procellariiformes, of which the albatrosses are the best example (Hindell *et al.* 1999), and seems to apply to this study in which Barau's Petrel is the largest of the two Procellariiforme species and shows the highest levels.

The equal distribution of Cu between liver and muscle in all three studied species is an unexpected result. This accumulation pattern has, however, already been observed, although not explained, in the Common Diving Petrel (*Pelecanoides urinatrix*), the South Georgian Diving Petrel (*P. georgicus*), and the Antarctic Prion (*Pachyptila desolata*) from the Kerguelen archipelago (Bocher *et al.* 2003). Se also differed from the usual distribution pattern because it was mostly accumulated in kidney of the studied birds. There are few data on the distribution of Mn among seabird tissues. It was found mainly in liver and kidney of Procellariiformes studied by Kim *et al.* (1998) and mainly in liver of Reunion birds. Further studies are needed to determine the bioaccumulation pattern of Mn in seabird tissues.

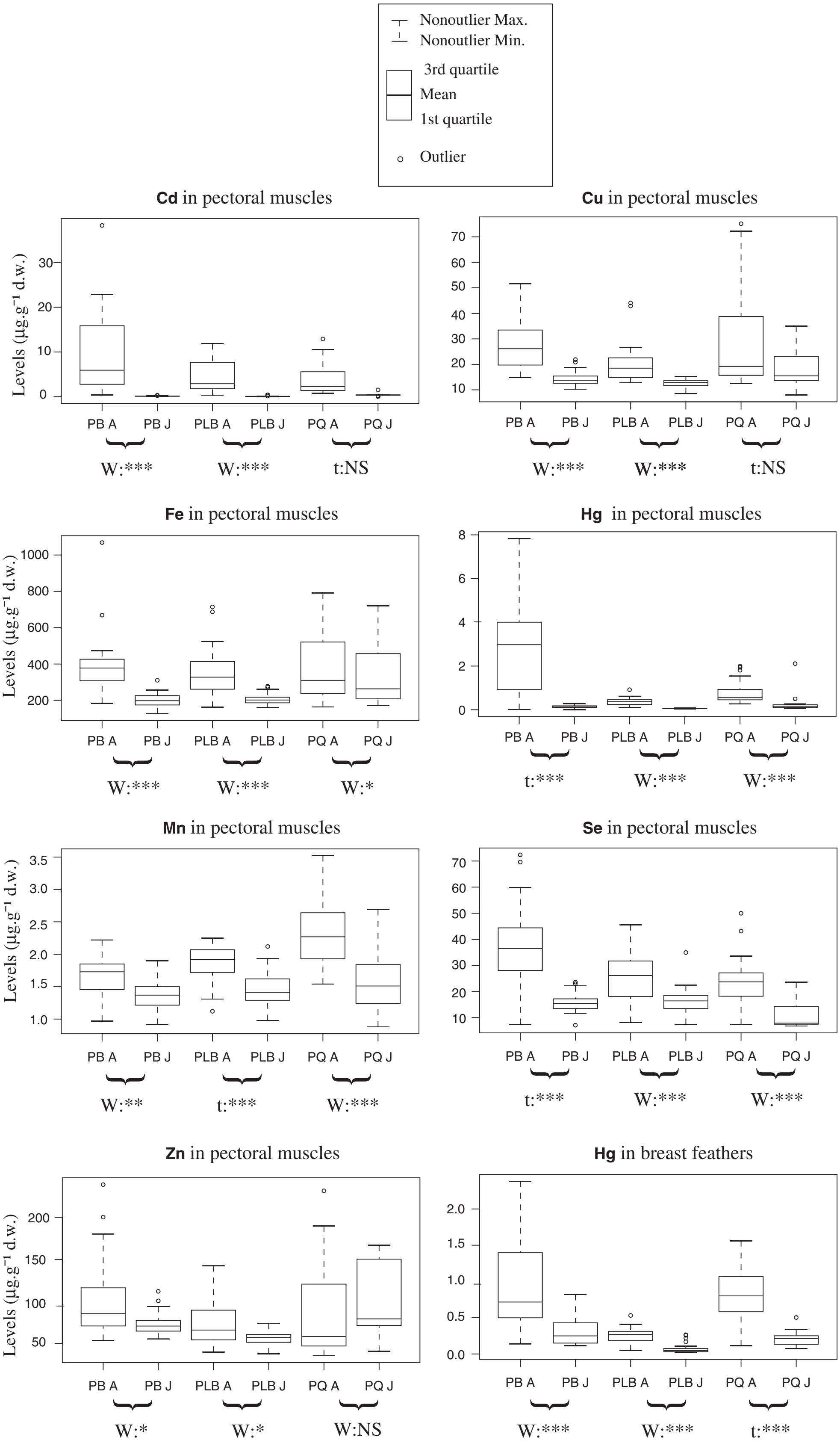
Influence of phylogeny. Tropicbirds belong to the Phaethontidae family, whereas the other two species are from the Procellariidae family. Only in the tropicbirds did juvenile birds have higher essential metal levels than adult birds. This same pattern has already been observed in various species (Kim et al. 1998; Stewart *et al.* 1999), in which the young accumulate essential elements to meet particularly high nutritional needs (Thompson *et al.* 1996). This strategy is required to survive the fast caused by the fledgelings abandonment by their parents several days before their first flight. Because young Procellariiformes also undergo a long fasting period before fledging, but do not have high essential elemental levels, the White-Tailed Tropicbird may differ from the two Procellariiforme species in the way its metabolism deals with the accumulation of such essential elements.

