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Mercury exposure in relation to foraging ecology and its impact on the oxidative status of an endangered seabird

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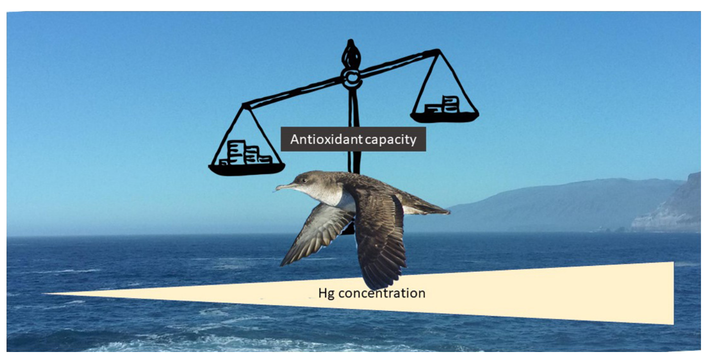
HIGHLIGHTS

- We quantified the blood Hg concentrations of adult black-vented shearwaters.
- Hg concentrations were moderate and similar between sexes.
- The foraging habitat explained variation in Hg among birds.
- The individual trophic level did not explain Hg exposure.
- Hg concentration was negatively correlated with blood antioxidants.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury is a natural element extensively found in the Earth's crust, released to the atmosphere and waters by natural processes. Since the industrial revolution, atmospheric deposition of Hg showed a three-to-five-fold enrichment due to human activities. Marine top predators such as seabirds are recognized valuable bioindicators of ocean health and sensitive victims of Hg toxic effects. Hg negatively affects almost any aspect of avian physiology; thus, birds prove valuable to study the effect of Hg exposure in vertebrates. The Black-vented Shearwater is endemic to the North-Eastern Pacific Ocean, where it forages along the Baja California Peninsula during the breeding period. The area has no industrial settlement and is in the southern portion of the California Current System (CCS). After observing possible contamination effects in eggshells, we decided to quantify the exposure of breeding birds to Hg and test for possible effects on oxidative status of the species. The concentration of Hg in erythrocytes averaged 1.84 μg/g dw and varied from 1.41 to 2.40 μg/g dw. Males and females had similar Hg concentrations. The individual trophic level (reflected by $\delta^{15}N$) did not explain Hg exposure. In contrast,

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Baja California peninsula

individuals foraging inshore had higher Hg concentrations than those foraging more offshore (reflected by $\delta^{13}C$). Shearwaters having higher concentrations of Hg had lower activity of the antioxidant enzyme glutathione peroxidase and showed lower non-enzymatic antioxidant capacity. Levels of plasma oxidative damage, superoxide dismutase and catalase were not associated with Hg. Our results indicate that (i) the foraging habitat is the factor explaining Hg exposure and (ii) there is some evidence for potential harmful effects of Hg exposure to this seabird species of conservation concern.

Capsule: The foraging habitat is the factor explaining Hg exposure in seabirds and we observed potential harmful effects of Hg exposure in a seabird species of conservation concern.

1. Introduction

One of the most toxic elements to human health and wildlife is Mercury (Hg), especially in its methylated form Me-Hg (Eagles-Smith et al., 2018), which accumulates in the tissues of living organisms (Mason et al., 1995). Several studies have reported ecologically relevant concentrations of Hg in wildlife in North America (Scheuhammer et al., 2016; Weiss-Penzias et al., 2016; Zhang et al., 2016) and, to a lesser extent, in Central and South America (Di Marzio et al., 2019; Sebastiano et al., 2016; Sebastiano et al., 2017a). In Mexico, most studies have focused on the Gulf of California, especially for the high presence of industrial, agricultural, and mining activities (Sánchez-Rodríguez et al., 2001). Recent work on 14 fish taxa from Mexico revealed low Hg levels; thus, Hg is not expected to cause health issues to local fish-eating birds (Elliott et al., 2015). However, several studies reported the presence of relatively high concentrations of Hg in aquatic birds from this region (Lerma et al., 2016; Ruelas-Inzunza et al., 2009). To the extent of our knowledge, no studies have to date investigated both the presence and the effect of Hg exposure on local wildlife. Under the predictions that environmental concentrations of Hg will rise in coming years and the impact of Hg exposure is likely to be exacerbate by climate change (Krabbenhoft and Sunderland, 2013; St. Pierre et al., 2018; Stern et al., 2012), it is crucial to investigate the concentration of this ubiquitous element in seabird tissues and to provide early warning of its effects on their health status. Indeed, although the detrimental effects of Hg exposure in captive birds have long been known, we still have a poor understanding of the effects of sublethal Hg concentrations on individual health of free-living birds (Whitney and Cristol, 2017), and their consequences at the population level (Goutte et al., 2014a; Goutte et al., 2014b), particularly in bird species of conservation concern (Pacyna et al., 2017; Tsao et al., 2009).

