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Patterns of mercury exposure and relationships with isotopes and markers of oxidative status in chicks of a Mediterranean seabird

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Abstract: The Mediterranean basin is a hotspot of mercury (Hg) contamination owing to intense anthropogenic emissions, volcanic activity and oligotrophic conditions. Little work has been done to assess the sources of Hg exposure for seabirds and, particularly, the physiological consequences of Hg bioaccumulation. In this study, we (i) describe the individual and temporal variation in blood concentration of total Hg (THg) over three breeding seasons, (ii) identify the factors that affect the THg exposure and (iii) determine the individual- and population-level connections between THg and blood-based markers of oxidative status in chicks of Scopoli's shearwaters (*Calonectris diomedea*) breeding on the island of Linosa in the southern Mediterranean. We carried out the work on chicks near fledging because they are fed with prey captured near the colony, thus their Hg levels reflect local contamination. The concentration of THg in erythrocytes varied from 0.23 to 4.29 µg g^{-1} dw. Chicks that were fed upon higher trophic level prey (i.e., higher $\delta^{15}N$ values) had higher THg levels. Individual variation in THg concentrations was not explained by parental identity, sex nor $\delta^{13}C$ values. There was significant variation in THg among chicks born from the same mother in different years. We found significant correlations between THg and markers of oxidative status; however, these correlations were no longer significant when we took into account the annual variation in mean values of all metrics. Males with higher values of body condition index had higher blood THg, while THg and body condition index were not correlated in females. Our data indicate that THg levels were moderate to high if compared to other seabirds. However, there is little evidence for harmful short-term detrimental effects owing to THg exposure.

Keywords: Antioxidant, Methylmercury, Oxidative damage, Shearwater, Trophic level

Capsule: We found moderate to high levels of blood mercury, which were explained by trophic level of prey given to chicks. We did not detect any short-term harmful effects.

1. Introduction

Mercury (Hg) is a highly toxic non-essential metal of major concern for wildlife and human health. Hg is released into the environment from both natural and anthropogenic sources (Eagles-Smith et al., 2018). The UNEP estimated that the largest sources of Hg emissions from human activities are artisanal and small-scale gold mining, and coal burning (UNEP, 2018). Global Hg emissions and concentrations in the environment have grown slightly in the period 2010-2015 because declines of commercial Hg use in the developed world have been matched by increases in Hg release into the environment by developing countries (Science for Environment Policy, 2017; Streets et al., 2019). This scenario is further worsened by the capacity of Hg to spread easily across environments because it can travel long distances far from emission sources and remains in the atmosphere for up to a year (Science for Environment Policy, 2017; Streets et al., 2019). In aquatic ecosystems, Hg is converted into methylmercury by microorganisms, allowing its efficient bioaccumulation and its biomagnification up the food chain. Once accumulated into the body, Hg may cause a range of adverse effects on neurobehavioural, endocrine, immune or reproductive functions (Tan et al., 2009; Whitney & Cristol, 2018). These evidences have urged the adoption of new regulations to protect human health and the environment from anthropogenic emissions and releases of Hg into the environment (e.g., the Minamata Convention on Hg, signed in 2013 and entered into force in 2017, http://www.mercuryconvention.org/).

The Mediterranean basin is significantly affected by Hg contamination because geochemical processes related to volcanic activity and cinnabar deposits together with industrial and agricultural activities release large amounts of Hg into the environment (Rajar et al., 2007; Žagar et al., 2007; Sunderland and Selin, 2013; Salvagio Manta et al., 2016; Science for Environmental Policy, 2017; Streets et al., 2019). Furthermore, oligotrophic conditions of the Mediterranean Sea favour the biomagnification of Hg in food webs

(Chouvelon et al., 2018). Mediterranean marine organisms are therefore exposed to high risks of Hg contamination. Piscivorous birds are particularly prone to accumulate Hg because of the biomagnification (Eagles-Smith et al., 2018; Whitney & Cristol, 2018). Levels of Hg in seabirds breeding in Mediterranean islands are actually significantly higher than those recorded in seabirds from Atlantic islands (e.g., Renzoni et al., 1986; Ramos et al., 2009).

