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Trace elements in tissues of white-chinned-petrels (*Procellaria aequinoctialis*)
from Kerguelen waters, Southern Indian Ocean

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Abstract:

The use of seabirds to assess marine contamination by trace elements in areas remote from pollutant emission points has already been done at various latitudes. Nevertheless, little information is available concerning the Southern Indian Ocean. Determining the contaminants levels there appears necessary not only due to several deleterious effects reported in literature, but also as previous studies have highlighted elevated concentrations of cadmium (Cd) and mercury (Hg) in mollusks, crustaceans and fish. Within this context, the white-chinned-petrel appears as a key species due to its lifespan, diet and trophic position. Thirty three accidentally killed (collision with lights/bycatch in longline vessels) individuals collected in Kerguelen waters were analysed for Cd, copper (Cu), Hg, selenium (Se) and zinc (Zn) in liver, kidney, pectoral muscle, feathers and for mature males, testis. Elevated Hg concentrations (average: $58.4 \mu\text{g g}^{-1}$ dw in liver) are likely due to the presence of mesopelagic prey in the diet of *P. aequinoctialis*. Cd concentrations (average of $65.7 \mu\text{g g}^{-1}$ dw in kidney) can be attributed to a high level of fisheries offal consumption, as well as crustacean and squid ingestion. Correlation of Hg with Se indicates its detoxification by co-precipitation, and correlation of Cd with Zn suggests its displacement by Cd on metallothioneins binding sites. This work also indirectly confirms ecological data (range and diet composition) from the wintering period of the species, which is rather scarce. Seasonal diet change and moulting accounted more for the obtained results than sex of the birds.

Keywords: Heavy metals, seabirds, Procellariiformes, Southern Ocean, subantarctic islands

1. INTRODUCTION

Contamination of the marine environment is a problem of concern at the worldwide scale. Oceanic ecosystems are often considered less impacted than coastal ones because they are not directly submitted to river influxes, runoff, and point-source pollution from urban, industrial and agricultural emissions (Mailman, 1980). Nonetheless, many of these contaminants, e.g. persistent organic pollutants or mercury (Hg) are transported by the atmosphere all over the world and they deposit into oceanic environments (Fitzgerald and Engstrom, 1998). In the aquatic environment, contaminants such as trace elements are more mobile than in terrestrial ones (Selin, 2009), and species foraging in these environments could, therefore, be considered more vulnerable.

Trace elements encompass essential elements involved in physiological and biochemical processes (Abdulla and Chmielnicka, 1990) that can however be toxic when exposure and assimilation are excessive. Others, such as cadmium (Cd) and Hg, are non-essential elements that are potentially toxic even at relatively low concentrations. Exposure of marine organisms is governed by various factors, including foraging location and trophic position (Anderson et al., 2010). Among trace elements, Cd and Hg are known to bioaccumulate in food webs, resulting to high concentrations in long-lived top predator species such as marine birds. This process is particularly exacerbated at high latitudes (Dietz et al., 1998; Elliott and Scheuhammer, 1997), where the baseline concentrations are probably higher than at more temperate latitudes (Bocher et al., 2003). Food constitutes the main pathway of exposure of seabirds to contaminants (e.g., Ochoa-acuña et al., 2002). Nevertheless, numerous factors such as phylogeny, moult pattern, sex, life span and diet composition are likely to influence trace element bioaccumulation in seabird tissues, with Cd and Hg concentrations showing the largest variation among seabird species (Stewart et al., 1999; Walsh, 1990). Because seabirds are long-lived species situated at the top of the food

