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David Costantini • Alizée Meillere • Alice Carravieri • Vincent Lecomte • Gabriele Sorci • Bruno Faivre • Henri Weimerskirch • Paco Bustamante • Pierre Labadie • Hélène Budzinski • Olivier Chastel

D. Costantini
Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
Institute for Biodiversity, Animal Health and Comparative Medicine, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK
E-mail: david.costantini@uantwerpen.be
Telephone: +32 032652259

A. Meillere • A. Carravieri • V. Lecomte • H. Weimerskirch • O. Chastel
Centre d'Etudes Biologiques de Chizé (CEBC), UPR 1934-CNRS, F-79360, France

G. Sorci • B. Faivre
Université de Bourgogne, UMR – CNRS 6282 Biogéosciences, 6 Boulevard Gabriel, F-21000 Dijon, France

P. Bustamante
Littoral Environnement Société (LIENSs), UMR 7266-CNRS, Université de La Rochelle, 2 rue Olympe de Gouges, F-17000 La Rochelle, France

P. Labadie • H. Budzinski
UMR 5805 EPOC-LPTC, Université Bordeaux, 351 Cours de la Libération, F-33405 Talence Cedex, France

Author Contributions: OC, DC, VL, GS and H.W. designed research; VL performed field work; DC, AM, AC, VL, GS, BF, HW, PB, PL and HB and OC performed research; DC analysed the data; and DC and OC wrote the paper.

Abstract: Reproduction is a demanding activity for animals, since they must produce, and in some cases protect and provision, their young. It is often overlooked that demands of reproduction may also be exacerbated by exposure to contaminants. In this study, we make use of an exceptional long-term dataset to perform a cross-sectional study on the long-lived wandering albatross (*Diomedea exulans*) in order to test the effects of reproduction, persistent organic pollutants [POPs: pesticides and polychlorinated biphenyls (PCBs)], mercury, individual age (3–47 years), and sex on the levels of plasma oxidative damage and inflammation. The results of our study support the hypothesis that oxidative damage may be a physiological cost of reproduction and that individuals carrying higher levels of organic or non-organic contaminants have higher oxidative damage. Levels of the inflammatory protein haptoglobin were similar between breeding and nonbreeding birds, with the exception of breeding males which had the lowest levels of haptoglobin. Our data also show an effect of age and of organic contaminants on the plasma oxidative damage level, but not on plasma haptoglobin. In addition, plasma oxidative damage level increased with red blood cell mercury concentration in females but not in males. Hence, our study highlights that the harmful effects of contaminants may come through interaction with factors like life stage or gender, suggesting potential for high variation in susceptibility to contamination among individuals.

Keywords: Ageing • Inflammation • Mercury • Oxidative stress • POPs • Reproduction • Seabirds

Introduction

Reproduction is a critical and demanding phase of animals' lives. Transition from a non-reproducing to a reproducing state is associated with hormonal changes that may incur in various metabolic costs to the individual (Ketterson and Nolan 1992). Reproductive effort (e.g., egg production, parental care) may also have costs that are paid later in life in terms of reduced survival or fecundity (Stearns 1992). Oxidative stress has been proposed as one cost associated with reproduction (Alonso-Alvarez et al. 2004; Wiersma et al. 2004; Costantini 2008; Metcalfe and Monaghan 2013). Oxidative stress is the rate at which oxidative damage is generated, which depends on a complex balance between pro- and antioxidant mechanisms, including repair systems (Halliwell and Gutteridge 2007). Such damage can contribute to cell senescence, loss in organ and organism performance, and may influence life-history strategies (Costantini 2008; Metcalfe and Alonso-Alvarez 2010). Progressive damage to biomolecules (e.g., oxidation of DNA, lipids or telomeres) may also trigger an inflammatory response, a mechanism used by the organism to protect itself from a stressful agent (Sorci and Faivre 2009; Jelena et al. 2013). Inflammation-inducible proteins, like haptoglobin, can limit the spread of oxidative damages across tissues by binding molecules with pro-oxidant activity (Jelena et al. 2013).

