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Heavy Metals Content in Great Shearwater (*Ardenna Gravis*): Accumulation, Distribution and Biomarkers of Effect in Different Tissues

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Abstract

The purpose of this study was to explore the usefulness of Great Shearwater (*Ardenna gravis*) as a bioindicator for biomonitoring programs for metal pollution. Three different metals were analysed in liver, kidney, and feathers, including cadmium, lead, and zinc. Glutathione-S-transferase, malondialdehyde, reduced glutathione, and catalase were assessed as oxidative stress biomarkers. Sex-related trends in metal accumulation also were evaluated. In liver and kidney, the mean concentrations of Zn (146.1 ± 5.14 and 108 ± 2.70 mg/kg, respectively) and Pb (0.19 ± 0.01 and 0.13 ± 0.01 mg/kg, respectively) in *A. gravis* were generally comparable to values reported in other studies. However, animals presented slightly higher concentrations of Cd (9.67 ± 0.65 in liver and 17.41 ± 0.84 mg/kg in kidney) than those reported in the same species sampled in Southern Atlantic waters. The slightly higher levels of Cd found in this study compared with other studies are probably affected by the location in Northern Atlantic waters (with different diet intake). In feathers, levels of Zn (70.70 ± 1.76 mg/kg) were lower than in other *Ardenna* shearwaters, whereas higher levels were found for Cd (0.16 ± 0.01 mg/kg) and Pb (0.84 ± 0.06 mg/kg). The lack of differences found between males and females could be influenced by the migration status, because both sexes stay in similar physiological conditions, with no laying eggs. Levels found in the feathers of the present study were related to concentrations in internal tissues below those which cause adverse effects in birds. Thus, feathers would appear as a potential noninvasive tool for metals biomonitoring in seabirds, because it is possible to quantify them. Baseline data of oxidative stress levels have been reported, both in liver and kidney, presenting no correlations with the levels of metals in these tissues. The low internal metal levels and the lack of correlations between oxidative stress metrics suggest a low risk of the environmental concentrations for seabirds.

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The use of bioindicators is a well-known way for monitoring the qualitative and quantitative environmental consequences of inorganic elements. Seabirds are among the top predators in the marine ecosystem, making them at risk from the bioaccumulation of toxic chemicals from both natural and anthropogenic sources (Bond and Lavers 2011). For this reason, birds are recognized as a significant tool in environmental management, considering them of high relevance for biomonitoring purposes (Nardiello et al. 2019). Their use will help to identify food chain contaminants, determine levels of environmental contamination and adverse effects on the animals themselves, and finally, assess human health risks (García-Fernández 2014).

Shearwaters (order Procellariiformes) are among the most widespread, abundant seabirds in the world's oceans, being a group of pelagic species that sometimes undertake transequatorial migrations that encompass both the northern and southern Atlantic Oceans (Haman et al. 2013). Their behavior makes these species of high interest for being used as potential sentinel species, acting as good bioindicators of metals in the marine environment.

Pelagic species have higher levels of some heavy metals as Hg and Cd than other inshore species, being in this case pointed out natural sources rather than pollution the cause of their appearance in tissues (Muirhead and Furness 1988). However, atmospheric transport of metals and selective uptake or storage by marine organisms could cause increases in pelagic ecosystems (Bryan 1984). Exposure to metals can harm species, for example, by impairment of biological function of proteins, enzymes, and cell damage. Seabirds are relevant to evaluate bioaccumulation processes, because top predators consume preys containing high levels of environmental contaminants (Champoux et al. 2015), show relatively high resistance to toxic effects, and they can live for many years (Nardiello et al. 2019).

Great Shearwater (*Ardenna gravis,* O'Reilly 1818) leaves the breeding grounds in South Atlantic waters in April–May and migrates N to NW Atlantic. In July–November, the birds fly E and SE towards W Europe and NW Africa waters and then S to South Atlantic (Cramp and Simmons 1977). They often are seen behind fishing boats or where whales are actively feeding. Unfortunately, this behavior makes them vulnerable for entangling and eventually drowning in fishing gear while actively foraging (Haman et al. 2013).