web, they are also submitted to the process of biomagnification of contaminants (Furness and Camphuysen, 1997). For these reasons, marine birds are considered to be useful bioindicators of environmental contamination (Furness and Camphuysen, 1997; Monteiro and Furness, 1995). They seem to be particularly appropriate to survey the health status of marine ecosystems as they generally display lower coefficients of variation of contaminant levels than fish or marine mammals (Gilbertson et al., 1987). Furthermore, they are reflecting large areas integrating contaminants even from remote places that would be otherwise difficult to sample (Stewart et al. 1999; Carravieri et al., 2013). The use of seabirds to monitor marine contamination by trace elements in areas remote from pollutant emission points has already been done at various latitudes (Anderson et al., 2010; Fitzgerald and Engstrom, 1998; Kojadinovic et al., 2007). However, information concerning the Southern Indian Ocean is still scarce. Only one study reported trace element concentrations in liver, kidney and muscle for 5 small petrels, prions and diving-petrels from the Kerguelen Islands, all of them mainly feeding on zooplankton (Bocher et al. 2003). The study highlighted that the diet was a key factor driving the exposure and trace element levels in the tissues of these birds. More recently, Hg concentrations were determined in feathers from a large number of species, revealing a strong influence of the feeding behaviour on Hg concentrations (Blévin et al., 2013; Carravieri et al., 2013). Identification of high levels of Cd and Hg in mollusks, crustaceans and fish from the Kerguelen waters (Bocher et al., 2003; Bustamante et al., 2003, 1998) would result in elevated exposure of seabirds. Moreover, several deleterious effects of such elements have already been reported in birds, for instance: decrease in egg laying, egg hatchability and increase in embryonic mortality; and besides neurotoxicity, there is also evidence for immunotoxic effects, such as decreased immune response and histological changes (Scheuhammer et al., 2007; Goutte et al. in press) and several endocrine effects as well (e.g. Tan et al., 2009; Tartu et al. 2013). In this context, the monitoring of the

contaminants in the Southern Indian Ocean appears therefore a matter of importance.

Procellaria aequinoctialis, even though one of the most abundant seabirds in the Southern Ocean is classified as Vulnerable in the IUCN red list since literature presents historical declines of populations within the Southern Ocean, namely at Crozet (Barbraud et al. 2008) and South Georgia (Berrow et al. 2000), but not conclusively in Kerguelen Islands (Barbraud et al. 2009). Very high rates of incidental mortality in longline fisheries have been recorded in recent years (Birdlife International, 2013). The probability that these circumstances will continue and its susceptibility to predation and the degradation of breeding habitat indicate that a rapid and on-going population decline is likely (Birdlife International, 2013). Specifically for the Kerguelen waters, the mortality caused by fisheries constitutes a serious threat for *P. aequinoctialis* at least at the regional scale of the Southern Indian Ocean (Barbraud et al., 2009). A similar conclusion was taken for the Crozet Islands population, even though estimates of the number of birds killed by the longline fishing vessels remained within the threshold levels (estimated around 8000 individuals per annum in this specific case) after 2003 (Barbraud et al., 2008). Association to commercial fisheries is due whether because *P. aequinoctialis* target the same areas of high productivity than fishermen and/or because fisheries appear to represent an additional potential food source for these opportunistic seabirds in the form of offal and discards of non-target species (Delord et al., 2010). In their wintering grounds, i.e., the eastern boundary upwelling system of the Benguela current (Péron et al., 2010), *P. aequinoctialis* population's from the Kerguelen waters mainly feed on offal, i.e., fish heads and guts scavenged from commercial trawlers (Jackson, 1988). Nevertheless, their diet also includes “natural” pelagic fish prey, crustaceans and cephalopods, in this order. Prey given to chicks during the breeding season is mainly composed of fish, followed by cephalopods and then crustaceans (Delord et al. 2010). In the Crozet Islands (3.2 degrees north from Kerguelen), the diet of *P. aequinoctialis* is somewhat

similar with fish prevailing, especially mesopelagic species (Catard et al., 2000; Connan et al., 2007; Ridoux, 1994). However, crustaceans and cephalopods are important prey as well.

In this study, we examine the levels of Cd, copper (Cu), Hg, selenium (Se), and zinc (Zn) concentrations in the feathers, liver, kidney and muscle tissues (and also testis in a smaller extent) of the white-chinned-petrel *P. aequinoctialis* from Kerguelen waters using only incidentally killed animals. The second objective of this work was to make the relationship between trace element levels in internal tissues, reflecting the risk of toxicity for the birds, and their levels in the feathers that, if related to the internal organs, could provide a wider non-destructive monitoring basis (Agusa et al., 2005).

2. MATERIALS AND METHODS

2.1. Sampling

Sample collection occurred from October/1998 to March/2000 at Kerguelen Archipelago, Southern Indian Ocean. Thirty three white-chinned petrels were collected as bycatch of longline fishing vessels or found recently dead on land killed following collision with the Scientific Station lights. Only intact or almost intact birds were considered for this study. The petrels were weighed in the field, immediately frozen and kept at -20°C until analyses. Birds were thawed overnight and dissected for liver, kidney, muscle (pectoral), testis (only on three mature males) and body feather removal, as body feathers (mainly pectoral ones, avoiding brood patch areas) give the best measure of the mercury contamination of a bird (Furness and Camphuysen, 1997). They were also sexed by visual observation of the gonads: 6 individuals could be identified as females and 12 as males.