Procellariiformes have been cited for their particular ability to concentrate trace elements, resulting in much higher element burdens than many other seabirds (Walsh 1990). However, within the Procellariiformes order, phylogeny is thought to explain relatively little of the interspecific variations (Fiona *et al.* 1999). Other than intrinsic differences in bioaccumulation linked to phylogeny, several characteristics of a species may explain part of the interspecific variability.

Size and life span obviously have an impact on the quantity of trace element concentrated in the body because of a dilution effect and because longer-living species have more time to accumulate larger burdens. Barau's Petrel is the largest of the 3 species (400 g) and very likely the longest living seabird because its phylogenetically and geographically closest parent, the Galapagos Petrel *P. phaeopygia*, lives > 25 years. Barau's Petrel is followed by White-Tailed Tropicbird, 260 g, and Audubon's Shearwater, 200 g, both having a life span > 20 years (Weimerskirch 2001). This pattern is respected for most trace element levels analyzed here, especially nonessential elements.

Diet is considered to be one of the most important factors of trace element variability because it is the major contamination path in marine vertebrates (Stewart *et al.* 1999; Thompson *et al.* 1998; Kim *et al.* 1998). Of the three Reunion seabird species, Barau's Petrel has by far the highest Hg content in the studied tissues. Fish prey are known to be a source of contamination for top predators because they accumulate high levels of Hg (Honda *et al.* 1990), mainly in a bioavailable form, *i.e.*, methylmercury (Bloom 1992). However, Barau's Petrel includes only 2% fish in its diet but feeds on cephalopods of the *Taonius* genus (Le Corre, 2001, unpublished data). These are mesopelagic squids that live in depths ranging from 500 to 700 m (Clarke 1986; Nesis 1987). It is thought that Barau's Petrel feeds on their floating carcasses. The deep, poorly oxygenated waters promote the methylation of Hg, which is easily transfered to living organisms (Thompson *et al.* 1998). Recently, relatively high Hg levels were reported in mesopelagic cephalopods from the northeastern Atlantic waters, in which a major proportion of Hg was in an organic form (Bustamante *et al.* 2006). These findings are consistent with the fact that Hg levels found in pelagic marine birds are higher than those found in terrestrial birds (Monteiro *et al.* 1996; Thompson *et al.* 1998). High Hg levels in *Taonius* sp. could be responsible for the higher Hg levels found in Barau's Petrel.