An individual that reproduces in a polluted environment is also faced with the need of controlling the action of contaminants because they can influence the immune activity, increase stress levels, or reduce survival of wild animals (Dell'Omo 2002; Koivula and Eeva 2010; Isaksson 2010). Interest in the interactions between contaminants and organism adaptation in a changing world has dramatically grown in the last few decades. It is, however, sometimes forgotten in ecological and behavioral studies that some of the among individuals or species variation in life-histories or physiological parameters may simply be the by-product of contaminant exposure (Carere et al. 2010; Frederick and Jayasena 2011; Tartu et al. 2013). For example, several persistent organic pollutants (POPs) can cause reproductive failure in free-living birds through different mechanisms, such as eggshell thinning, embryo mortality, or alterations of reproductive behaviors (e.g., Burger and Gochfeld 2004; Bustnes et al. 2003, 2012). Biomagnification of the non-essential metal mercury in food webs is also a great cause of concern, because it causes detrimental effects on behavior, neurology, endocrinology, or development in humans and wildlife (Scheuhammer et al. 2007; Tan et al. 2009; Frederick

and Jayasena 2011; Tartu et al. 2013). Moreover, it has been shown in some studies that both organic and non-organic contaminants can increase generation of oxidative damage (Metodiewa and Dunford 1990; Whysner and Wang 2001; Isaksson 2010). Therefore, contaminants and oxidative stress might interact, possibly exacerbating costs of reproduction and of other life history stages. This may be especially true for top predators (e.g., seabirds, birds of prey) in which accumulation of contaminants in the body is higher (biomagnification).

In this study, we assessed the effects of reproduction, persistent organic pollutants (organochlorine pesticides and PCBs), mercury, individual age (3–47 years), and sex on the levels of plasma oxidative damage and inflammation. To this end, we performed a cross-sectional study on known-age free-living wandering albatrosses (*Diomedea exulans*), a large and very long-lived species (≈50 years). We make use of an exceptional long-term dataset (Weimerskirch et al. 1997) to identify the history of each bird. The non-breeding part of the albatross population is composed of young, immature birds that have never bred before and middle-aged or old birds that have considerable breeding experience but did not breed over the course of the field study (Weimerskirch et al. 1997). Using the non breeding component of a population offered us the opportunity to have a control group for the breeding season during which we carried out the field work; this is a crucial aspect that field studies have invariably failed to include in tests of the oxidative cost of reproduction (Metcalfe and Monaghan 2013). Furthermore, wandering albatrosses bear high levels of some contaminants in their tissues (Hindell et al. 1999; Blévin et al. 2013), a feature that makes these seabirds a suitable species to investigate the effects of POPs and mercury on plasma oxidative damage and inflammation.

Materials and methods

Study area and birds

The field study was carried out on Possession Island in the southwestern Indian Ocean (46.8°S, 51.8°E), where 300–400 pairs of wandering albatrosses nest each year. Wandering albatrosses return to their breeding grounds in December and females lay a single egg in late December–early January. Both parents incubate alternatively until hatching in March. All birds had been ringed as part of a long-term mark–recapture program (Weimerskirch et al.

1997), with nestlings being ringed since 1965. From 21 December 2007 to 4 March 2008, we captured wandering albatrosses on the breeding grounds and a sample of venous blood was taken from the tarsus within 3 min of capture with a 1-ml heparinized syringe and a 25-gauge needle. The volume of the blood draws never exceeded 0.05 % of the bird's body mass (8–12 kg). The blood was centrifuged to separate plasma (for POPs, oxidative damage, and inflammation analyses) from red blood cells (for total mercury and molecular sexing), which were then stored at −20 °C until laboratory analyses. Sample sizes varied across analyses because we could not collect all the data from each individual: breeding males were 40–46; breeding females were 27–33; non-breeding males were 20–33; non-breeding females were 7–11 (for sample sizes, see also figure captions). Birds were sexed by molecular sexing according to Fridolfsson and Ellegren (1999). Data on oxidative damage and inflammation have been previously published by our group in an article in relation to aging of albatrosses (Lecomte et al. 2010). Here, we are revisiting this database in order to simultaneously analyze the effects of breeding activity and body contaminant levels on oxidative damage and inflammation.