2.2. Trace element analyses

Analyses were performed as described by Bocher et al. (2003): for Cd, Cu and Zn determination, two aliquots of approximately 200–300 mg of each homogenised dried sample were digested in 5 ml of suprapure 14 N nitric acid at 60°C on a hot plate until the solution was clear. After evaporation, the residues were dissolved in 10 ml of 0.3 N suprapure nitric acid. Trace elements were determined by flame Atomic Absorption Spectrophotometry (AAS) with a Varian spectrophotometer Spectra 250 Plus with a deuterium background correction (high Cd concentrations, Cu and Zn) and by graphite furnace AAS (Hitachi Z-5000) with Zeeman background correction (low Cd concentrations and Se). Feather analyses included a previous cleaning step in order to remove surface lipids and contaminants, by using a 2:1 chloroform:methanol solution followed by two successive methanol rinses. After cleaning, body feathers were oven dried for 48 h at 50 °C.

Mercury analysis was carried out with an Automatic Mercury Analyser spectrophotometer, ALTEC AMA 254, which does not require an acid-digestion of the samples. Aliquots ranging from 10 to 50 mg of dried sample were directly analysed after being inserted in the oven of the apparatus. After drying, the samples were heated under an oxygen atmosphere for 3 min, and the Hg liberated and subsequently amalgamated on an Au-net. The net was then heated to liberate the collected Hg, which was measured by AAS.

Accuracy and reproducibility of the methods were tested using dogfish liver (DOLT-2) and muscle (DORM-2) and lobster hepatopancreas (TORT-2) (National Research Council, Canada) reference standards. Standard and blanks were analysed along with each set of samples and recoveries of the certified values and recoveries of the metals ranged from 92 to 105%. Measurements were also validated by the IAEA Intercalibration Exercise (Coquery et al., 2001). Concentrations are expressed in dry weight in order to compensate eventual moisture loss during freezing and to facilitate comparison between tissues and with other

studies. Blanks were analysed at the beginning of each set of samples and the detection limit of the method was $0.005 \mu\text{g g}^{-1}$ dry mass.

2.3. Statistical analyses.

Tests were performed using Microsoft Excel and Statsoft Statistica. Before analyses, data was checked for normality of distribution and homogeneity of variances using Shapiro-Wilk and Brown-Forsythe tests, respectively. Parametrical (Tukey HSD/ANOVA) and non-parametrical tests (Kruskal-Wallis/ANOVA) followed accordingly. Comparison between sexes was performed by means of Wilcoxon-Mann-Whitney test. Statistically significant results were set at $\alpha=0.05$.

3. RESULTS

Cd, Cu, Hg, Se and Zn concentrations in the tissues of *P. aequinoctialis* are presented in Table 1. No significant correlations (Pearson's product-moment) were detected between tissue concentrations (both in wet weight and dry weight basis) and total body weight of the birds. The results overall showed that liver and kidney are the main sites for bioaccumulation of non essential trace elements, with mean values of $58.4 \pm 33.4 \mu\text{g g}^{-1}$ dw of Hg in the liver and $65.7 \pm 22.2 \mu\text{g g}^{-1}$ dw of Cd in the kidney. In these tissues, Cd and Hg concentrations were one order of magnitude higher than those of muscle and feathers, which were the only tissues that did not present statistically significant difference for both contaminants. In contrast, Cd concentrations in the testis were as high as in the kidney.

Cu presented similar concentrations and ranges in liver, kidney, muscle and testis. Nevertheless, Cu variability was always lower than 30% in the 3 former tissues whereas it

was above 70% in the testis, mostly due to the small sampling number (n=3) that precluded further statistical analyses. Feathers showed the lowest concentrations in average, but with a relatively high variability (coefficient of variation of 43%). Se concentrations were the lowest in the liver and highest in the kidney, with average concentrations of 6.2 ± 2.0 and 16.1 ± 4.8 $\mu\text{g g}^{-1}$ dw, respectively. Nevertheless, the variation of Se concentrations remained always < 35%. For Zn, the concentrations were the lowest in muscles and highest in feathers in which the variability was relatively high (coefficient of variation of 73%). Nevertheless, this difference was statistically significant. Only muscle and liver, and liver and kidney (paired) did not present such differences. The other tissues, liver, kidney and testis, showed similar concentrations that were intermediate between those of muscle and feathers.