In the present study, the levels of Zn, Cd, and Pb in the kidneys, livers, and feathers of *A. gravis* were determined by means of ICP-MS, considering the possible influence of sex on the element concentrations. Glutathione-S-transferase (GST), malondialdehyde (MDA), reduced glutathione (GSH), and catalase (CAT) also were evaluated, as oxidative stress biomarkers, to assess any potential effect derived from metal accumulation. Moreover, to validate the potential use of feathers as nondestructive samples, the correlation between the element content in internal tissues (liver and kidney) and feathers was evaluated. With those considerations, the final goal of the study was to provide baseline data regarding metal levels in the tissues and feathers of *A. gravis*.

Materials and Methods

Field Procedure and Sampling

In October 2006, 107 *A. gravis* were accidentally caught during their prebreeding, southward migration by a longline fisherman in the Shannon Sea area, west of Ireland (52º 30 'N, 12º 00′ W). The specimens were first frozen in the longline cellars, and then at – 20 °C in freezers at the Faculty of Sciences (University of A Coruña, Galicia) for further study.

Necropsies (external and internal postmortem analyses of birds) were performed at this University in 2007 with defrosted specimens following the standard protocols of Jones et al. (1982), Van Franeker (1983), Camphuysen (1995), and Camphuysen and van Franeker (2007).

At the time of the necropsies, the birds were in good condition, fresh despite remaining 7–10 months frozen, which allowed their detailed study. From a subsample of 46 corpses, a portion of approximately 10 g of liver and kidney tissue was taken, placed individually in plastic bags, and stored at – 20 °C. Similarly, body feathers were pulled out from the lower back (approximately 20 g) and stored in plastic bags. For each individual, different feathers were pooled to limit potential interfeather differences.

During the necropsy, some parameters, such biometrics, physical condition, and state of internal organs were registered for collateral studies. Moreover, birds were sexed during necropsies. The sex and degree of development of the gonads were determined by measuring the width and length of the left testicle and that of the larger ovarian follicle and were subsequently divided (males/females, 29/17). After sampling, the remains were removed hygienically by incineration, under current European legislation.

Reagents

Nitric acid (69%) and hydrogen peroxide (30%) were purchased from TraceSELECT™, Fluka (Seelze, Germany). Multielement Calibration Standard 3 solution was supplied by PerkinElmer Inc. (Shelton, CT). The Institute for Reference Materials and Measurements provides the certified sample of lyophilized bovine liver. Praxair (Madrid, Spain) supplied the collision gas and the argon (both with a purity of 99.999%).

Determination of Metal Levels

A digestion procedure, consisting in a microwave assisted acid, adapted from that reported by Fromant et al. (2016) and Morton et al. (2017), was followed to obtain the metal content. Two grams of each sample were weighed into Teflon PTFE flasks and 6 mL of a freshly prepared mixture (3:1, v/v) of concentrated HNO_3 (69%) and H_2O_2 (30%) were added. The flasks were maintained closed for 12 h at room temperature as a previous step of predigestion. Then, vessels were sealed and microwave-digested (following a 15-min ramp of temperature up to 180 °C, and maintained for 5 min). Finally, the flasks were cooled down to room temperature, and the resulted sample was diluted to 10 mL with deionized water. A flask without sample was digested following the same protocol and used as a blank. All sample solutions were clear. A second set of identical samples from the same individuals was dried at 80 °C until constant weight in order to calculate the percentage of humidity in each sample (average humidity of 74.02%, 68.22%, and 20.85% in kidney, liver, and feathers, respectively) to avoid losses of volatile elements. A standard reference material (BCR® certified reference materials—ref. 185R, Community Bureau of Reference, EU) was used to test the accuracy of the microwave digestion method. Four replicates were done on NIST SRM 1577b Bovine liver to check the accuracy; the results were in agreement with the certified material. Feathers were washed before analytical determination to remove external contamination from their surface: subsequently using tap water, distilled water, Milli-Q water, and acetone (Jaspers et al. 2004).