The Environmental Quality Standards Directive (2008/108/EC modified by Directive 2013/39/EU) of the European Union determined quality standards for Hg levels in both water and fish in order to protect piscivorous animals from its toxic effects. In piscivorous animals, a long-term accumulation may lead to chronic effects. Once accumulated in the body, Hg may affect fundamental fitness traits, such as reproduction and lifespan (e.g. Tartu et al., 2013, 2014, 2015, 2016) with negative consequences at the population level (Evers et al., 2008; Goutte et al., 2014a,b). The number of studies that addresses the mechanistic underpinnings of Hg effects on marine animals is, however, still limited (Whitney and Cristol, 2018). There is a need for more studies that assess the effects of Hg on wildlife, both at individual and population level, in order to understand fully its impact on ecosystems. A growing body of evidence indicates that Hg exposure induces molecular oxidative damages and changes in antioxidant molecules in a number of seabird species (Henny et al., 2002; Hoffman et al., 2005, 2009; Costantini et al., 2014; Gibson et al., 2014). The hypothesis that exposure to Hg is linked to increased oxidative stress is also supported by direct biochemical evidences. The underlying mechanisms of action of Hg involve (i) toxic effects via bonding to sulfhydryl groups of proteins and impairment of the glutathione system and (ii) formation of reactive oxygen species mainly via Fenton reaction, finally producing mutagenic and carcinogenic end-products of oxidative stress cascade (Koivula and Eeva, 2010; Jomova and Valko, 2011).

In this study, our aims were to (i) describe the individual and temporal variation in blood concentration of total Hg (THg), (ii) identify the factors that affect Hg exposure (parental

identity, trophic level and foraging area) and (iii) determine the individual- and population-level connections between Hg and blood-based markers of oxidative status in chicks of Scopoli's shearwaters (*Calonectris diomedea*) breeding on the island of Linosa in the southern Mediterranean. The island lies in the Strait of Sicily at ca. 250 km from Augusta bay, which is one of the most contaminated areas with Hg in the Mediterranean Sea (Salvagio Manta et al., 2016). In addition, we also analysed the correlation between blood Hg and body condition index of chicks because Hg may cause loss of energetic stores (e.g., fat) in young birds (Scheuhammer, 1987). Prior work in Linosa carried out in 1982 showed that the Hg concentration in eggs (mean ± SD) was 5.90 ± 1.45 µg g^{-1} dw and in tissues collected from adults varied from 0.87 ± 0.17 µg g^{-1} dw in fat to 86.16 ± 99.16 µg g^{-1} dw in liver (Renzoni et al., 1986). These concentrations were high compared to those recorded in Atlantic shearwaters (Renzoni et al., 1986). However, there are no recent data on Hg exposure, nor on the potential effects on the physiological conditions in birds. We carried out the study on chicks at fledging age because (i) the impact of Hg on the organism may be especially relevant when contamination occurs in early life, (ii) they provide a valuable snapshot of the local contamination because they are fed by parents that forage in the vicinity of the breeding colony (e.g., Carravieri et al., 2020) without any interference of Hg accumulated by adult shearwaters in the wintering areas of West Africa (Müller et al., 2014) and (iii) the influence of Hg exposure within the egg is negligible owing to the growth dilution effect (Ackerman et al., 2011).

2. Materials and Methods

2.1. Study species and study area

The Scopoli's shearwater is a pelagic seabird that breeds in the Mediterranean. Scopoli's shearwaters consume a wide range of prey including pelagic fish (e.g., sardines), planktonic crustaceans, fish larvae and discards from fisheries (Grémillet et al., 2014; Cianchetti-Benedetti

et al., 2018). In Linosa, the fishing areas of adults are located near the island (Cianchetti-Benedetti et al., 2018). Recent work on the Linosa population showed little concern of exposure to several persistent organic pollutants for the conservation status of this shearwater population (Costantini et al., 2017). However, populations of the Scopoli's shearwater are estimated to be in slow decline (www.iucnredlist.org). BirdLife International (2015) provided an estimate of 30,500-48,100 pairs for the European population. Defos du Rau et al. (2015) estimated the presence of 141,000-223,000 breeding pairs in the whole Mediterranean basin. Scopoli's shearwaters have an estimated lifespan of ca. 25 years (Fransson et al., 2010) and start breeding when they are around 5 years old (Thibault et al., 1997). Shearwaters lay one egg in May and chicks leave the colony at around the end of October. We carried out the fieldwork on the island of Linosa (35°52' N, 12°52' E) in the Sicily Channel (Figure 1). All adults and chicks have been tagged with permanent metal rings since 2007. Each nest was also tagged with a permanent number and georeferenced; the maximum linear distance between nests included in the present study was ca. 750 m (Figure 1).

2.2. Sampling

Blood samples were collected from chicks near fledging in the period 27 September to 10 October 2016, 4 to 14 October 2017, and 30 September to 12 October 2018. Upon capture, we first collected a sample of venous blood (0.5 ml) from the tarsal vein and then we measured the body mass, and both the beak length and beak width of each chick. When back to the field station, within 1-2 h from blood collection, we centrifuged (4'000 rpm for 4 minutes) the tubes containing the whole blood in order to separate the serum from the erythrocytes. Both serum and erythrocytes were stored in separate tubes at -20 °C in the field station and at -80 °C in the laboratory.