Males and females have shown significant contaminant differences in only one case: Cd in muscle was higher in males. All other comparisons were not statistically significant.

Inter and intra-tissue correlations are shown in Table 2. Significant intra-tissue correlations appeared mostly within liver and kidney and to a lesser extent in muscle, whereas no correlation was found in feathers. In liver, Cd was positively correlated to Hg, Se and Zn, Cu was positively correlated to Zn, and Se to Hg. In kidney, Se was positively correlated to Cd, Cu and Zn and Zn was positively correlated to Cd, Cu, and Se. In muscle, only Cu was negatively correlated to Zn and Se positively correlated to Hg.

Almost no significant inter-tissue correlations were detected for Cu and Zn, with only concentrations in the liver being negatively correlated to those in the feathers. Significant inter-tissue correlations appear mostly between the liver and kidney for non essential elements but also for Se. Thus, Cd concentrations in the liver correlated positively with those in the kidney but negatively with those in the feathers. Concerning Hg, concentrations in the liver positively correlated to those in kidney and in muscle. Similar correlations were found for Se, as well as a significant correlation between liver and muscle Se concentrations.

In order to better understand detoxification processes, other correlations were also investigated for non essential elements (Fig. 1 & 2). Figure 1 presents the liver Hg concentrations as a function of kidney Cd concentrations. The relationship between both non essential elements in their main tissues of bioaccumulation was however not significant. Figure 2 presents the molar relationship between Se and Hg. It highlighted a significant increase of the number of atoms of Se with those of Hg in the liver, but not in the kidney.

4. DISCUSSION

Limited information is currently available for trace element concentrations in the tissues of the *P. aequinoctialis* with, to the best of our knowledge, only three studies reported such levels for feathers (Blévin et al. 2013), for soft tissues (Anderson et al., 2010) and for both (Stewart et al., 1999). The concentrations of trace elements in liver and kidney of *P. aequinoctialis* from Kerguelen waters were overall in the same order of magnitude than those reported for *P. aequinoctialis* collected in 1995 and 1996 as bycatch of tuna fisheries around New Zealand (Stewart et al., 1999). Moreover, it presents the same distribution pattern between liver and kidney tissues for all elements (except Se which was not analysed by these authors). However, Hg concentrations in the feathers of adult birds from the present study were obviously higher (4.2 times) than those in chicks from the same location as a result of longer term exposure in adults (Blévin et al. 2013).

In birds from Kerguelen waters, Cu concentrations in feathers were half those of *P. aequinoctialis* collected in Bird Island, South Georgia, during the 2001/02 austral summer (Anderson et al., 2010). In contrast, Zn values from the present study were 1.5 times higher when compared to Bird Island seabirds. Even though trace elements contamination is known to have several deleterious effects in birds testis (Scheuhammer, 1987), data for analyses of such tissue is scarce. Kim et al. (1998) present data with similar distribution and, in a general

way, within the same orders of magnitude as the present work. Elevated Cu variation in this tissue might be linked to body condition, as suggested by Malinga et al. (2010). These authors also suggested that the variation of Cu concentrations could be due to different stages of development of the gonads, which was visually confirmed in the present study. More generally, sex might play a role in trace element distribution in some seabird species (Kim et al., 1998). Sexual size dimorphism or different age composition in each gender (see Bugoni and Furness, 2009) could affect contaminant levels, but the main driving sex-related factor is the excretion of potentially toxic materials into the egg that reduce the contaminant burdens of breeding females (Stewart et al., 1999). Combined to the different turnover rates of the different tissues, it is most likely that the significantly higher Cd muscle concentrations in males than in females of white-chinned petrels from the Kerguelen waters could be explained by the muscle turnover possibly matching the egg laying period, since it is long known that this tissue has a slower turnover rate than liver (Hobson & Clark, 1992). Moreover, no dilution with growth or emaciation effect was detected, as previously stated. So, further discussion considers the whole grouped dataset, with no sex separation.