A platform collision cell inductively coupled plasma mass spectrometer ICP-MS 7900 equipped with an integrated autosampler (Agilent Tech) was used for element detection (Table 1). For an optimal nebulization of the sample, a Peltier-cooled (2 °C) cyclonic chamber (Elemental Scientific, Omaha, NE) and a low-flow (0.25 mL/min) Meinhard concentric nebulizer (LGC, London, UK) was employed.

The ICP-MS was daily calibrated to obtain the highest values of intensity indicated by the ratios CeO/Ce < 2.5%, Ce^{++}/Ce < 3% and background (220) < 1 cps. The instrumental detection limits were 0.005 mg/kg for all the elements. Calibrating solutions were freshly prepared from a 10-mg/L Multi-element Calibration Standard 3 solution (PerkinElmer, Inc., Shelton, CT). The quality of the analytical procedure was checked with the same certified sample of lyophilized bovine liver previously indicated. The values obtained for these elements were consistent with certified reference values, and the recovery yields varied between 89% for Cd and 96% for Zn. Limit of detection (LOD) and of quantification (LOQ) were determined according to the ICH-Q2 guideline on method validation (Singh 2015), after analysing repeated blanks with the same procedure used for the samples and determining the standard deviation. The final values of both parameters were calculated taking into account the samples dilution factor and the weight and were in all cases lower than 0.003 and 0.009 mg/kg for LOD and LOQ, respectively. The coefficients of variation for replicate samples ($n = 5$) were determined to be lower than 5.3%. All samples were run in batches that included analytical blanks.

Metal concentrations are expressed as mg/kg dry weight (dw), because dry values are considered to be more reliable and consistent compared to wet weight values (ww) (Adrian and Stevens 1979).

Biomarkers Analyses

The enzymatic GST activity was measured at 340 nm according to Habig et al. (1974) using CDNB as a substrate. MDA as a marker for LPO was measured at 532 nm by using 2-thiobarbituric acid (Recknagel et al. 1982). GSH levels were measured by using the Hissin and Hilf fluoromethric method (1976) at excitation of 350 nm and emission of 425 nm. Catalase activity (CAT) assay, consisting of the spectro-photometric measurement of 10 mM H_2O_2 breakdown at 240 nm was evaluated according to the method of Clairborne (1985). Specific activities are related to mg protein, which was estimated by the Bradford method (Bradford 1976) protocol, using bovine serum albumin as standard.

Statistical Analysis

Data were analyzed using statistical software Prism 6 (version 6.02) (GraphPad Software Inc., La Jolla, CA). The concentrations of metals were presented as mean values ± SEM, median and range. Shapiro–Wilk normality test was performed to determine whether the data were normally distributed. Due to the nonnormal distribution of the data, a nonparametric Kruskal–Wallis test was used to determine the influence of the considered tissue on the metal concentrations. Similarly, a Mann–Whitney *U* test was used to assess the influence of sex and age. Moreover, a Spearman test was performed to determine the correlations among metal levels. The level of statistical significance was set as $p < 0.05$.

Results and Discussion

The main statistical parameters corresponding to the Zn, Cd, and Pb levels found in the organs (liver and kidney) and feathers of Great Shearwater are shown in Table 2. Not only the means ± standard errors are shown but also the ranges and coefficients of variation (CV), as well as the number of samples below the level of detection. In this sense, while Zn was detected in all the samples, Cd was not detected in 4.3% (2/46) of the feather samples. Similarly, Pb was under the limit of detection in 6.5%

Table 1 Operation conditions for the ICP-MS

Potency RF (W)	1550
Plasma mode	General purpose
Omega bias (V)	−120
Omega lens (V)	9.3
Extract 2 (V)	−245
Deflect lens (V)	1.0
Energy discrimination (V)	5
Collision gas (mL/min)	5
Cell entrance (V)	−40
Cell exit (V)	−60