Seabirds are long-lived top predators of marine food webs, bearing high levels of Hg (Rowe, 2008); thus they prove valuable to study the effects of Hg exposure in birds. A recent review pointed out that Hg might negatively affect almost any aspect of avian physiology (Whitney and Cristol, 2017). However, little work has assessed so far the effects of Hg on physiological traits of wildlife. One way through which Hg might impact on organism function is through the increase of molecular oxidative damages and disruption of antioxidant defences (Ercal et al., 2001). Because of its great molecular affinity for thiols and selenium (Ralston and Raymond, 2018), Hg may directly impact on the redox mechanisms involving glutathione (reviewed in Whitney and Cristol, 2017), such as antioxidant enzyme activity of glutathione peroxidase (GPx). Previous works on seabirds have shown that exposure to Hg might increase oxidative damage to lipids (increased oxidative damage, Costantini et al., 2014; increased antioxidant oxidation and enzymatic antioxidant activity, Kenow et al., 2008), thus oxidative status markers provide a fundamental tool to determine the impact of Hg on seabirds.

During our long-term monitoring project on the Black-vented Shearwater (*Puffinus opisthomelas*) breeding on Natividad Island, we observed some cases of reproductive failure. Therefore, we hypothesized that the shearwater population might be exposed to Hg concentrations of concern that may affect their health status.

This study main goal was thus to quantify short-term Hg exposure using blood samples of adult Black-vented Shearwaters during the breeding season. We further assessed the antioxidant status and oxidative damage levels to evaluate whether Hg impacts on the oxidative status. Finally, using carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, respectively) as proxies, we determined the main routes of contamination.

2. Materials and methods

2.1. Species and study site

The Black-vented Shearwater is a burrow-nesting seabird with nocturnal colony attendance and a single egg clutch. It is endemic to Mexico and distributes along the Pacific coasts of North America, and IUCN (International Union for Conservation of Nature) consider it as near threatened (Birdlife-International, 2016). Generally, it feeds ashore on the continental shelf in high productivity areas, mainly on anchovies, sardines, and squid (Keitt et al., 2000a). According to the described diet of the species (Keitt et al., 2000b), the Black-vented Shearwater expected isotopic values should be between −20 and − 16‰ of $\delta^{13}C$. The species owns a very restricted area of distribution especially during the breeding season, where 95% of the global population breeds sympatrically on Natividad Island, Mexico (27° 86′ 25.59″ N, 115° 17′ 14.18″ W; Fig. 1), within the El Vizcaino Biosphere Reserve. Birds arrival to the colony starts in December with prospecting individuals reinforcing pair bonds before egg laying, usually occurring from February through March. Eggs hatch in late April–May and chicks are ready to fledge in July (Keitt et al., 2000a; Keitt et al., 2003). Natividad Island is of conservation interest, hosting "globally significant populations" of the Black-vented Shearwater, according to the IBA criteria (BirdLife-International, 2010).