Influence of trophic ecology on trace element concentrations

Stewart et al. (1999) classified procellariiform seabirds in function of their pattern of accumulation of Hg in the liver and Cd in the kidney. White-chinned petrels from Kerguelen fall into the same category as *Procellaria* petrels (including the white-chinned petrel) from New Zealand that are characterized by moderately high liver Hg and high kidney Cd concentrations. No significant relationship between liver Hg and kidney Cd was found, which can be attributed to the distinctive biochemistry of the two metals (Stewart et al. 1999). Elevated Hg concentrations in the tissues of *P. aequinoctialis* are likely due to the presence of mesopelagic prey in its diet that is mainly composed of fish and cephalopods in Kerguelen

waters (66 and 17% by mass, respectively; Delord et al., 2010). In marine predators, trophic position is not always the main factor in determining Hg concentrations, because mesopelagic prey was shown to increase disproportionately their Hg burdens (Ochoa-acuña et al., 2002; Thompson et al., 1998). Indeed, mesopelagic fish have elevated Hg concentrations as the result of enhanced Hg methylation in deep waters (Chouvelon et al., 2012; Monteiro et al., 1996). Cephalopods can also be considered as an important predator Hg source since they mainly contain Hg under organic forms, which is highly bioavailable for the upper trophic levels (Bustamante et al., 2006). Long-term Hg exposure of *P. aequinoctalis* population from Kerguelen waters is also influenced by the species feeding habits outside the breeding period. For instance, *P. aequinoctalis* winters in the Benguela current, with associated dietary changes (Jackson, 1988). In this area, fish is still the main prey class (73% by mass), but it includes an important proportion (36%) of offal, mainly fish heads and guts discarded by fishing vessels that keep aboard the commercially interesting part, i.e. the muscle. Muscle is one of the main tissues in fish with the lowest concentrations of trace elements (except Hg and arsenic; Bustamante et al., 2003; Kojadinovic et al., 2007; Metian et al., 2013). Because trace element concentrations in muscle are below concentrations in the whole fish, the consumption of offal could represent a higher intake of trace elements, especially Cd. In Kerguelen fish, liver contains up to 87% of the whole Cd body burden with concentrations $>10 \mu\text{g g}^{-1} \text{ dw}$ (Bustamante et al. 2003). Therefore, interactions with fisheries both during and outside the breeding season would lead to an increase intake of Cd in addition to Cd contamination resulting from squid consumption (Bustamante et al., 1998a; Muirhead and Furness, 1988). Indeed, Cd levels in *P.aequinoctalis* tissues were in the same range as those of species with a higher proportion of cephalopods in their diets, such as albatrosses. For example, the Laysan albatross (*Phoebastria immutabilis*) presents average renal Cd levels of $59.7 \mu\text{g g}^{-1} \text{ dw}$ (Honda et al., 1990) and Southern Ocean albatrosses (*Diomedea exulans*, *D.*

epomorpha, *D. cauta*, *D. bulleri* and *D. melanophris*) show renal Cd levels ranging from 74 to 132 $\mu\text{g g}^{-1}$ dw (Stewart et al. 1999). Crustaceans are also likely to contribute to Cd exposure in *P. aequinoctalis*. For instance, the amphipods *Themisto* spp. (notably *T. gaudichaudii*) is naturally enriched in Cd (Ritterhoff and Zauke, 1997; Bocher et al., 2003) and may represent a significant source of this element *via* both direct consumption (Catard et al., 2000; Delord et al., 2010) and secondary ingestion, since this crustacean contributes significantly to the diet of several petrel prey (Anderson et al., 2010). Assuming that offal have a Cd concentration of 10, “natural prey” fish of 0.1, cephalopods of 25, *Themisto* spp. of 40 and other crustaceans of 1 $\mu\text{g g}^{-1}$ dw, an estimation of prey contribution in the Benguela waters suggest that cephalopods, offal, crustaceans and natural fish prey would be responsible for approximately 51.8%, 43.5%, 1.2% and 0.4% of the total Cd intake, respectively. Therefore, the hypothesis of offals influencing greatly the birds’ Cd levels is likely to be verified. Such calculations were made from diet analysis by mass in the Benguela Region (Jackson, 1988) together with available Cd concentrations of prey from the southern Ocean (Bocher et al., 2003; Bustamante et al., 2003; Ritterhoff & Zauke, 1997).