Table 2 Main statistical parameters corresponding to Zn, Cd, and Pb concentrations, expressed in mg/kg of dry weight in *Ardenna gravis* ($n = 46$) liver, kidney, and feathers

Sample	Element	Median ± SEM	Range (CV)	n < LOD
Liver	Zn	146.1 ± 5.140	76.90–196.6 (24.22)	0
	Cd	9.670 ± 0.647	1.650–16.37 (47.44)	0
	Pb	0.191 ± 0.011	< LOD–0.300 (42.64)	3
Kidney	Zn	108.0 ± 2.703	62.39–124.4 (18.16)	0
	Cd	17.41 ± 0.844	5.180–25.06 (35.55)	0
	Pb	0.131 ± 0.009	< LOD—0.239 (45.61)	9
Feathers	Zn	70.70 ± 1.758	49.63–92.24 (17.06)	0
	Cd	0.158 ± 0.009	< LOD–0.270 (41.16)	2
	Pb	0.844 ± 0.058	0.063–1.575 (62.84)	0

SEM standard error of the mean, *CV* coefficient of variation (%), *LOD* limit of detection

(3/46) and 19.6% (9/46) of the liver and kidney samples, respectively.

The highest concentration of Zn was found in the liver, whereas for Cd was found in the kidney, and feathers presented the highest levels of Pb. Pooling all samples, Zn presented the highest values in all tissues.

Zn is an essential element, being necessary for the metabolism. Zinc acts as a cofactor of 200 enzymes, having an important role in the antioxidant defense system, ameliorating the effects of environmental stress (Sahin et al. 2005). However, excessive concentrations of this metal in the organism can cause adverse effects, as it happens with other essential metals. The threshold level of Zn toxicosis in birds was established in 1,200 mg/kg (Gasaway and Buss 1972), and poisoning has been described in birds when levels of this metal exceed 2,100 mg/kg in the liver and kidney (Eisler 1993). It has been described that zinc toxicosis can affect the renal, hepatic, and the hematopoietic tissues, although the exact toxic mechanisms of zinc is not known (Richarson 2006). In the present study, Zn levels were at least 10 times below the level associated with Zn toxicosis. Concentrations of Zn were higher in the liver (146.1 mg/kg) and kidney (108 mg/kg) than in feathers (70.7 mg/kg), denoting this metal a tendency to accumulate in internal organs. The concentrations of Zn agree with those found in other close related seabird species, as Short-tailed Shearwater (*Ardenna tenuirostris*) from the Bering Sea (105.2 ± 32.8 mg/kg in liver and 103.4 ± 9.5 mg/kg kidney) (Ishii et al. 2017). Zn levels in feathers (70.7 ± 1.76 mg/kg) were found lower than those reported by Bond and Lavers (2011) in Flesh-footed Shearwater (*Ardenna carneipes*) from Eastern Australia (96.1 ± 4.91 mg/kg). To our knowledge, there were not more studies performed to assess metal content in feathers of *Ardenna* spp.