2.2. Spatial analysis

Using dataloggers specifically designed for this species (Axy-Trek, Technosmart Europe, Rome, Italy), 11 breeding Black-vented Shearwaters were tagged. Dataloggers were attached to the back feathers of the birds (Tesa® 4651, Tesa SE, Hamburg, Germany) using 4 strips of marine tape, weighing a total of 11 g (9 g of the instrument plus 2 g of tape; < 5% of body mass). Every night, until the bird returned, the colony was visited, and no GPS equipped bird failed to return to its burrow. Data loggers were included in this study to identify foraging areas of males and females during the breeding season. Only three birds of those blood sampled were equipped with dataloggers. We assumed the foraging areas of the sampled individuals being the same of the foraging areas used by the tracked ones.

2.3. Data and sample collection

As part of the long-term monitoring program of the Black-vented Shearwaters, we captured and ringed adult birds at their nests ($n = 20$) at the end of the incubation period (April). We measured the following traits: right-wing length (to the nearest millimeter using a

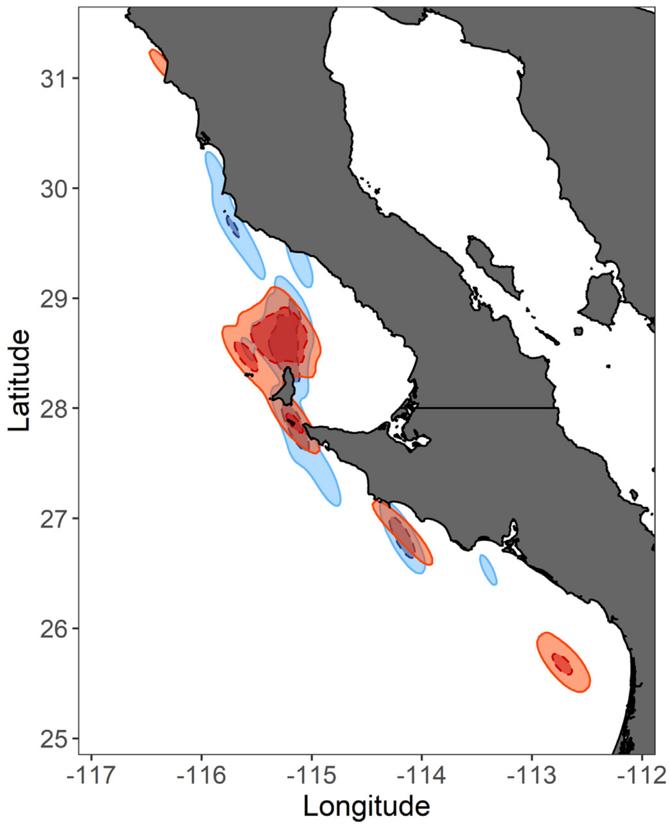

Fig. 1. Foraging areas of males (light blue identifying 25% Kernels and dark blue identifying 75% Kernels) and females (orange identifying 25% Kernels and red identifying 75% Kernels) according to tracks obtained during the breeding period. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ruler), length of right tarsus from the middle of midtarsal joint to distal end of tarsus-metatarsus (to the nearest 0.1 mm with a calliper), head plus bill length, bill length (nearest 0.1 mm), and body mass (nearest 0.5 g using a Pesola spring balance). We collected 100 μL of blood from the tarsal vein using heparinized syringes and needles; we stored the samples in an ice refrigerated carrier and transported to the field lab. We centrifuged samples after <30 min from sampling for 3 min at 12000 rpm at room temperature and we stored plasma and erythrocytes in separated tubes at −20 °C while on the field, and at −80 °C in the laboratory. A drop of blood (5 μL) was collected on filter paper and stored at −20 °C for molecular sexing using a previous protocol (Albores-Barajas et al., 2010), at Dr. Adrian Munguia Vega Lab, La Paz, Baja California Sur.