Procellariiformes are also known for having high Cd levels because of their diet, which is essentially composed of cephalopods, and cephalopods have been shown to be responsible for the high Cd levels in top predators (Smith *et al.* 1984;


Nonoutlier Max.
Nonoutlier Min.
3rd quartile
Mean
1st quartile
Outlier
Cd in pectoral muscles
Cu in pectoral muscles
Fe in pectoral muscles
Hg in pectoral muscles
Mn in pectoral muscles
Se in pectoral muscles
Zn in pectoral muscles
Hg in breast feathers
Levels (µg.g^{-1} d.w.)
PB A
PB J
PLB A
PLB J
PQ A
PQ J
W:***
W:***
t:NS
W:***
W:***
t:NS
W:***
W:***
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t:***
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t:***
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W:***
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W:NS
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Fig. 3. Comparison of elemental levels ($\mu g.g^{-1}$ *dw*) in pectoral muscles and breast feathers of adult (A) and juvenile (J) seabirds. Outliers are represented as individual points. For each species, the significances of the level differences between juveniles and adults are indicated below the boxplots. (PB - *P.baraui*; PLB - *P. l. balloni*; PQ - *P. lepturus*); *t*: *t*-test; *W*: Wilcoxon test.

Bustamante *et al.* 1998a, 1998b). This seems to be the case in Reunion Procellariiformes and especially in Barau's Petrel becasue 98% of its diet is composed of cephalopods (Gigan & Lebon, 2001, unpublished data). The study of Hg and Cd burdens of the various prey species of the three birds would be useful in confirming these hypotheses.

Interindividual differences. The high individual fluctuations observed for Cd, Cu, and Hg could have many causes, such as individual variation in diet (see 4.1.2), body condition, and age (within an age class).

White-Tailed Tropicbird was the only species for which the individual variations of Hg burdens were higher in juvenile than in adult birds. This may be explained by a difference in their breeding cycle. Contrary to the Procellariiformes species, which breed on Reunion Island at definite periods, tropicbirds breed individually thoughout the year. Because Hg intake originates from diet, young tropicbirds collected during various periods of the year would show more interindividual variations than young petrels or shearwaters, which were all collected roughly at the same time. Furthermore, these large interindividual differences would not be as apparent in adult tropicbirds, which would have integrated Hg during a longer period, thus minimizing variability.

Individual fluctuation in elemental levels was also found to be induced by variability in the nutritional condition of Common Guillemots *(Uria aalge)* at death (Debacker *et al.* 2001b). The influence of MC and BC on elements levels was studied to determine whether the body condition of these birds had an influence on the results. As a general trend, most significant differences in trace element levels existed between the MC indices 1 and 3, which are two extreme cases, *i.e.*, well-fed and starving birds respectively ($P_{Wilcoxon}$: *). Only renal Fe, muscular Mn, and hepatic and renal Se seemed unaffected by the bird's muscular condition. The higher elemental levels in the starving birds were most probably caused by loss of tissue weight rather than by an increase in element levels. Surprisingly, few significant correlations were found between the BC index, which ranged from 0.7 to 4.5, and trace element levels.

It is not possible to estimate the age of adult seabirds unless they are marked, yet trace elements are known to accumulate in an organism over the lifetime. Age can therefore be considered a possible cause of individual fluctuation among adults of each species.

Relationships between trace element levels. The evolution of Hg tissue-to-tissue correlations in juvenile and adult Barau's Petrels is a good illustration of the bioaccumulation of this element. Indeed, significant correlations of Hg levels were observed between feathers, liver, kidney, and muscle in juvenile petrels ($r = 0.371$* to $r = 0.717$***; $n = 32$). In adults, Hg levels in liver, kidney, and muscle remained correlated ($r = 0.58$** to $r = 0.786$***; $n = 20$), whereas feather Hg levels were no longer significantly correlated to the others. Once the feather is formed, blood irrigation subsides, implying that no further metal is deposited. In adult Barau's Petrel, Hg burden in the feathers had not evolved since the last plumage growth, whereas Hg levels in the other organs had increased with Hg accumulation.