The distribution of Cd in liver and kidney followed the usual pattern, being higher in the first organ, as shown in a recent review work (Vizuete et al. 2019). It has been already reported that high levels of Cd in kidney, respect to liver, are indicative of chronic exposure to low concentrations of this metal (Scheuhammer 1987). A high Cd accumulation in kidney could demonstrate the role of this organ in the detoxification process and storage of nonessential elements (Lucia et al. 2008). According to Wu et al. (2001), an exposure to Cd derives in skeletal demineralization, due to the consequent increase of calcium excretion, thus leading to increases in bone fragility and risk of fractures. Background levels of Cd have been reported at < 3 and < 8 mg/kg dw in liver and kidney respectively, which might indicate increased environmental exposure. Also, the threshold level of Cd has been considered above 3 mg/kg (dw) in the liver of birds (Scheuhammer 1987; Burgat 1990). The levels of Cd found in the present study in liver (9.67 mg/kg) and kidney (17.41 mg/kg) are higher than this threshold, which may indicate a strong exposure to this metal of *A. gravis*. Barbieri et al. (2007) showed lower heavy metal levels (Cd and Pb) in *A. gravis* that breed on South Atlantic Ocean islands and migrate off the Brazilian coast in their post-breeding migration (June-July) reporting that this region presumably has negligible or nonexistent local heavy metal pollution. These authors found Cd levels of 7.8 mg/kg in liver and 13.31 mg/kg in kidney, being slightly higher in individuals captured in their North Atlantic feeding area (present work). Other species from Northern latitudes presented levels of Cd in the same range of concentrations, such as *A. tenuirostris* from the Bering Sea (6.06 ± 4.06 mg/kg in liver and 27.4 ± 13.4 mg/kg in kidney) (Ishii et al. 2017). Regarding feathers, Cd levels (0.16 ± 0.01 mg/kg) also were higher than those found in *A. carneipes* from Eastern Australia (0.065 ± 0.008 mg/kg) (Bond and Lavers 2011).

Regarding Pb, in the present study, levels observed in liver where higher than those found in kidney, in contrast to Cd. Only a few studies on Pb levels in seabirds as gulls have been published, revealing that Pb values are higher in the kidney compared with the liver and feathers (Vizuete et al. 2019). The normal background of Pb in seabirds has been considered ranging from 0.5 to 5.0 mg/kg dw in the liver and from 1.0 to 10.0 mg/kg dw in the kidney (Scheuhammer 1987; Kehrig et al. 2015). Similarly, the threshold level of toxicity for this metal in feathers has been established in 4 mg/kg dw (Burger and Gochfeld 2000). The concentrations found in *A. gravis* from the present study for Pb in the liver, kidney, and feathers can be considered low (0.19, 0.13, and 0.84 mg/kg, respectively), with no toxicological relevance. Kim and Oh (2014) described a negative effect in behavior and growth of gull chicks where liver Pb levels of

6.2 mg/kg were detected. These effects were not expected in the sampled *A. gravis*, because Pb levels are far below this threshold value. Contrary to the issue reported for Cd, there were not differences when the present results of Pb levels were compared with those found in the same species from the South Atlantic (0.18 mg/kg in liver and kidney) (Barbieri et al. 2007) or close-related species as Manx Shearwater (*Puffinus puffinus*) from Brazil (0.1 mg/kg in liver) (Cardoso et al. 2014). Relatively high Pb deposition fluxes are encountered in areas of the northern hemisphere close to anthropogenic sources (Burger and Gochfeld 2000). However, the present work shows low Pb levels that can be explained in a pelagic species, such like *A. gravis*, because it lives in medium waters or near the surface, limiting its contact with the seabed or the coast as much as possible.

In feathers, the levels of Pb were the highest of the analyzed elements. These levels (0.84 ± 0.06 mg/kg) agree with those found in other seabird species, such as Yellow-legged Gull, *Larus michahellis* (0.83 ± 0.37 mg/kg) (Otero et al. 2018) or Northern Gannet, *Morus bassanus* (0.40 ± 0.05 mg/kg) (Nardiello et al. 2019). Within other *Ardenna* species, lower levels were found in *A. carneipes* (0.42 ± 0.05 mg/kg) (Bond and Lavers 2011). *A. gravis* arrive in Greenland and Iceland in July–August, being there where they are finishing the molting (Ginn and Melville 1983). According to the above, by mid-October the adult *Ardenna gravis* would already have all the complete molt both in the remiges flight feathers (primary and secondary) and the rectrices. However, although unlikely, 26.2% of the 107 sampled birds had active molt, demonstrating that the reported molting period would be dilated, at least according to the rectrice feathers. Nevertheless, the high amount of already finished molting birds demonstrates that the metal levels found in feathers should come from recent acquisition, being related to environmental levels appeared in the living area.