2.3. Mercury

We quantified the THg (expressed as µg g^{-1} dw dry weight) in freeze-dried erythrocytes (aliquots ranging from 0.33 to 2.7 mg) following the protocol described in Chouvelon et al. (2009). We measured the THg in blood because prior work on several vertebrates showed that (i) it is highly and positively correlated with the blood concentration of methylmercury, (ii) most of the Hg occurs in the methylated form within the body tissues, and (iii) blood THg reflects short-term exposure and is a valuable nonlethal predictor of Hg concentrations in internal tissues (e.g., Skerfving, 1988; Thompson and Furness, 1989; Monteiro, 1996; Sakamoto et al., 2002; Eagles-Smith et al., 2008; Yates et al., 2014). Briefly, we used blanks and the certified reference material (CRM) TORT-3 Lobster Hepatopancreas (NRC, Canada; certified Hg concentration: 0.292 ± 0.022 µg g^{-1} dw) to check the analytical quality. The average value (± SD) of CRM was 0.285 ± 0.002 µg g^{-1} dw (n = 15) with a recovery of 98 %. The quantification limit of the method was 0.5 ng. The average variation coefficient of samples analysed in duplicate was 0.76 %.

2.4. Stable isotopes

The isotopic niche of seabirds was used as a proxy of their ecological niche: $\delta^{13}C$ values indicate the foraging habitat (inshore/benthic vs. offshore/pelagic), while $\delta^{15}N$ values indicate the trophic level (Newsome et al., 2007). An amount of 0.30 ± 0.05 mg of dried erythrocytes were weighed in tin cups for stable isotope analyses. Carbon and nitrogen stable isotope ratios were measured using an elemental analyzer (Flash 2000, Thermo Scientific, Milan, Italy) coupled with a 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 $\delta^{15}N$) following the formula: $\delta^{13}C$ or

$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. The analytical imprecisions were < 0.10 ‰ for carbon and < 0.15 ‰ for nitrogen.

2.5. Oxidative status markers

We measured four markers of oxidative status following previous protocols (e.g., Costantini et al., 2013). Briefly, we measured the concentration of protein carbonyls (PCs, marker of protein oxidative damage) in serum using the Protein Carbonyl Colorimetric assay (Cayman Chemical Company, Ann Arbor, MI, USA); the activity of the antioxidant enzyme glutathione peroxidase (GPx) in erythrocytes using the Ransel assay (RANDOX Laboratories, Crumlin, UK); the activity of the antioxidant enzyme superoxide dismutase (SOD) in erythrocytes using the Ransel assay (RANDOX Laboratories, Crumlin, UK); the serum non-enzymatic antioxidant capacity (OXY) against the hypochlorous acid (HOCl, oxidant naturally generated in cells) using the OXY-Adsorbent test (Diacron International, Grosseto, Italy). We used the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA) with albumin as a reference standard to quantify the concentration of proteins in serum and erythrocytes. Quality controls were included in all assays performed. Values of markers were expressed as nmol/mg proteins for PCs, units/mg proteins for both GPx and SOD, mmol/l of HOCl neutralized for OXY.

2.6. Statistical analyses

All analyses were carried out using SPSS Version 23 (Armonk, NY, USA). First, we carried out a model selection to identify the best-fitting linear mixed model to Hg concentration data from a set of candidate models using the Akaike Information Criterion (AIC). We used AIC values unadjusted for sample size because these provided very similar results to AIC values adjusted for sample size (data not shown). Models were first ranked according to AIC values. We then calculated ΔAIC and Akaike weights. The ΔAIC is the difference of each model AIC

value relative to the AIC of the best model. A ΔAIC < 2 suggests substantial evidence for the model (Burnham and Anderson, 2002). Akaike weights are obtained by transformation of AIC values and indicate the probability that the model is the best among all candidate models (Burnham and Anderson, 2002). THg values were square-root transformed to achieve a normal distribution of model residuals. As fixed factors, we considered the sampling year and the sampling date to control for any among and within year variation in environmental parameters that might have affected the condition of chicks, $\delta^{13}C$ and $\delta^{15}N$. The female identity was included as a random factor in all tested models (similar results were obtained if the male or the nest identify was included instead of female identity). In preliminary analyses, we also entered into the models the factors longitude and latitude of the nest or quadratic terms for latitude and longitude to account for nonlinear relationships and spatial autocorrelation (Legendre, 1993). All factors were not significant, thus they were not further considered in the final analyses. We then tested correlations between Hg and all markers of oxidative status by using Pearson correlation coefficient (similar results are obtained using square-root transformed Hg values). Finally, we tested the correlation between THg and body condition index (body mass normalised by body size index obtained from principal components analysis, explained below) using partial correlation. To do so, body mass was included in the partial correlation model as dependent variable, and both THg concentration and body size index as independent variables. This approach enabled to measure the strength and direction of the relationship between Hg and body mass whilst controlling for the effect of body size. The body size index was estimated using the PC1 from a PCA (60.5 and 56.7 % of variance for males and females, respectively) on beak length and beak width for males (loadings: 0.75) and females (loadings: 0.78), separately. These analyses on body condition index were carried out for males and females, separately, because they differed in both body mass and body size metrics.