Correlations between elements—such as Cu, Fe, and Zn in muscle and Fe and Zn in liver—of the three species suggests that uptake and pathways of metabolism and storage are relatively similar for these trace elements.

Comparison With Other Species and Locations

Table 2 draws a parallel between elemental levels in liver, kidney, and feathers in seabirds from Reunion Island and phylogenically related species from other locations in the world. Elemental burdens in juvenile and adult birds are presented separately.

Most ecotoxicologic works are based on the study of adult animals because they have integrated environmental contamination for a longer period of time. Metal levels obtained for adult birds in this study are generally comparable with those reported for petrels and shearwaters studied in other oceanic islands (Muirhead and Furness 1988; Stewart *et al.* 1994; Stewart *et al.* 1997; Stewart *et al.* 1999). Nevertheless, Se and Zn values observed in these birds were fairly high. In kidney, Se levels exceeded 3.6 to 4.5 times the value of 10 $\mu g.g^{-1}$ *wet weight* (ww) (approximately 40 $\mu g.g^{-1}$ *dw)* considered as the level above which Se may have harmful effects on birds (Thompson *et al.* 1996). This indicates that regulation mecanisms must exist in Reunion birds. Similar Se levels have been observed in other wild seabird, such as Leach's Storm-Petrel *(Oceanodroma leucorrhoa,* 78 $\mu g.g^{-1}$ *dw*) from the Atlantic coast of Canada (Elliott *et al.* 1992), the Black-footed Albatross *(Diomedea nigripes*, 113 $\mu g.g^{-1}$ *dw*) from the Northern Pacific, as well as birds from pristine area, such as the Grey Petrel *(Procellaria cinerea*, 100 $\mu g.g^{-1}$ *dw)* from the Southern Indian Ocean (Kim *et a1.* 1996, 1998).

The *Phaethon* genus has received limited attention regarding elemental bioaccumulation. Feather Hg levels in Reunion's White-Tailed Tropicbird are 7 times inferior to those found in Red-Tailed Tropicbirds from Midway Island (Burger and Gochfeld 2000). In Barau's Petrel feathers, Hg levels fit in the 0.6 to 2.7 $\mu g \cdot g^{-1}$ range reported for petrels and shearwaters (Burger 1993). For Hg in feathers, a range of 5 to 40 $\mu g.g^{-1}$ *dw* can be considered associated with adverse effects (Eisler 1987; Burger and Gochfeld 1997). In this study, the highest Hg level recorded among all birds was 5 times inferior to this toxicity limit. It can therefore be presumed that the marine birds studied here do not suffer from any adverse effects of Hg.

Contrary to Hg, means for renal Cd were above the 10 $\mu g.g^{-1}$ *ww* adverse-effects level determined by Eisler (1985). However, Furness (1996) suggested that levels above which adverse effects occur in pelagic seabirds may be higher than for other birds and that no adverse Cd effects have been documented in wild seabirds. Cd and Hg levels in soft tissues of adult Barau's Petrels are close to those found in the

Table 2. Trace element levels (mean ± SD, μg.g^{-1} *dw*) in seabirds from different biogeographic areas[a] 1: Tropical; 2: Subtropical; 3: Temperate; 4: Subpolar. * designates species for which data have been converted from "wet weight" to "dry weight" assuming a tissue water content of 70%.