Diet is the main pathway of incorporation of non-essential but also essential elements in seabirds. Studies on trace elements in prey showed generally higher values for Cu and Zn in cephalopods when compared to mesopelagic fish (Anderson et al., 2010; Bocher et al., 2003; Bustamante et al., 2006a, 2003). Moreover, the antagonism between Cd, Cu and Zn could modify absorption, retention and distribution of the other two (Underwood, 1977). Nevertheless, homeostatic control of internal essential elements plays a major role regulating their absorption in vertebrates, which depends on the individual nutritional requirements (Walsh, 1990). In *P. aequinoctalis* from the present study, the correlations of Cu with Zn would reflect their nutritional status. Thus, low intraspecific variation of Cu and Zn concentrations should be expected in the tissues of marine birds in good health. In *P.*

aequinoctialis from Kerguelen waters, Cu and Zn averages were close to the majority of other Kerguelen species (authors' unpublished data), thus highlighting their homeostatic regulation, which is also confirmed by their comparatively low relative standard deviations.

Inter- and intra-tissues relationships between trace elements

Inter- and intra-tissue correlations between elements reflect the different processes of regulation of essential elements and of detoxification of non essential elements.

In the case of intra-tissue analyses, in the liver, Cd positively correlated with Zn, Se and Hg, and Hg positively correlated with Se, which is consistent with previous data for procellariiforms seabirds such as the black-footed albatross *Diomedea nigripes* from Ryukyu Islands (Kim et al., 1998). In regard to Cd and Zn co-accumulation, their correlation is likely to reflect Cd detoxification by metallothioneins (MTs) as already reported for different seabird groups (e.g., Kojadinovic et al., 2007; Ninomiya et al., 2004). Indeed, the great affinity of Cd for MTs leads to the competition with Zn for binding sites and to a reduction of the toxic effects of Cd (Klaassen et al., 1999), whereas the Zn displacement would induce MT synthesis, increasing the number of binding sites within the cells. In regard to the co-accumulation of Hg with Se, their correlation (the highest coefficient found in the whole study) is indicative of Hg detoxification mechanism involving the biomineralization and storage of non-toxic Se-Hg complex and formation of liver granules when elimination through the feathers cannot match the individual's dietary Hg intake (Nigro and Leonzio, 1996). Such a process seems to be operative in *P. aequinoctialis* from Kerguelen waters in the liver but not in the kidney (Figure 2).

Moreover, in the kidney, correlation of Se with Zn could be associated with their interaction in detoxifying processes, even though according to Norheim (1987) Se plays a more important role. Se correlation with Cu could be inferred from the consumption of some

crustacean prey, notably *E. superba* and *T. gaudichaudii* (see Anderson et al., 2010), since the physico-chemical form in which Se is sequestered by different prey may vary and affect its subsequent bioavailability. Hence, it would appear that intra-specific variation in blood Se concentrations in seabirds most likely reflects individual differences in prey selection (see standard deviation in diet composition in Delord et al., 2010).

Besides Hg, Se is involved in detoxification of other heavy metals such as Ag, Cd, Cu and Zn (Ikemoto et al., 2004). In regard to inter-tissue analyses, Se significant positive correlations between liver, muscle and kidney are probably linked to the detoxifying mechanisms it is involved in, which is corroborated by its significant positive correlation with other elements, notably Hg in liver and muscle tissues. Hg significant positive correlation in liver with muscle tissue was already reported for several bird species (e.g. Borgå et al., 2006) and is assumed to result from the bioaccumulation of this element.

The significant positive correlations of Cd and Hg in both the liver and kidney confirm the role of these tissues as the main sites for detoxification and long term storage (e.g. Bocher et al., 2003). The negative correlation between Cd concentrations in liver and Cd concentrations in feather could be related to a different diet during the non breeding period (higher offal proportion) when moulting normally occurs (Anderson et al., 2010) and in the breeding period, when samples were collected. Indeed, liver has a high protein turnover rate (Cherel et al., 1991) that reflects diet a few days before collection. The Zn negative correlation between liver and feather could result from the same processes, which is confirmed by its significant correlation with Cd in the liver. This shift towards liver co-accumulation can happen in periods as short as one or two weeks, as demonstrated by Levengood and Skowron (2007). In other words, Cd and Zn are co-accumulated in the liver (emphasised by the significant intra-tissue correlation) and before being released from the liver to the feathers (emphasised by the negative inter-tissue correlation between liver and

feathers). Even though, Zn in feathers presented large variation. This could be due to either molecular interactions between Cd, Cu, and Zn or to Zn affinity for liver metallothioneins (Bocher et al, 2003).