3. Results

The concentration of THg in erythrocytes averaged 0.81 µg g^{-1} dw and varied from 0.23 to 4.29 µg g^{-1} dw (median = 0.72 µg g^{-1} dw, Table 1). One chick was excluded from the following statistical analyses because it was an influential outlier owing to a very high concentration of Hg (4.29 µg g^{-1} dw). The best-fitting model included sampling year and $\delta^{15}N$ as main predictors (Table 2); no other models were within 2 ΔAIC of the top model. The THg concentration was significantly higher in 2018 than in both 2016 and 2017, while it was similar between 2016 and 2017 (Table 3). Chicks that were fed upon higher trophic level prey (i.e., higher $\delta^{15}N$ values) had higher THg levels (Table 3, Figure 2). The maternal contribution was not significant (Wald = 0.01, P = 0.99), indicating that the variation in THg among related nestlings born in different years from the same mother was similar to that among unrelated nestlings. We found a similar result when the model was restricted to only those females that contributed a nestling in each of the three study years (data not shown). Neither the $\delta^{13}C$ values nor the sex explained the among chick variation in blood THg because models including these two factors were not supported (Table 2).

We found significant correlations between THg and protein carbonyls (r = 0.18, p = 0.004), GPx (r = -0.17, p = 0.005) or OXY (r = -0.13, p = 0.021) using individuals sampled in all three years. Blood THg and SOD were not significantly correlated (r = 0.06, p = 0.19). All correlations were no longer significant (p ≥ 0.21) when we used z-score transformed data to remove any effect owing to among year variation in both THg and marker values. Finally, we found that males with higher values of body condition index had also higher blood THg (partial correlation, r = 0.18, p = 0.04), while we did not find any correlation between blood THg and body condition in females (partial correlation, r = -0.03, p = 0.78). When we used z-score transformed values of Hg, body mass, and body size, the correlation between body condition

index and THg in males was even stronger (partial correlation, r = 0.28, p = 0.004), while we did not find any correlation between blood THg and body condition in females (partial correlation, r = 0.06, p = 0.56).

4. Discussion

4.1. THg variation and comparison with other studies on seabirds

We found large variation among chicks and sampling years in the blood concentration of THg. We sampled the chicks around three months after hatching (phase of asymptotic growth), thus we can reasonably exclude a significant contribution of Hg excreted by mothers into the single egg they lay because prior work on the related Cory's shearwater (*Calonectris borealis*) showed that chicks have a fast excretion rate mainly owing to body and plumage growth (growth dilution effect, Monteiro and Furness, 1995). Cory's shearwater chicks may, for example, excrete around 40-60 % of the Hg intake through the plumage (Monteiro and Furness, 2001b). Also, Hg has a half-time in blood of around six days owing to storage in internal tissues and excretion from the body (Monteiro and Furness, 2001b). The exponential phase of growth in chick Scopoli's shearwaters occurs in the first two months of life, thus it was ended a few weeks before we collected the blood samples. This suggests that the tHg concentrations reflected mainly the current exposure through the food owing to a modest excretion capacity.

Prior work showed that levels of Hg in eggs of Scopoli's shearwaters collected in three Mediterranean islands (Crete, Linosa, and Majorca) in the period 1982-1984 were 2.5-3.5 times higher than those recorded in eggs of Cory's shearwaters collected from Selvagen island in the Atlantic Ocean (Renzoni et al., 1986). Hg values in the three Mediterranean stations were quite homogeneous. Hg concentrations of all body tissues analysed were also higher in the Mediterranean stations than in the Atlantic. In particular, the liver Hg levels in Scopoli's shearwaters were four to six times higher than those in Cory's shearwaters (Renzoni et al.,

1986). Similarly, Ramos et al. (2009) found that the concentration of Hg in feathers collected from Mediterranean shearwaters in 2001 was significantly higher than in those collected from Atlantic shearwaters in the same year.