Species	*n*	Area	Age class	[Cu] liver	[Cu] kidney	[Zn] liver	[Zn] kidney	[Cd] liver	[Cd] kidney	[Sc] liver	[Hg] liver	[Hg] kidney	[Hg] feathers	[Mn] liver	References
Barau's Petrel *P. baraui*	32	1	J	29.3 ± 4.3	11.7 ± 3.1	118 ± 46	137 ± 30	6.3 ± 4.3	27.8 ± 13.8	36.1 ± 7.7	1.1 ± 0.3	0.55 ± 0.28	03 ± 0.1	7.37 ± 2.56	This study
Barau's Petrel *P. baraui*	20	1	A	20.2 ± 23.5	19.5 ± 5.1	316 ± 172	235 ± 61	66.8 ± 31.8	145 ± 70	81.7 ± 30	243 ± 14.1	24.2 ± 18.5	0.96 ± 0.31	10.3 ± 2.8	This study
Kerguelen Petrel *P. brevirostris**	14	3	A	21.3 ± 10.7	19.6 ± 4.6	146 ± 43	180 ± 24	49.9 ± 13.6	180 ± 56		15.3 ± 5				Muirhead & Furness 1988
Atlantic Petrel *P. incerta**	13	3	A	16.3 ± 8	24.4 ± 5.6	150 ± 29	248 ± 24	63.3 ± 33.3	244 ± 80		93.2 ± 36.7				Muirhead & Fumess 1988
Soft-Plumage Petrel *P. mollis**	18	3	A	17.3 ± 6.1	23.6 ± 4.4	143 ± 26	200 ± 44	49.9 ± 28	192 ± 60		69.9 ± 76.6				Muirhead & Furness 1988
Great-Winged Petrel *P. microptera*	1	2/3	A	12.9	15.2	247	141	39.8	130		21.3	28.3			Stewart *et al.* 1999
White-Chinned Petrel *Procellaria aequinoctialis*	3	4	A						47.7 ± 32.7		34.5 ± 10.9				Kim *et al.* 1996
Grey Petrel *Procellaria cinerea*	5	4	A						100 ± 55		110 ± 109				Kim *et al.* 1996
Audubon's Shearwater *P. bailloni*	38	1	J	11.1 ± 3.2	8.55 ± 4.72	209 ± 76	110 ± 56	3.29 ± 2 57	12.6 ± 11.5	48.5 ± 13.7	0.26 ± 0.07	0.17 ± 0.06	0.07 ± 0.01	11.1 ± 23	This study
Audubon's Shearwater *P. bailloni*	23	1	A	16.5 ± 5.9	15.4 ± 5	288 ± 129	244 ± 58	53 ± 38	147 ± 55	57.3 ± 18.9	1.72 ± 1.32	1.16 ± 0.51	0.25 ± 0.04	13.4 ± 3.9	This study
Greater Shearwear *P. gravis**	12	3	A	19.6 ± 3.3	24.4 ± 7.8	126 ± 12	184 ± 32	49.9 ± 19.3	296 ± 80		6.66 ± 5.53				Muirhead & Furness 1988
Little Shearwater *P. assimilis**	13	3	A	29.3 ± 8.4	23.2 ± 21.9	133 ± 23	200 ± 36	46.6 ± 21.3	172 ± 60		3.99 ± 1.03				Muirhead & Furness 1988
Sooty-Shearwater *P. griseus*	7	2/3	A	16.7 ± 3.8	19 ± 2	91.3 ± 17.3	144 ± 26	28.2 ± 15.3	151 ± 65		2.5 ± 1.8	1.7 ± 1.1			Stewart *et al.* 1999
Cory's Shearwater *Calonectris diomedea*	17	3	A	20.6 ± 10	37.6 ± 6.5	156 ± 33	155 ± 21	23.4 ± 9.4	111 ± 36						Stewart & Furness 1996
Cory's Shearwater	35	2/3	J	13.3 ± 7.4	12.6 ± 3.5	176 ± 48.6	115 ± 24.4	2.03 ± 2.78	9.31 ± 10.1						Stewart & Furness 1996