Measurement of plasma oxidative damage

The Cayman's TBARS assay (Cayman Chemical, Ann Arbor, USA) was used to measure the thiobarbituric acid reactive substances in plasma. This method provides a general quantification of oxidative damage molecules that occur in the plasma, such as those generated by lipid peroxidation or carbonylation. The principle of the assay is based on the formation of an adduct between the thiobarbituric acid and the oxidative damage molecules under high temperature (90–100 °C) and acidic conditions, which generates a color directly proportional to the concentration of oxidative damage molecules. First, 10 µl of each plasma sample or standard were added to 10 µl of sodium dodecyl sulfate into 500-µl vials, and mixed. Then, 400 µl of color reagent (132.5 mg of thiobarbituric acid diluted into 12.5 ml of an acetic acid solution and 12.5 ml of a sodium hydroxide solution) were added to each plasma solution, and capped vials were kept in boiling water for 1 h. After 1 h, the vials were removed from boiling water and immediately put on ice for 10 min in order to stop the reaction. Finally, 150 µl of each solution were randomly pipetted in well plates and readings were taken at 530 nm. Standard curves were obtained from serial dilutions of an initial

standard of MDA (from 0 to 50 μM). The coefficient of variation of measures was 9.0 %.

Measurement of haptoglobin

Plasma haptoglobin (inflammation-inducible protein) was measured using a colorimetric assay (Tri-Delta Development, Ireland) based on hemoglobin-binding reaction. In plasma, haptoglobin binds free hemoglobin released from erythrocytes, so inhibiting its pro-oxidative activity. First, 7.5 μl of each plasma sample or standard were randomly pipetted in well plates. To each well, 100 μl of a solution of hemoglobin and 140 μl of a solution of chromogen were added, respectively. Plates were then agitated and left to incubate for 5 min at room temperature. Then, solutions were read at 630 nm. Standard curves were obtained from serial dilutions of an initial standard (0–2.5 mg/ml). The coefficient of variation of measures was 3.7 %.

Measurement of mercury

Total mercury was analyzed in red blood cells, which indicates short-term exposure (Kahle and Becker 1999; Tartu et al. 2013; Goutte et al. 2014). Total mercury (Hg) was quantified with an Altec AMA 254 spectrophotometer (aliquots mass: 5–10 mg dry weight, dw; see also Bustamante et al. 2006). All analyses were repeated 2–3 times until having a relative standard deviation <5 %. Accuracy was checked using a certified reference material (CRM; Tort-2 Lobster Hepatopancreas, NRC, Canada; certified Hg concentration: 0.27 ± 0.06 μg/g dw). Our measured values were 0.24 ± 0.02 μg/g dw, n = 31. Blanks were analyzed at the beginning of each set of samples and the detection limit of the method was 0.005 μg/g dw. Mean values of replicates were used in statistical analyses.

Measurement of POPs

The targeted compounds included seven indicator PCBs (CB-28, -52, -101, -118, -138, -153, and -180), and 10 organochlorine pesticides. The compounds chosen for further investigation were CB-99, -105, -118, -128, -138, -153, -180, -183, -187, and -194, and the Σpesticides (*p*,*p*-DDE, HCB, *cis*-chlordane, *trans*-nonachlor). Certified solutions containing all analytes at