THg blood levels in shearwater chicks may be considered as moderate to high if compared to levels detected in other seabirds, some of which living in areas highly contaminated with Hg (e.g. Sebastiano et al., 2016, 2017; Carravieri et al., 2017, 2020). Blood THg concentrations in Scopoli's shearwaters were (i) higher than those recorded in blood of chicks of *Larus canus* in north Germany (Kahle and Becker, 1999), *Catharacta skua* on Shetland and Western Isles (Bearhop et al., 2000), *Hydropogne caspia* in San Francisco bay, California (Eagles-Smith et al., 2008), seven seabird species on Machias Seal Island in Canada (e.g., *Sterna paradisaea*, *Fratercula arctica*, *Somateria mollissima*; Bond and Diamond, 2009), *Sula nebouxii* on Sinaloa in the Gulf of California (Lerma et al., 2016), four tern species and *Leucophaeus atricilla* in French Guiana (Sebastiano et al., 2017), *Catharacta maccormicki* on Adélie land (Carravieri et al., 2017) and seven seabird species on Adélie land, Mayes island, Possession island and Amsterdam island (e.g., *Aptenodytes forsteri*, *Pagodroma nivea*, *Halobaena caerulea, Eudyptes chrysocome*; Carravieri et al., 2020), (ii) similar to those in blood of chicks of *Himantopus mexicanus* in San Francisco bay, California (Eagles-Smith et al., 2008), *Fregata magnificens* in French Guiana (Sebastiano et al., 2016, 2017) and five seabird species on Possession island and Amsterdam island (e.g., *Aptenodytes patagonicus*, *Thalassarche carteri*; Carravieri et al., 2020) and (iii) lower than those in blood of chicks of *Catharacta lonnbergi* on Kerguelen, Crozet and Amsterdam island (Carravieri et al., 2017) and *Diomedea amsterdamensis* on Amsterdam island (Carravieri et al., 2020). Finally, levels of blood THg of shearwater chicks were also comparable to those recorded in adults of two seabird species from Arctic and Antarctica that were likely high enough to interfere with major endocrine mechanisms regulating reproductive behaviour (black-legged kittiwake *Rissa*

tridactyla in Svalbard: Tartu et al., 2013, 2016; snow petrel *Pagodroma nivea* in Antarctica: Tartu et al., 2014, 2015). This large among species variation in blood THg appears to be mainly explained by geographic/spatial factors rather than trophic niche and also by unidentified characteristics of the species themselves (e.g., capacity of excretion, life-history), which deserve comparative models to be unraveled (Carravieri et al., 2020).

4.2. Sources of Hg contamination

Scopoli's shearwaters consume a wide range of prey (Grémillet et al., 2014) including discards from fisheries (Cianchetti-Benedetti et al., 2018). Accordingly, we found moderate variation in $\delta^{15}N$ among chicks; values ranged from 7.4 to 9.6 ‰ and the chick excluded from statistical analyses because of its very high THg concentration (i.e., outlier) also had the highest $\delta^{15}N$ value. Chicks had values of $\delta^{15}N$ similar to those recorded particularly in sardines, but also in squids and anchovies in Mediterranean (Cardona et al., 2015; Albo-Puigserver et al., 2016), suggesting that shearwaters were feeding on these particular prey. Prior work showed that sardines from the Sicily channel had Hg levels significantly higher than in sardines from other areas of the Mediterranean (Copat et al., 2012; Brambilla et al., 2013; Llull et al., 2017). Our results show that the trophic level of prey estimated by $\delta^{15}N$ values was significantly related to blood THg concentrations of chicks. This finding is in agreement with prior work, which showed that the blood concentration of THg is significantly linearly related to its intake in both chick and adult Cory's shearwaters (Monteiro and Furness, 2001a,b). Our results are also in line with work on other seabirds. Bearhop et al. (2000) found that some variation in blood THg of chick great skuas was explained by dietary specialisation because chicks that were fed upon higher trophic level prey had higher levels of THg in blood (correlation between blood THg and $\delta^{15}N$: Shetland, $r = 0.48$; Western Isles, $r = 0.46$). Similarly, chicks with higher $\delta^{15}N$ values had higher blood THg in six seabird species in French Guiana ($r \geq 0.44$, Sebastiano et al.,

2017). In the Kerguelen islands community, species-related Hg variations in chick feathers of 21 seabird species were highly and positively linked to $\delta^{15}N$ values (Blévin et al., 2013). This highlights the occurrence of efficient Hg biomagnification processes within marine trophic webs.

$\delta^{13}C$ values (range: -18.5 to -19.6 ‰) showed little variation, indicating that the foraging range of adult shearwaters was rather small. It is therefore not surprising that the levels of THg were not explained by the foraging area, as values of $\delta^{13}C$ were not associated with blood THg concentrations. In a previous GPS-tracking study of the same population, we actually found that adults go fishing mostly in the south of the island with a maximum linear distance of 75 ± 44 km (mean ± SD; Cianchetti-Benedetti et al., 2018). This result further supports the assumption that our data reflect local exposure.