Table 2. Continued

Species	*n*	Area	Age class	[Cu] liver	[Cu] kidney	[Zn] liver	[Zn] kidney	[Cd] liver	[Cd] kidney	[Se] liver	[Hg] liver	[Hg] kidney	[Hg] feathers	[Mn] liver	References
C. diomedea															
Wedge-Tailed Shearwater *P. pacificus*	20	1	A										3.85 ± 0.35		Burger & Schreiber 1992
Wedge-Tailed Shearwater *P. pacificus*	12	1	J										1.91 ± 0.20		Burger & Schreiber 1992
Christmas Shearwater *P. nativitatis*	2	2	A										0.34 ± 0.01		
White-Tailed Tropicbird *P. lepturus*	17	1	J	31.5 ± 13.5	18 ± 6	528 ± 198	193 ± 50	4.53 ± 3.06	19.5 ± 32.5	43.7 ± 18.4	1.26 ± 2.47	0.86 ± 1.29	0.29 ± 0.02	27.9 ± 10.8	This study
White-Tailed Tropicbird *P. lepturus*	32	1	A	29.3 ± 22.6	24.2 ± 28.5	305 ± 190	241 ± 101	47 ± 28	117 ± 58	685 ± 23.2	1.89 ± 1.23	1.88 ± 1.12	0.84 ± 0.10	17.9 ± 7.7	This study
Red-Tailed Tropicbird *P. rubicauda*	23	2	A										6.41 ± 0.53		Burger & Gochfeld 2000

squid-eating Great-Winged Petrel *(P. macroptera)* from New Zealand. Metal burdens in Audubon's Shearwaters are similar to those found in the piscivorous Cory's Shearwaters from the Azores islands (Stewart *et al.* 1997). Although seabirds feeding on similar prey tend to show closer metal burdens than species whose diets are different, the geographic origin of the bird is important. For example, the Atlantic Petrel *(P. incerta)* from Gough Island is close to the Great-Winged Petrel in size and is also a squid-eater. However, its hepatic Hg (respectively renal Cd) levels are almost 4 (respectively, 2) times higher than those found in the Great-Winged Petrel and Barau's Petrel. These comparisons suggest the existence of large differences in elemental levels even among nonindustialized, isolated oceanic islands. Besides, these values remain far below levels that can be attained in polluted areas, such as the Saint Lawrence Gulf, where small, 50-*g*, plankton-eating Leaches Storm Petrels accumulate 3 to 4 times more renal Cd (183 ± 65 $\mu g.g^{-1}$) than the Reunion birds, which are larger and feed at a higher trophic level.

Conclusion

Our study has revealed some similarities and differences that exist among Barau's Petrel, Audubon's Shearwater, and White-Tailed Tropicbird in terms of intake, pathways of metabolism, and storage of certain elements among age-classes. Diet stood out as a major factor of element level variation. Among prey, cephalopods appeared to contribute importantly to the Cd and Hg intake of Reunion marine birds. The study of the excretion and storage of Cd, Cu, Fe, Hg, Mn, Se, and Zn in these three seabirds would be most valuable to understand the detoxification strategies employed by these birds. Indeed, Reunion seabirds bioaccumulated fairly high levels of Cd, Hg, Se, and Zn, especially considering the remoteness of Reunion Island with respect to anthropogenic activity. However, for each element, similar or higher levels have been observed in parent species originating from other pristine areas. In this context, two scenarios can be considered: the levels of trace elements measured during this study correspond (1) to backround levels whose sources are natural (volcanism. erosion, *etc.*) or (2) to the echo of distant pollution that has reached this part of the Western Indian Ocean by aereal and/or marine currents or indirectly though the migration of prey species from polluted zones. Physiologic studies of the Reunion seabird species would be useful to distinguish healthy birds from those in bad physiologic condition in relation to the BC index (Wenzel and Adelung 1996) to better apprehend the influence of the bird's physiologic condition on elemental levels.

Acknowledgments. This research was supported by the *Conseil Général de La Réunion.* J. K. also benefited from support of the *Conseil Régional de La Réunion* and the *European Social Fund* through a doctoral grant. The authors would like to thank E. Robert, M. Rouquette, P. Grondin, and N. Ghanem in La Réunion for their help with tissue preparation and C. Churlaud, *Centre Commun d'Analyse* (La Rochelle), for her assistance with the analyses on the ICP-AES. We would also like to thank the numerous volunteers who participate in the SEOR Petrel rescue program. We are also grateful toward the referees for helpful comments on the manuscript.

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