2 ng/µl each were obtained from LGC Standards (Molsheim, France). To each plasma sample of 100 µl, internal standards (1 ng each) were added gravimetrically: PCBs 30, 103, 155, and 198 were used to quantify PCBs and *p,p′*-DDT-d8 was used to quantify pesticides, respectively. All standards were provided by either Dr Ehrenstorfer or Cambridge Isotope Laboratory (via Cluzeau Info Labo, Sainte-Foy-La-Grande, France). POPs were extracted using 1 ml of pentane:dichloromethane (90:10; v/v); after centrifugation (2,000 rpm, 2 min at 4 °C), the organic layer was collected and the operation was repeated. Both extracts were combined and purified on an acid silica gel column (40 % sulphuric acid, H_2SO_4). After extract loading, analytes were eluted with 3 × 5 ml of pentane/dichloromethane (90/10; v/v). The extract was then concentrated using a Rapid-Vap vacuum evaporation system from Labconco (Kansas City, MO, USA) to a volume of 1 ml; it was then further concentrated under a gentle stream of nitrogen (40 °C) after addition of 100 µl of isooctane as solvent keeper. A syringe standard (octachloronaphtalene, 1 ng) was finally added to quantify internal standards and to assess their recovery rate for each sample (68–108 %). Final extracts were analyzed by gas chromatography coupled with electron capture detection (GC-ECD) as described elsewhere (Tapie et al. 2011). The quality control was done by means of the analysis of procedural blanks (clean and empty glass tubes treated like a sample, one run for 8 samples). Chicken plasma samples (Sigma-Aldrich, St Quentin Fallavier, France) spiked at 3 ng/g were also analyzed; the recovery rates of PCBs and organochlorine pesticides were in the range 77–103 % with coefficients of variation lower than 17 % (n = 5), except for CB-52 (22 %) and mirex (29 %). POPs levels were blank corrected and the detection limit (LoD) was set at two times the mean blank value; for analytes that were not detected in blanks, LoD was determined as the concentration with a signal to noise ratio of 3. Overall, LoDs ranged from 0.09 to 0.76 ng/g wet weight. Additionally, serum total lipids were measured on an aliquot of 10 µl by the sulfo-phospho-vanillin (SPV) method for colorimetric determination (e.g., Houde et al. 2006). Ecological effects of POPs will be fully discussed in a separate article.

Statistical analyses

Analyses were carried out using STATISTICA (v.10; Stat-Soft, Tulsa, OK, USA). We used generalized linear models with a backward removal (critical *P* value at 0.05) to build the

minimum model significantly explaining the observed variance. A normal distribution and identity link function was used for the plasma oxidative damage; a normal distribution and log link function was used for haptoglobin. In each model, we included the individual reproductive status at the time of bleeding (breeding vs. non-breeding) and the sex as main factors, and the individual age as a covariate. We also included the concentration of mercury or an index of organohalogenated contaminant concentration as covariate. Two-way interactions were also included. In the non-breeding cohort of our population, we included both individuals that were sexually mature or were not (i.e., <6 years of age; Weimerskirch et al. 1997) because they did not differ in oxidative damage nor in haptoglobin ($P \geq 0.26$). In the breeding cohort, we had some pairs (14–17); however, we did not include it in the models because preliminary analyses showed that a pair never significantly explained variation in oxidative damage nor in haptoglobin ($P \geq 0.85$). Importantly, breeding and non-breeding birds did not differ in age in all databases used for the statistical analyses (*t* test: $P \geq 0.11$; e.g., in the larger database, the mean age and standard error were 23.9 ± 1.3 years and 23.1 ± 2.4 years for breeding ($n = 71$) and non-breeding ($n = 44$) birds, respectively). In our dataset, there was no multicollinearity because the variance inflation factor was always <2.