Finally, in regard to the use of feathers as a non destructive monitoring tool, it is noticeable that they presented significant inter-tissue correlation in only two occasions: Zn and Cd, both with liver and both negative as well. No significant correlation to other trace elements was found for none of the tissues, possibly due to two main factors: firstly, the sampling of the birds occurred in different times of the year and not only that, but consequently in different time shifts in regard to the moult. This might result in a degree of fluctuation in the trace element concentration of the internal tissues and, therefore, the static concentration of the feathers may no longer be correlated to those. The second hypothesised factor is that moult occurs normally during the non breeding period (Anderson et al., 2010), when diet is different (Jackson, 1988), therefore the variation in internal tissues could be enhanced due to a variation in exposure *via* diet. A further study with birds sampled in a narrower time interval and with a homogenous set of animals in regard to the last moult could better answer the question whether feathers can be used to monitor trace elements in this species and under what conditions.

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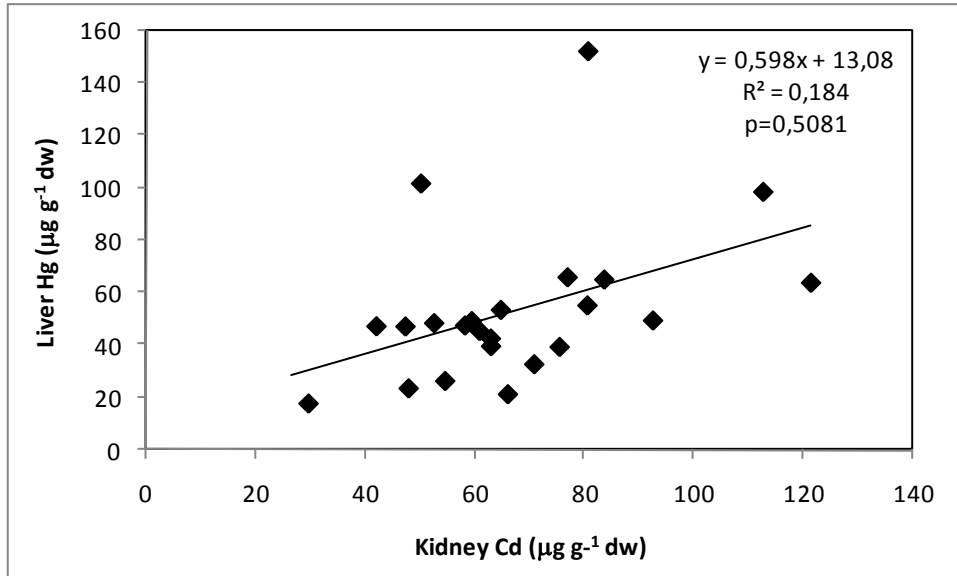


Figure 1 – Concentrations of liver Hg versus kidney Cd ($\mu\text{g}\cdot\text{g}^{-1}$ dw) in white-chinned petrels *P. aequinoctialis* from Kerguelen waters.