2.4. Oxidative status markers

The non-enzymatic antioxidant capacity was determined using the Ferric Reducing Ability of Plasma (FRAP) test applied to erythrocytes (i.e., Ferric Reducing Ability of Erythrocytes, FRAE) (Benzie and Strain, 1996); it was expressed as μmol Trolox/g relative to the fresh weight). We measured three antioxidant enzymes in erythrocytes: *i)* the activity of superoxide dismutase (SOD) was determined by measuring the inhibition of nitroblue tetrazolium reduction at 560 nm. It was further expressed as U/mg protein per minute; *ii)* the activity of catalase (CAT) was assayed by monitoring the decomposition rate of H_2O_2 at 240 nm and expressed as μmol H_2O_2/mg protein per minute; and *iii)* glutathione peroxidase (GPx) activity, determined by a spectrophotometric method and expressed as μmol NADPH/mg protein per minute. We also used the Thiobarbituric Acid Reactive Substances (TBARS) assay to quantify plasma lipid peroxidation. Values are expressed as nmol of Malondialdehyde (MDA) equivalents/mL of plasma. We used established protocols for vertebrates to perform all the analyses (Sebastiano et al., 2017b; Sebastiano et al., 2018). Detailed protocols are provided in the supplementary material.

2.5. Mercury and stable isotopes

The analysis of isotopic ratios of carbon ($^{13}C/^{12}C$ or $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) is a powerful tool to identify the foraging habitat and trophic position of wildlife (Hobson, 1999, Maruyama et al., 2001, Rubenstein and Hobson, 2004). The $\delta^{15}N$ increases at each trophic level, with consumers' tissues having values between 3 and 5‰ greater than the prey they are synthesised from (DeNiro and Epstein 1978, Hobson and Clark, 1992, Bearhop et al., 2002). Values of $\delta^{13}C$ decrease from coastal to oceanic habitats, making them useful proxies for assessing habitat use in marine organisms (France 1995, Hobson et al., 1997, Newsome et al., 2010).

We quantified the total concentration of Hg and both the stable nitrogen and carbon isotope ratios in freeze-dried erythrocytes following previous protocols (Sebastiano et al., 2016, 2017a). Briefly, we quantified stable nitrogen and carbon isotopes using an elemental analyzer (Flash 2000, Thermo Scientific, Milan, Italy) together with an isotope ratio mass spectrometer (Delta V Plus with a Conflo IV interface, Thermo Scientific, Bremen, Germany). Values were expressed in the δ unit notation as parts per mille (‰) deviation from standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and N_2 in air for $\delta^{15}N$). The analytical imprecisions were <0.10 ‰ for carbon and <0.15 ‰ for nitrogen. Hg was measured in erythrocytes (aliquots ranging from 0.9 to 1.4 mg) using a direct mercury analyzer AMA-254 from Altec. The quality control/quality assessment of Hg determination was evaluated by the analyses of procedural blanks and of CRM (certified reference material) TORT-3 Lobster Hepatopancreas from the NRC, Canada. CRM were analysed at the beginning, at the end of each analytical cycle and every 10 samples. Certified Hg concentration of the CRM is 0.292 ± 0.022 μg/g dw and the average value (±SD) obtained in the present study was 0.285 ± 0.002 μg/g dw ($n = 15$). Thus the recovery of the CRM was 97.7 ± 0.7%. The detection limit of the AMA was 0.5 ng. We expressed the Hg concentration as μg/g dw (dry weight). Because blood is also measured and reported on a wet-weight basis, the formula blood (ww) = blood (dw) ∗ 0.21 can be used to convert dry-weight values to wet-weight values (i.e. assuming an average 79% of moisture) as previously done (Ackerman et al., 2016).

2.6. Statistics

We used the software STATISTICA 10 (Tulsa, OK, USA) to run all statistical analyses. All blood samples were collected at the same site in a single day, thus these factors were not considered. First, we analysed the foraging ecology of species using data from GPS and isotopes. We used a Bayesian framework to analyse stable isotope data (Jackson et al., 2011). We calculated the standard ellipse area corrected for small sample sizes (SEAc), which contains approximately 95% of the data within a set of bivariate data, in order to quantify niche width and then compare it between sexes. For this, we used the SIBER library for R (Jackson et al., 2011). We performed Spatial analyses using R 3.3.1 (R_Core_Team, 2019). Applicable significance level was set at $\alpha = 0.050$ for all the analyses. Second, we ran general linear models including sex, $\delta^{13}C$ and $\delta^{15}N$ as main factors and blood Hg as dependent variable. General linear models were also run to test the association between blood Hg and oxidative status markers (all as dependent variables in separate models) while controlling for sex, which was included as a main factor. We ran similar models to test the association between blood Hg and body mass (dependent variable). In this case, sex was included as a main factor and body size index as a covariate. In so doing, the model calculates the strength and direction of the relationship between Hg and body mass, while controlling for the effect of body size on body mass. Thus, the model normalises the body mass by the variation among individuals in body size, while testing the covariation; this approach is preferable to the use of residuals to estimate body condition index (García-Berthou, 2001; Green, 2001). The index of body size was calculated using the PC1 from a PCA on wing length, tarsus length, head and beak length and beak width.