As an index of organohalogenated contaminant concentration, we used the first or the second principal component that we extracted from a principal component analysis (PCA) on the correlation matrix of organohalogenated toxicants. We excluded from the statistical analyses levels of contaminants that had low loadings in a preliminary PCA or that had values below the detection limit. In the end, 13 organohalogenated chemicals met our requirements to enter in the final models. Two PCAs were run for oxidative damage and haptoglobin databases, separately. In both cases, the first two principal components explained 67 % of the total variance of contaminants for the oxidative damage database (PC1 = 44 %, PC2 = 23 %) and for the haptoglobin database (PC1 = 45 %, PC2 = 22 %; Table 1), respectively. Of the 13 compounds, only γ-HCH and CB-180 had low loadings on the two main axes of variation. We have therefore tested their effect on oxidative damage or inflammation separately. Given that their effect was not significant (data not shown), we have not discussed them. Since blood volume was not always sufficient to carry out all physiological and contaminant analyses, and since outliers were excluded from the database, sample sizes differed among statistical models (see figure captions).

Results

Plasma oxidative damage and mercury

Breeding albatrosses had higher levels of plasma oxidative damage than non-breeding birds (Fig. 1; Table 2); older non-breeding individuals tended to have a higher oxidative damage level than younger individuals, but the relationship between age and oxidative damage did not appear in breeding birds (Fig. 2; Table 2). Differences between males and females in oxidative damage were affected by the individual load of mercury: females having high mercury concentration suffered higher oxidative damage ($r = 0.34$, $P = 0.038$), while males showed an opposite, although non-significant pattern ($r = -0.20$, $P = 0.078$; Fig. 3; Table 2).

Plasma oxidative damage and persistent organic pollutants

In the model where the PC1 was included as a covariate (Table 2), similar differences between breeding and non-breeding individuals emerged as for the model of mercury; moreover, birds with high levels of organohalogenated toxicants had higher levels of oxidative damage (Fig. 4). In the model with PC2 as a covariate (Table 2), a difference again emerged between breeding and non-breeding birds in oxidative damage level.

Haptoglobin and mercury

Mercury did not affect the concentration of haptoglobin. The final minimum model showed only a significant interaction between reproductive status and sex (Table 2): while haptoglobin concentration in non-breeding individuals was similar between males and females, haptoglobin was higher in breeding females than in breeding males (Fig. 5).

Haptoglobin and persistent organic pollutants

PC1 and PC2 did not significantly influence the concentration of haptoglobin. As for the model with mercury, only the interaction between reproductive status and sex was significant (Table 2).

Discussion

The results of our study show for the first time in a free-living vertebrate that breeding individuals may have higher plasma oxidative damage than non-breeding individuals. Levels of the inflammatory protein haptoglobin were similar between breeding and non-breeding birds, with the exception of breeding males which had the lowest levels of haptoglobin. Our data also show an effect of age and contaminants on the plasma oxidative damage level, but not on plasma haptoglobin. The cost of reproduction is a central paradigm of life-history theory (Stearns 1992). We currently know very little about the currency of costs of reproduction. It has been suggested that oxidative stress may be one key cellular mechanism underlying the costs of reproduction (Costantini 2008; Metcalfe and Alonso-Alvarez 2010). However, there is not a general consensus (Metcalfe and Monaghan 2013). The few studies in which reproductive effort was manipulated, for example, found that enzymatic and non-enzymatic antioxidant defences may be altered, possibly sacrificed in favor of investment in reproduction (Alonso-Alvarez et al. 2004; Wiersma et al. 2004; Losdat et al. 2011) or up-regulated in response to an increase in free radical production (Garratt et al. 2013). Moreover, while the increase in egg production effort may induce an increase in plasma oxidative damage (Travers et al. 2010), experimental increases of offspring rearing effort did not result in an increase in oxidative damage (Garratt et al. 2013). Metabolic costs of reproduction may also be induced by increases in sexual hormones that accompany the transition from a non-reproducing to a reproducing state (Alonso-Alvarez et al. 2007; Casagrande et al. 2012). Our data were collected in a way that does not allow us to disentangle the relative contributions of changes in hormonal status and effort in egg production or chick rearing. Regardless of mechanisms, our findings provide evidence, at least in this particular species, that reproductively active individuals may have higher plasma oxidative damage than individuals of comparable age that are not breeding. In contrast to oxidative damage, results on haptoglobin apparently provided a different picture. While reproducing and non-reproducing females did not differ, reproducing males had lower levels of plasma haptoglobin than non-reproducing males and all females. Haptoglobin is a well-known acute phase protein that indicates an ongoing inflammatory response, and is found in a wide range of taxa, including birds. Haptoglobin normally circulates at low levels, but concentrations increase during inflammatory responses. It exerts an antioxidant activity because it binds free hemoglobin