Normally, the studies developed with seabirds show that levels of Pb are higher than Cd levels in feathers, as shown by Agusa et al. (2005) in Black-tailed Gulls (*Larus crassirostris*) from Japan, Mansouri et al. (2012) in Heuglin's or Siberian Gull (*Larus heuglini*) from Iran, or Nardiello et al. (2019) in *M. bassanus* from Spain. However, Franklin's Gulls (*Leucophaeus pipixcan*) of North America presented higher concentrations of Cd than Pb in their feathers (Burger 1996). This difference can be associated to the specific pollution appeared in the living area. The west coast of Ireland presents several fishery ports, which bouts can release substances to the water. These substances can derive from petrol and antifouling paintings, allowing their exposure to seabirds, which can present accumulation of elements like the reported in the present study.

Following the breeding season, adults and juveniles migrate to the Northern Hemisphere to feed primarily on fish, krill, and squid in the pelagic North Atlantic Ocean

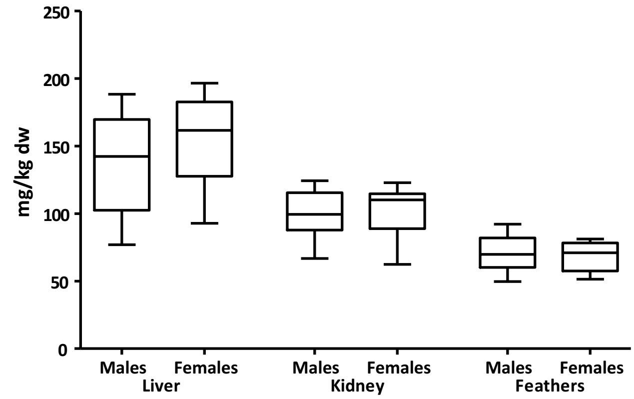

Fig. 1 Zn levels (mg/kg of dry weight) in liver, kidney and feathers of *Ardenna gravis*, according to sex (males/females, 29/17). Box plots represent median values and 25 to 75% percentile ranges

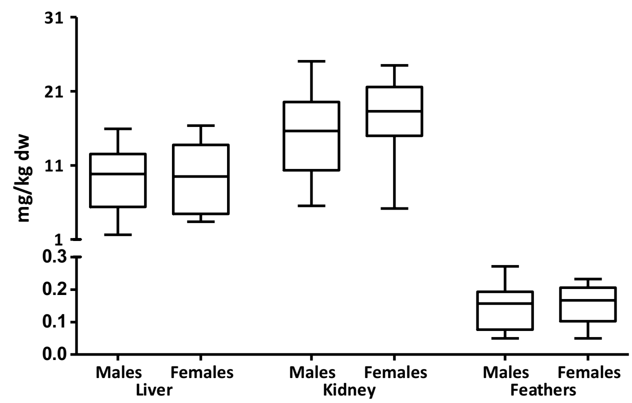

Fig. 2 Cd levels (mg/kg of dry weight) in liver, kidney and feathers of *Ardenna gravis*, according to gender (males/females, 29/17). Box plots represent median values and 25 to 75% percentile ranges

during the boreal summer (Haman et al 2013). Food may be a source of heavy metals and an explanation for the high levels found in the sampled birds. Indeed, it has been reported that the squid *Gonatus fabriccii* appears to be an important transfer of Cd (31.68 mg/kg found) and Zn (137.55 mg/kg found) in the North Atlantic pelagic food web (Lischka et al. 2020).

As shown in Figs. 1, 2, and 3, whereas other aquatic fish species showed difference between males and females, *A. gravis* did not present such differences. Usually, females present lower levels of heavy metals due to the release during the egg-laying period (Burger and Gochfeld 1997).