Finally, we did not detect any differences in THg levels between male and female chicks. Similar results were obtained in previous work on adult Cory's shearwaters (Monteiro and Furness, 2001a). However, while males in better body condition had higher blood THg concentrations, the body condition of females was not correlated to their blood THg concentrations. We do not know the reasons for this difference between sexes. For example, if males receive more food than females, we might expect them to be more exposed to THg. Prior work on chick Cory's shearwaters (Monteiro and Furness, 2001b) did not find any effect of Hg administration on body condition; however, males and females were pooled together in the statistical models. This sex effect deserves further investigation.

4.3. Hg and blood oxidative status

We found little evidence for any possible short-term effects of Hg exposure on blood-based oxidative status markers. We found a positive correlation between the blood concentration of THg and protein oxidative damage and negative correlations between blood THg and two

antioxidant markers (GPx and OXY). These results would indicate that chicks suffer higher oxidative stress when being exposed to higher Hg. However, these correlations were affected by variation among sampling years in the mean values of these metrics. This result suggests little evidence for any possible short-term effects of Hg on the oxidative status at individual level. However, we cannot rule out the possibility that the among-year variation would indicate an effect of Hg on oxidative status at population level. This might be possible if (i) the within-year variation in THg was not large enough to induce visible effects or (ii) other environmental factors that also affect the oxidative status varied among years. These hypotheses deserve further work to be elucidated.

The correlation between THg and markers at population level are in agreement with the literature. Prior work on other seabirds found that the activity of GPx decreased with increased Hg in double-crested cormorants *Phalacrocorax auritus* (Henny et al., 2002), great egrets *Ardea alba* (Hoffman et al., 2005), and snowy egrets *Egretta thula* (Hoffman et al., 2009); the expression of the antioxidant genes GPx-3 and glutathione *S*-transferase μ3 increased with Hg exposure in female double-crested cormorants (Gibson et al., 2014); an increase of lipid oxidative damage was also related to high blood concentrations of Hg in wandering albatrosses *Diomedea exulans* (Costantini et al., 2014).

5. Conclusions

We found large within- and among-year variation in the blood concentration of THg of chicks, but not between male and female chicks. THg levels may be considered as moderate to high if we compare them to those detected in other seabirds. The correlations between blood THg and markers of oxidative status or individual body conditions indicate little concern for short-term harmful effects. In our population, most hatched chicks (> 90 %) survive until fledging, thus

they appear to tolerate these levels of Hg. However, we cannot exclude that any possible effects might emerge later in life.

Prior work on other seabirds showed that adults had levels of blood THg from 3 to 14 times higher than those recorded in their nestlings (e.g., Bearhop et al., 2000; Sebastiano et al., 2017). If this is also the case for Scopoli's shearwaters in our study area, taking the average of 0.81 µg g^{-1} dw as a reference for chicks, we might expect adults to have levels of blood THg ranging from ca. 2.43 to a very high concentration of 11.34 µg g^{-1} dw. This indicates that it will be important in future work to determine the THg levels of adults and if these are harmful to their physiological or reproductive functions.

Finally, it has been predicted that the new EU regulations would have caused a reduction of the total mass of Hg in the Mediterranean in 2020 by about 3-12 % compared to the levels measured in 2005 (Rajar et al., 2007). Although in vicinity of our study area, there are still active significant sources of anthropogenic Hg (Salvagio Manta et al., 2016), it will be of interest to verify if Hg exposure in shearwaters follows such expectations. Given the widespread diffusion of the Scopoli's shearwater in the Mediterranean, easy access to nests, fidelity of the birds to the breeding site and its habits of wintering in West Africa (Müller et al., 2014), it could be used for a prolonged biomonitoring of Hg exposure along a geographic and latitudinal gradient.

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Table 1. Descriptive statistics of mercury, stable isotopes and markers of oxidative status reported for each sampling year, separately. Values are shown as mean ± standard error; sample sizes are reported between brackets.

	2016	2017	2018
Hg (µg g^{-1} dw)	0.85 ± 0.05 (97)	0.56 ± 0.03 (79)	1.03 ± 0.06 (73)
$\delta^{15}N$ (‰)	8.46 ± 0.04 (97)	8.01 ± 0.03 (79)	8.48 ± 0.05 (73)
$\delta^{13}C$ (‰)	-18.89 ± 0.02 (97)	-18.87 ± 0.02 (79)	-18.97 ± 0.02 (73)
Protein carbonyls (nmol/g proteins)	2.33 ± 0.11 (95)	1.26 ± 0.14 (60)	2.80 ± 0.12 (73)
Glutathione peroxidase (units/mg proteins)	0.24 ± 0.02 (96)	0.37 ± 0.02 (75)	0.20 ± 0.03 (73)
Superoxide dismutase (units/mg proteins)	3.65 ± 0.09 (96)	1.89 ± 0.10 (77)	2.36 ± 0.11 (73)
OXY (mmol/l HOCl neutralised)	168 ± 4 (96)	217 ± 5 (78)	183 ± 5 (73)

Table 2. List of candidate models. $\delta^{13}C$ and $\delta^{15}N$ were not included in a same model because they were significantly correlated ($r = -0.47$, $p < 0.001$), thus raising an issue of collinearity. AIC = Akaike Information Criterion.