3. Results

We obtained 11 GPS tracks (four males and seven females) during incubation and chick rearing period. We recorded 1493 dives for males and 1666 for females, respectively allowing us to identify foraging areas used (Fig. 1). On the basis of 75% Kernel Density Estimation of diving points, we identified three core foraging areas: a) in shallow waters (<200 m depth) along the northern coast of El Vizcaino Bay, and at the edge of the continental slope (200 m isobath); b) north from Isla Cedros; and c) south of the colony toward Ulloa Gulf. The distribution range of the species during the breeding period is also outlined by the 25% Kernel Density Estimation in Fig. 1. We obtained no significant effect of individual or sex on latitude reached (individual: $F_{1,12} = 0.002$, $p = 0.962$; sex: $F_{1,12} = 0.033$, $p = 0.858$), this let us assume that the distribution obtained is representative of the breeding period.

As expected, the $\delta^{13}C$ values for the Black-vented Shearwater reflected an average of −18.72 ‰ $\delta^{13}C$, ranging from coastal waters with a maximum of −18.02 ‰ $\delta^{13}C$ to more oceanic waters with a minimum of −19.83 ‰ $\delta^{13}C$. Trophic niche did not differ significantly between sexes, with females showing a larger range toward oceanic waters than males (female SEAc = 0.600; male SEAc = 0.404, overlap = 0.278). Neither mean values of oxidative status markers nor their variances differed between males and females (*t*-test, $p \geq 0.07$; Levene test, $p \geq 0.24$).

The concentration of total Hg in erythrocytes (Table 1) averaged 1.84 ± 0.28 μg/g dry weight, varied from 1.41 to 2.40 μg/g dry weight (corresponding to an average of 0.39 ± 0.06, range of 0.30–0.50 in wet weight). Males and females had similar blood Hg (GLM, $p = 0.30$). The foraging area as estimated by $\delta^{13}C$ was significantly correlated with blood Hg: shearwaters fishing closer to the mainland had higher Hg concentrations than shearwaters fishing offshore (GLM, coeff. estimate ± se: 0.34 ± 0.09, $p = 0.002$, Fig. 2). In contrast, the trophic level of shearwaters as estimated by $\delta^{15}N$ did not correlate significantly with blood Hg (GLM, $p = 0.49$, Fig. 2).

General linear models showed that sex was never a significant predictor of any oxidative status marker or body condition ($p \geq 0.06$), thus it was removed in order to improve fitting of the models (based on Akaike Information Criterion) and the analyses were re-run. We found moderate and statistically significant associations between Hg and both FRAE (GLM, coeff. Estimate ± se: −7.75 ± 3.43, $p = 0.036$) and GPx (GLM, coeff. estimate ± se: −0.0003 ± 0.0001, $p = 0.036$) (Fig. 3), while Hg was not associated with TBARS ($p = 0.69$), CAT ($p = 0.99$), SOD ($p = 0.71$) nor with body mass normalised by the covariate body size ($p = 0.99$).

Table 1
Descriptive statistics of Hg (μg/g dw), $\delta^{13}C$ and $\delta^{15}N$ (‰) and markers of oxidative status measured in 20 Black-vented shearwaters.