released from erythrocytes, so inhibiting its pro-oxidative activity mediated by iron (Jelena et al. 2013). Studies on various bird species have found that haptoglobin concentration is a distinctive trait of an individual and has the capacity to predict endotoxin-induced changes in concentration of this acute phase protein (Matson et al. 2012). A decrease in circulating haptoglobin is often reported as an index of a status of haemolytic anaemia (Körmöczi et al. 2006). We do not know why haptoglobin in breeding males was lower than other birds. It might suggest that breeding males were in better health status (e.g., in cases in which the parasite load was low) than other birds. In particular, the high levels of haptoglobin in non-breeding birds might indicate that they skipped reproduction because of a poor health status. However, the previously described association between low haptoglobin concentration and hemolytic anemia (Körmöczi et al. 2006) requires caution in interpretation. Further studies will, therefore, be needed to ascertain the role of haptoglobin as a parameter of health status in albatrosses.

Our data also show that older individuals had a higher plasma oxidative damage level than younger individuals. However, the increase in plasma oxidative damage with individual chronological age only emerged in non-breeding birds. Previous work on the same population of breeding wandering albatrosses showed that individual age strongly affected foraging behavior and reproductive performance, but was unrelated to physiological measures like immune markers or hormones (Lecomte et al. 2010). Another study on a different population of wandering albatrosses revealed that declines in performance with age are followed by a striking increase in breeding success and a key parental investment trait at the final breeding attempt (Froy et al. 2013). Physiological changes typical of reproduction might therefore have hidden the relationship between age and accumulation/overproduction of oxidative damage. Breeding albatrosses tended to have higher levels of plasma oxidative damage than non-breeding birds regardless of individual age. When birds are around 40 years old, however, the differentiation in oxidative damage between breeding and non-breeding birds became less evident, probably because of a terminal increase in damage in non-breeding birds. From our dataset, it is unclear whether the increase in oxidative damage reflects senescence and why such an increase in older individuals was not observed in breeding birds. Individual age was strongly correlated with the number of lifetime individual breeding events ($r = 0.83$, $P < 0.001$). Hence, the increase in damage with age might also reflect reproduction-induced accumulation of damage. Our data are cross-sectional, which makes it impossible to separate

within-individual ageing patterns from between individual heterogeneity and any effects of differential survival among phenotypes. Our results suggest that a careful selection of relevant markers of physiological oxidative status is needed to test whether or not the observed terminal increases in reproductive investment in wandering albatrosses or other species are independent of individual state.

Individual contaminant load significantly contributed to explain variation in oxidative damage but not in inflammation. Albatrosses carrying higher levels of POPs had higher oxidative damage. Our data are in line with a recent meta-analysis showing a significant impact of pollution on various classes of antioxidants and oxidative damage in wild animals (Isaksson 2010). Although our analysis was restricted to a single biomarker of oxidative damage in plasma, Isaksson (2010) showed that thiobarbituric acid reactive substances in blood are a sensitive biomarker to contaminant exposure. POPs may cause oxidative stress through various routes, like increases in superoxide anion production (Metodiewa and Dunford 1990) or free iron release from metalloproteins (Whysner and Wang 2001). POPs might also cause oxidative stress through increases in corticosterone secretion (Costantini et al. 2011). This is relevant because studies on kittiwakes (*Rissa tridactyla*) and glaucous gulls (*Larus hyperboreus*) found that baseline corticosterone concentration was higher in those birds with higher POPs burden (Verboven et al. 2010; Nordstad et al. 2012). Preliminary data also show that corticosterone increases with POP levels in wandering albatrosses (Alizée Meillere and Sabrina Tartu, unpublished data). Previous studies on seabirds have also shown that exposure to POPs may cause changes in the oxidative balance (Hegseth et al. 2011; Bourgeon et al. 2012) and reduce survival (Erikstad et al. 2013). Overall, these findings suggest that oxidative stress might be a plausible mechanism involved in the reduction of longevity in individuals highly contaminated with POPs.