Model	AIC	ΔAIC	Weight
year + $\delta^{15}N$ + (female)	-203.9	0	0.944
$\delta^{15}N$ + (female)	-198.0	5.9	0.049
year + sex + $\delta^{15}N$ + (female)	-193.6	10.3	0.005
year + $\delta^{15}N$ + sampling date + (female)	-190.3	13.6	0.001
year + sex + $\delta^{15}N$ + sampling date + (female)	-180.2	23.7	0.000
year + (female)	-103.5	100.4	0.000
year + $\delta^{13}C$ + (female)	-102.3	101.6	0.000
year + sex + (female)	-95.1	108.8	0.000
year + sex + $\delta^{13}C$ + (female)	-93.7	110.2	0.000
year + sampling date + (female)	-90.5	113.4	0.000
year + $\delta^{13}C$ + sampling date + (female)	-89.3	114.6	0.000
year + sex + $\delta^{13}C$ + sampling date + (female)	-80.9	123.0	0.000
$\delta^{13}C$ + (female)	-59.0	144.9	0.000
sex + (female)	-44.6	159.3	0.000

Table 3. Outcome of the best-fitting model.

Factor	**Reference level**	**Level**	**Coefficient estimate**	**Standard error**	**t**	**p**
Intercept			-1.75	0.24	-7.4	< 0.001
Sampling year	2016	2017	-0.01	0.03	-0.3	0.79
	2016	2018	0.09	0.02	3.8	< 0.001
	2017	2018	0.10	0.03	3.5	0.001
$\delta^{15}N$			0.32	0.03	11.6	< 0.001

Figure 1. Distribution of nests on Linosa island. Birds breed inside crevices in the lava formation, and are mostly concentrated on the coast of Mannarazza, on the northern side of the island. Sources of images: Google Maps and Google Earth Pro, ©2018 Google.