	Mean	Median	Min.	Max.	Std. dev.
Hg	1.84	1.78	1.41	2.40	0.28
$\delta^{13}C$	−18.7	−18.5	−19.8	−18.0	0.5
$\delta^{15}N$	15.8	15.8	15.3	16.3	0.3
TBARS	96.9	101.2	51.9	131.5	20.4
FRAE	18.1	18.6	7.0	25.9	4.6
SOD	1.01	1.04	0.47	1.50	0.22
GPx	0.0007	0.0007	0.0005	0.0012	0.0002
CAT	0.61	0.66	0.15	0.89	0.23

4. Discussion

We report in the present study the first record of blood Hg concentrations in an endangered seabird from Baja California Peninsula, Mexico, the Black-vented Shearwater. The high inter-individual variation in Hg concentrations was partially explained by the foraging habitat but not by the individual trophic level. Our results also provide the first evidence that Hg exposure might impact the oxidative status of Black-vented Shearwaters during reproduction, one of the critical phases of life-history in birds.

High trophic level predators including large fish and fish-eating wildlife can show toxic concentrations of Hg in their tissues as a consequence of its biomagnification along food webs (Watras et al., 1998). However, we found no relationship between Hg levels and the nitrogen stable isotope $\delta^{15}N$, indicating that blood Hg concentration in the present species is not related to the trophic position. On the contrary, Hg was strongly associated with the carbon stable isotope ratio, suggesting that Hg concentrations may be driven by the feeding habitat of the species. The wide range in $\delta^{13}C$ indicates that Black-vented Shearwaters forage in diverse feeding habitats during the incubation period. Because the carbon signature is higher in coastal environments, our results suggest that birds bearing higher Hg levels in their blood are the ones feeding closer to the coast. Considering that turnover time in plasma and cellular component of blood vary from about 3 days to about 30 days, respectively (Hobson and Clark, 1993), we can assume that isotopes and Hg concentrations obtained from erythrocytes are representative of the breeding period and can be associated to the distribution range. Black-vented Shearwater core foraging areas lie within the California Current System (Soldatini et al., 2019). Breeding period northern tracks evidence foraging areas in the Vizcaino Bay and along the continental slope while southern tracks are mainly along the continental slope. Although the distance between these two systems is not large, their characteristics may be significantly different. These two systems provide nutrients from different origins in the foraging area of the Black-vented Shearwater that we can recognize in $\delta^{13}C$ differences obtained, representing simultaneously a coastal and oceanic origin for food ingested by shearwaters distributing in a rather reduced area. The inverse correlation of Hg with $\delta^{13}C$ suggests that shearwaters feeding in coastal waters are more exposed to Hg, resulting in higher Hg concentration in their blood. This may be due, for example, to the biomagnification potential of Hg or to an higher concentration of dissolved Hg in coastal waters. For instance, oligotrophic conditions associated with low productivity (Chouvelon et al., 2018) and/or simplicity of trophic food webs (Carravieri et al., 2020) largely influence Hg bioaccumulation and biomagnification. Furthermore, mesopelagic zones contain higher mercury and methyl-mercury concentrations than epipelagic (up to 200 m in depth) areas (Fitzgerald et al., 2007), resulting in enhanced Hg concentrations in mesopelagic fish (Blum et al., 2013; Chouvelon et al., 2012; Monteiro et al., 1996). Upwelling waters may thus represent an important source of Hg to surface waters (Conaway et al., 2009) and seabird feeding in more coastal areas may be more exposed to Hg.

Black-vented Shearwaters showed lower concentrations of Hg than Blue-footed Booby *Sula nebouxii* from the same region (Lerma et al., 2016), and than Caspian Terns *Sterna caspia* and Forster's Terns *Sterna forsteri* from San Francisco Bay (Eagles-Smith et al., 2008), but comparable blood Hg levels to that of Brown Noddy *Anous stolidus* and Cayenne Terns *Thalasseus sandvicensis* from another region (Sebastiano et al., 2017a). Overall, concentrations of Hg in shearwaters were similar to those reported to induce harmful effects in certain bird species. Hg might impact on organism function when blood concentration exceed 1.0 μg/g ww (Ackerman et al., 2016). However, sensitivity to Hg exposure may vary widely among species (Heinz et al., 2009). Some seabird species start to suffer detrimental effect of Hg exposure at very low concentrations (Ackerman et al., 2016), while other species show no apparent adverse effect even when exposed to higher concentrations of Hg