A. gravis nests in the archipelagos of South Atlantic between November and May, with the first spawning taking place in November and, especially, in December and migrates to the North Atlantic outside the breeding season. In mid-October, there is a notable migratory flow in Western Europe of *A. gravis* to the south from the nonbreeding zones of boreal waters. This species has differential

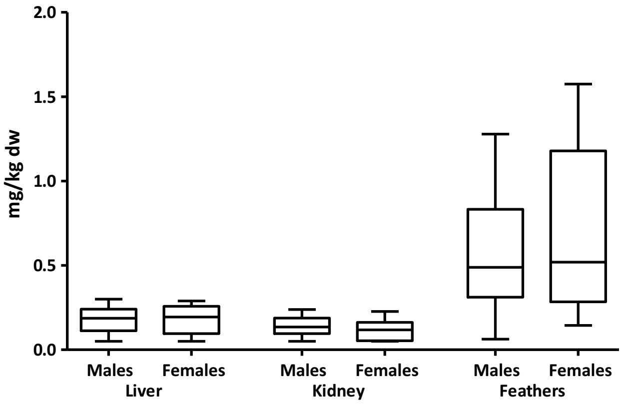

Fig. 3 Pb levels (mg/kg of dry weight) in liver, kidney and feathers of *Ardenna gravis*, according to gender (males/females, 29/17). Box plots represent median values and 25 to 75% percentile ranges

Table 3 Main statistical parameters corresponding to GST (nmol/min/mg protein), MDA (nmol/mg protein), GSH (nmol/mg protein), and CAT (μmol of H_2O_2/min) in Ardenna gravis ($n = 46$) liver and kidney

Biomarker	N	Liver (Median ± SEM)	Kidney(Median ± SEM)
GST	46	84.37 ± 14.88	45.25 ± 1.79
GSH	46	1.2 ± 0.07	16.65 ± 1.03
MDA	46	7.24 ± 0.5	3.44 ± 0.22
CAT	46	385.48 ± 26.25	465.32 ± 31.01

SEM standard error of the mean, *N* number of samples

migrations by sex, being usual that adult males arrive before females at the breeding colonies to delimit territories, although an overlap of migratory flow can occur with them (Romay et al. 2011). The lack of differences in our study can be explained by the season when the capture was performed, beginning of autumn. In October, animals are migrating and the new breeding has not arrived yet. Therefore, there is not a possibility for any transfer of metals from females by laying eggs. Moreover, the different pattern of behavior may explain the higher number of males ($n = 84$) compared with females ($n = 23$) in the 107 animals (29/17 in the subsample).

Several biomarkers also were evaluated in the processed samples of liver and kidney (GST, GSH, MDA, and catalase); results are shown in Table 3. These data are especially relevant due to the lack of information regarding the biomarkers of oxidative stress in this species and in general for seabirds. Moreover, it has been suggested that biomarkers of oxidative stress may provide an additional tool to assess the health status of individuals (Costantini and Dell'Olmo 2015). However, selection of biomarkers is not straightforward, and their diagnostic value might differ among species. When using these biochemical measures, it is necessary to use a multiple biomarker approach to obtain more information about basal levels (Beaulieu and Costantini 2014), as obtained with the present study. Similarly, it is interesting to examine how the biomarkers can relate to environmental contaminants. For example, it has been reported that, in mallards, lipid peroxidation increases with Pb exposure in blood, liver, bile, and brain, but decreases in nerves. It also was mentioned that GSH increases with Pb exposure in liver and bile (Mateo et al. 2003). However, the lack of correlations found in our study does not allow to confirm this statement in *A. gravis*.

Figure 4 shows the correlations study where the relationships between different organs were calculated for each one of the metals. It was possible to observe positive and significant correlations between feathers and liver for Pb and between feathers and kidney for Cd. These significant positive correlations appeared between internal organs and feathers suggest that the last may be used as a noninvasive tissue for better understanding metal exposure in seabirds during biomonitoring programs, as already described for other species (Nardiello et al. 2019). Moreover, metal content in feathers may indicate long-term exposure, because exposure and accumulation during the intermolt period can be derived from the element burdens in this tissue (Espejo et al. 2018). However, before considering the use of feather metal concentrations to predict element concentrations in soft tissues for biomonitoring purposes, there are several factors to be taken into account (Nam et al. 2005). First,