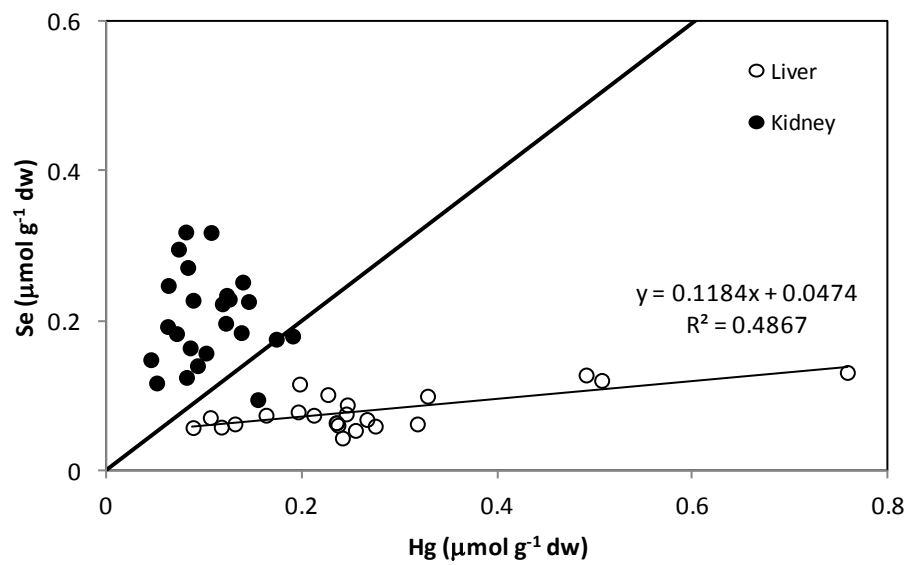


Figure 2 – Se and Hg molar concentrations ($\mu\text{mol g}^{-1} \text{ dw}$) in liver and kidney of white-chinned petrels *P. aequinoctialis* from Kerguelen waters. Bold line indicates the 1:1 molar ratio.

Table 1 – Trace element concentrations (mean \pm SD and range; $\mu\text{g}\cdot\text{g}^{-1}$ dw) in tissues of white-chinned petrels *P. aequinoctialis* from Kerguelen waters. Sampling numbers are given accordingly.

Tissue	n	Cd	n	Cu	n	Hg	n	Se	n	Zn
Kidney	29	65.7 ± 22.2	29	15.6 ± 4.4	27	23.6 ± 15.5	24	16.1 ± 4.8	29	131 ± 23
		26.4 – 121		11.8 – 35.7		8.91 – 91.8		7.5 – 25.2		96 – 200
Liver	26	20.5 ± 6.5	26	16.2 ± 4.6	26	58.4 ± 33.4	23	6.2 ± 2.0	26	127 ± 31
		7.43 – 37.0		10.6 – 31.8		17.6 – 152		3.4 – 10.4		80 – 193
Muscle	32	1.80 ± 0.82	32	12.0 ± 2.2	32	2.86 ± 0.80	31	9.7 ± 3.4	32	80 ± 24
		0.86 – 4.04		8.2 – 15.7		1.28 – 4.54		5.3 – 21.0		54 – 134
Feather	22	0.66 ± 0.39	22	6.0 ± 2.6	22	7.63 ± 3.87	-	-	22	196 ± 143
		0.18 – 1.53		2.8 – 11.8		2.30 – 14.2				100 – 797
Testis	3	42.7 ± 14.4	3	19.2 ± 13.7	-	-	-	-	3	145 ± 15
		30.8 – 58.8		11.3 – 35.0						128 – 156

Table 2 – Correlation (Pearson’s product-moment correlation coefficient) matrix for intra- and inter-tissue comparisons. Significant values at $\alpha=0.05$ are bold and asterisk marked.

	Cd	Cu	Hg	Se	Zn		kidney	liver	muscle	feather
<i>Kidney</i>						<i>Cd</i>				
Cd	1					kidney	1			
Cu	-0.244	1				liver	0.650*	1		
Hg	0.044	-0.070	1			muscle	0.387	0.332	1	
Se	0.413*	0.399*	-0.051	1		feather	-0.100	-0.635*	-0.188	1
Zn	0.382*	0.624*	0.063	0.552*	1	<i>Cu</i>				
<i>Liver</i>						kidney	1			
Cd	1					liver	0.320	1		
Cu	0.184	1				muscle	-0.334	0.480	1	
Hg	0.435*	-0.244	1			feather	0.029	-0.232	0.168	1
Se	0.607*	0.048	0.698*	1		<i>Hg</i>				
Zn	0.533*	0.525*	0.194	0.328	1	kidney	1			
<i>Muscle</i>						liver	0.584*	1		
Cd	1					muscle	0.419	0.579*	1	
Cu	0.236	1				feather	0.143	0.053	-0.119	1
Hg	-0.098	-0.050	1			<i>Se</i>				
Se	0.090	0.244	0.469*	1		kidney	1			
Zn	-0.164	-0.663*	-0.107	-0.225	1	liver	0.519*	1		
<i>Feather</i>						muscle	0.652*	0.728*	1	
Cd	1					feather	-	-	-	1
Cu	0.420	1				<i>Zn</i>				
Hg	-0.286	-0.135	1			kidney	1			
Se	-	-	-	1		liver	0.296	1		
Zn	0.005	-0.038	0.290	-	1	muscle	0.031	-0.259	1	
						feather	-0.316	-0.547*	0.352	1