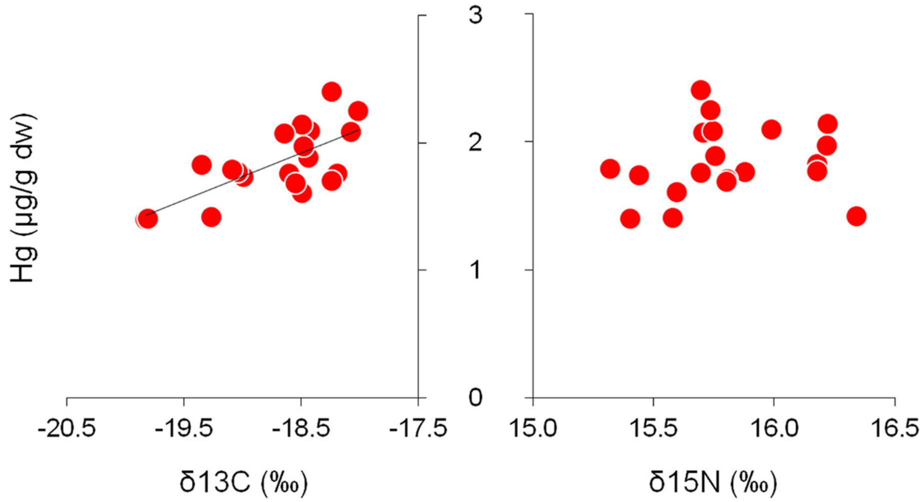

Fig. 2. Relationships between blood mercury concentrations (μg/g dry weight) and the stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$ (‰).The foraging area as estimated by $\delta^{13}C$ was significantly correlated with the blood concentration of Hg, while the trophic level of shearwaters as estimated by $\delta^{15}N$ did not correlate significantly with blood Hg.

(reviewed in Whitney and Cristol, 2017). This has been related to the protective action of Se against the toxicity of Hg, as shown in two skua species (Carravieri et al., 2017), which by interacting with Hg, may mitigate its toxic effects. More generally, the ratio between Se and Hg is used as an index to deduce potential toxicity risks (Scheuhammer et al., 2015), thus our results warrant further work to quantify Se levels in our species. While Hg levels in the Black-vented shearwater seem relatively moderated, Gibson et al. (2014) found that even low levels of Hg in blood altered the expression of oxidative stress-related genes in females Double-crested Cormorants *Phalacrocorax auritus*. Furthermore, in the Wandering Albatross *Diomedea exulans,* plasma oxidative damage increased with Hg contamination of red blood cells in females, but not in males (Costantini et al., 2014), further underlying that sensitivity to Hg may even vary among co-specific individuals and be exacerbated in females, especially during certain life-history stages as reproduction (Costantini et al., 2014).

Shearwaters exposed to higher Hg levels showed reduced non-enzymatic antioxidant capacity in erythrocytes and activity of GPx, suggesting that Hg might have impacted on certain pathways related to oxidative status regulation. Hg may impact on the oxidative status either by depleting antioxidant resources or by increasing production of reactive oxygen species, or both (Stohs and Bagchi, 1995). Because oxidative stress impairs important biological functions, including reproduction, birds with lower antioxidant defences might show a limited parental investment in reproduction (Bize et al., 2008). Although all individuals were able of breeding successfully, we cannot rule out that any harmful effects of Hg might emerge later in life or that more contaminated individuals that failed to