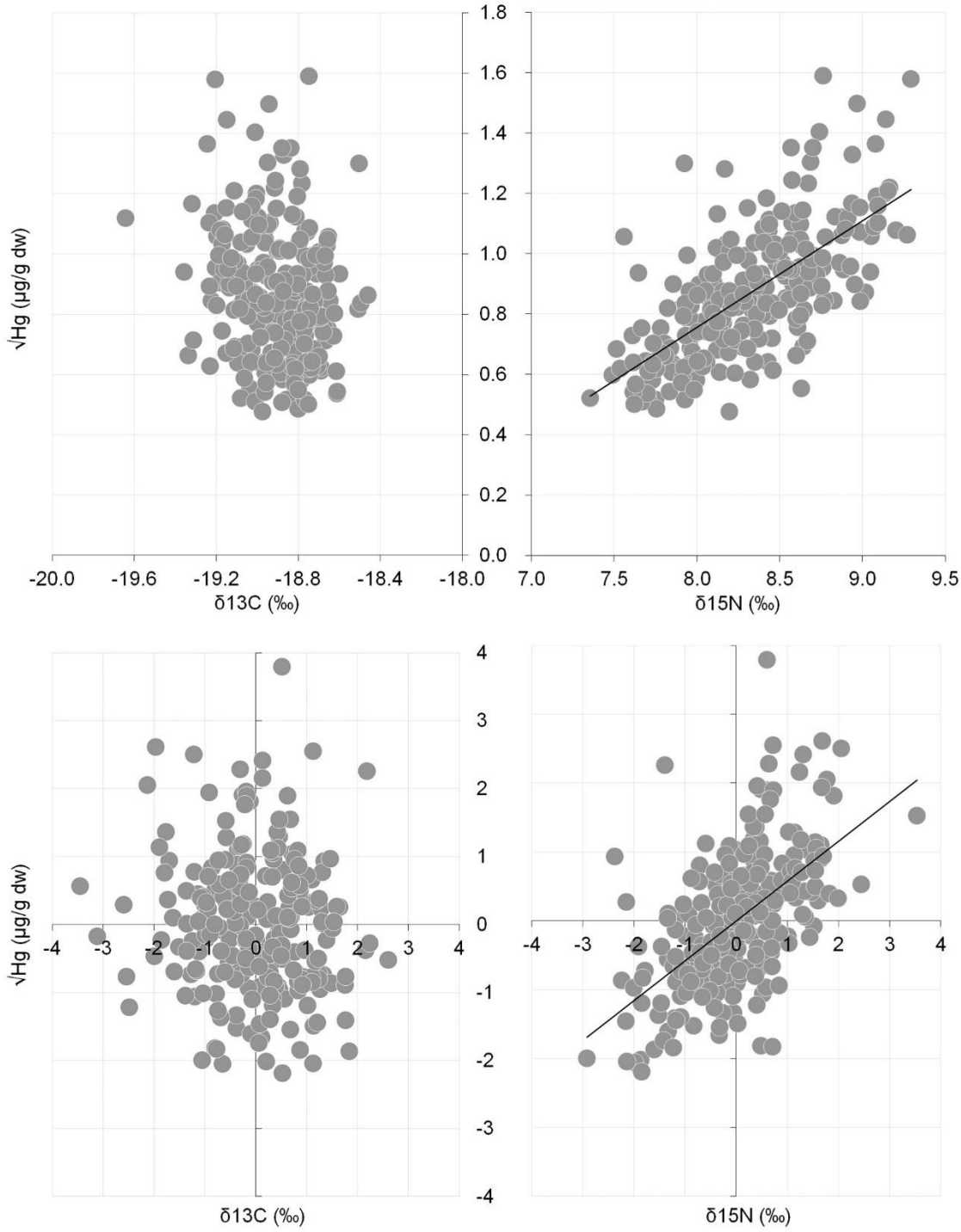

Figure 2. Relationships between blood Hg concentrations and the stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$. The regression line is shown only when the relationship was statistically significant. Upper graphs show untransformed values, while lower graphs show z-scores calculated to remove annual variation. The y-axis is placed on the right side because it is shared by both panels.

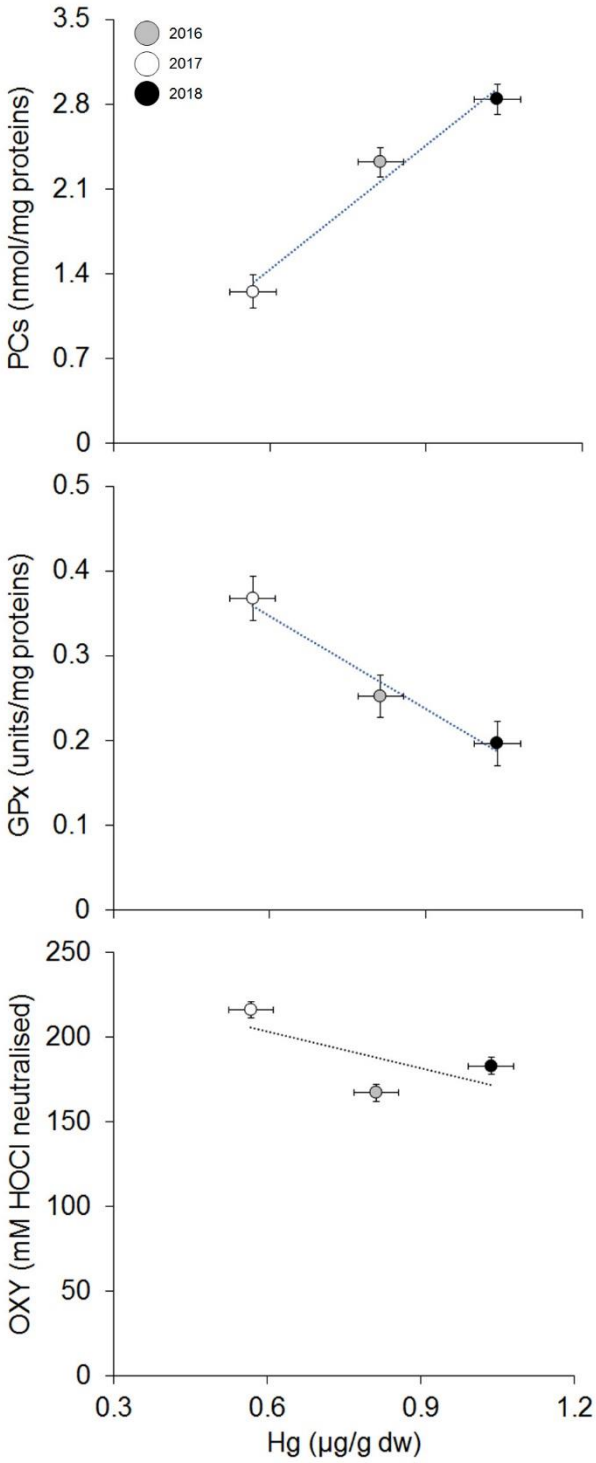

Figure 3. Relationships between population-level blood Hg concentrations and three markers of oxidative status: protein carbonyls (PCs), glutathione peroxidase (GPx) and serum non-enzymatic antioxidant capacity (OXY). Values are shown as means ± standard errors.