Fig. 4 Significant positive correlations in Pb concentrations between feathers and liver ($p < 0.01$), as well as in Cd between feathers and kidney ($p < 0.05$). Data are expressed in terms of dry weight

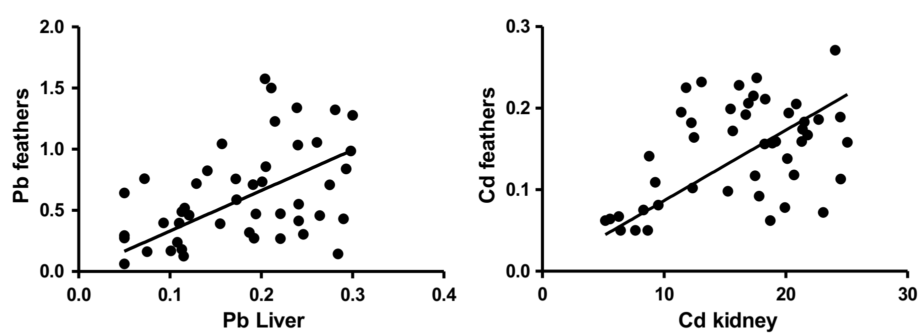

Table 4 Metal correlations found in a specific organ

Liver			Kidney			Feathers		
	Cd	Zn		Cd	Zn		Cd	Zn
Pb	*r: -0.08 p: 0.59*	*r: 0.04 p: 0.80*	Pb	*r: -0.05 p: 0.74*	*r: -0.01 p: 0.92*	Pb	*r: 0.2p: 0.12*	*r: -0.09 p0.57*
Cd		*r: 0.21 p: 0.16*	Cd		*r: -0.26 p: 0.08*	Cd		*r: -0.13 p:0.40*

Data expressed in terms of dry weight

an adequate cleaning of the samples must be considered; second, there is no exchange of blood with soft tissues once feathers growth is complete; and, finally, there is a seasonal cycle of some element accumulation and elimination in the soft tissues relative to feather molt (Nardiello et al. 2019).

Table 4 shows the correlations study where the relationships between different metals for a specific organ/tissue. In this case, no correlations were established for any of the metals studied. Other authors (Ishii et al. 2017) found positive correlations between Cd and Zn in liver samples of Short-shearwater. These heavy metals are expected to interact with a detoxification system, such as metallothionein (MT), a low molecular weight cysteine-rich protein involved in the homeostasis of essential metals, such as Zn. High levels of Cd induce the synthesis of MT, leading to the increase of the Zn uptake. In the present work, the slight correlation (not significant) observed (*r*: 0.21, *p*: 0.16) can explain the high levels of Zn found in all the organs/tissues. There were not correlations between metal levels and the tested biomarkers. Additionally, biomarkers of oxidative stress did not correlate with each other. These findings, together with the reported idea that correlations between metrics of oxidative stress occur only in severe pathological conditions (Costantini and Dell'Omo 2015), suggest that internal concentrations of Zn, Cd, and Pb in Great Shearwater were not high enough to trigger impairment at the oxidative stress level.

Conclusions

This study demonstrated the concentration profiles of Cd, Pb, and Zn in liver, kidney, and feathers of *A. gravis*. This study highlighted the use of *A. gravis* as a potential marker of current metal pollution. Generally, the concentrations of Zn and Pb in *A. gravis* were comparable to values reported in other studies. Animals presented considerable body burdens of Cd. In our study, *A. gravis* showed no differences between males and females, which may be explained by the migratory status, because females did not lay eggs yet. Positive correlations found between feathers and internal tissues for Pb and Cd suggest that the first can be a useful noninvasive tool for biomonitoring programs. The metal concentrations found together with the lack of correlations at the oxidative stress level suggest a low environmental risk for the metal content in the North-Atlantic for seabird species.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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