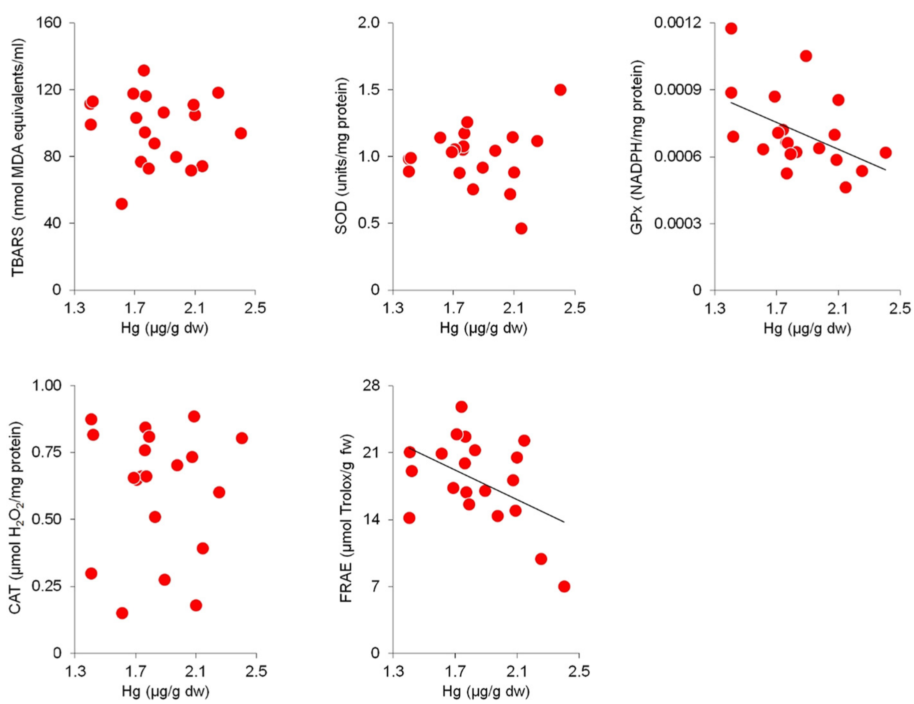

Fig. 3. Relationships among blood Hg (μg/g dry weight) and blood-based oxidative status markers. The regression line is reported only when the relationship was statistically significant. TBARS = Thiobarbituric Acid Reactive Substances, MDA = malondialdeyde, SOD = superoxide dismutase, GPx = glutathione peroxidase, CAT = catalase, FRAE = Ferric Reducing Ability of Erythrocytes.

breed were not included in the present study. Several studies found negative associations between oxidative stress and reproductive or survival perspectives (reviewed in Costantini, 2014). Biochemical evidences showed that Hg can bind to sulfhydryl groups of thiols, such as glutathione, and to interfere with selenoproteines (i.e. glutathione peroxidase). Previous work found that increasing hepatic concentrations of Hg were significantly associated with reduced GPx activity in Ruddy ducks *Oxyura jamaicensis* (Hoffman et al., 1998). Similar results have also been reported in mallards *Anas platyrhynchos* (Hoffman and Heinz, 1998). Thus, even low concentrations of Hg can cause a disruption of important physiological mechanisms. To conclude, our paper recalls the global focus needed for seabird conservation policies and the past effects of contaminants (Risebrough et al., 1968).

5. Conclusions

We found large individual variation in the blood concentration of Hg, which was partially explained by the foraging habitat, but not by the individual trophic level nor its sex. Hg concentrations may be considered as moderate to high when compared to those detected in other species of seabirds. The significant negative correlations we detected between Hg and two antioxidant markers indicate that Hg might have interfered with certain pathways of regulation of oxidative status. Given that worrying conservation status of the Black-vented Shearwater, we urge further work to understand whether the potentially negative effects we found may cause long-term effects on fitness traits of individuals exposed to higher Hg concentrations.

CRediT authorship contribution statement

Cecilia Soldatini: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Manrico Sebastiano:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **Yuri V. Albores-Barajas:** Conceptualization, Investigation. **Hamada Abdelgawad:** Formal analysis. **Paco Bustamante:** Formal analysis. **David Costantini:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics statement

National and institutional guidelines for the care and use of animals were followed. Fieldwork was carried out under permits SGPA/DGVS/00321/16, January 2016 and extension SGPA/DGVS/003843/16, April 2016 and renewal SGPA/DGVS/00404/17 January 2017.

Appendix A. Supplementary data

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