As regards mercury, females having high mercury concentration in red blood cells (Carravieri et al., unpublished data) suffered high plasma oxidative damage. In contrast, males showed an opposite but non-significant pattern. This difference between sexes in the relationship between mercury and oxidative damage is possibly explained by females (mean level of 10.3 μg/g dry weight) carrying higher levels of mercury than males (mean level of 6.3 μg/g dry weight). Similarly, Tavares et al. (2013) found that females had significantly higher levels of mercury in red blood cells than males in wandering albatrosses from South Georgia. Results on wandering albatrosses differ from those of previous studies on various fish-eating bird

species, which found that mercury concentrations in males are in most cases higher than females (Monteiro and Furness 1995; Robinson et al. 2012). Such difference in albatrosses might be driven by males and females foraging on different prey or different environments (Weimerskirch et al. 1993) that expose them to differing mercury threats. If foraging strategies differ between males and females, this may also contribute to explain the higher plasma oxidative damage of females if, for example, their diet is richer in unsaturated fatty acids or if the foraging cost for females is higher.

Mercury is a highly toxic non-essential metal that negatively influences humans and wildlife (Tan et al. 2009). Our data suggest that mercury might also negatively impact on females through an increase in oxidative stress. The increase in damage with mercury level was, however, similar between reproducing and non-reproducing birds.

In birds, mercury is excreted through its deposition in the feathers during the molt (Monteiro and Furness 1995). In this pathway, mercury binds to keratin sulfhydryl groups during synthesis of feathers. Seabirds with slow molt cycles, such as albatrosses, may, however, have a limited capacity for mercury elimination through feathers (Thompson and Furness 1989). Mercury may also be excreted from the body through the glutathione pathway. Mercury binds to the sulfhydryl groups on glutathione or on other thiols and forms a complex that is finally excreted in the feces (Ballatori and Clarkson 1985). Sulfhydryl groups like thiols are important molecules that regulate the oxidative balance and any depletion leads to disruption of redox signaling and control and an increase in oxidative stress (Jones 2006). High contamination with mercury might lead to a high depletion of thiols regardless of the pathway of excretion, which may compromise defenses against oxidation. Consequent depletion of glutathione to mercury contamination may, for example, compromise the activity of the enzyme glutathione peroxidase (Hoffman and Heinz 1998), which uses glutathione as a cofactor to detoxify the organism from peroxides and hydroperoxides. Importantly, the activity of selenium dependent glutathione peroxidase may be further compromised by depletion of selenium that is being used to biosynthesize mineral granules containing mercury and selenium in the liver (Nigro and Leonzio 1996). Our results raise the need of further studies to elucidate the consequences of oxidative stress induced by mercury in this threatened seabird species. In particular, further studies are needed to assess whether oxidative stress induced by mercury impinges on reproductive fitness or on other fitness-related traits.

In conclusion, results of our study support the hypothesis that oxidative stress may be higher

in reproducing individuals, possibly reflecting a transient increase in damage over the breeding phase. Our results also suggest that the individual oxidative stress level may be shaped by the interaction between exposure to contaminants and other factors, such as sex. Finally, our data provide evidence that plasma oxidative damage is higher in non-breeding older individuals. We highlight the need of future studies in order to tease apart the mechanisms (e.g., hormones, parental effort) that contribute to increase the oxidative damage level in breeding individuals. We also highlight the need of using additional biomarkers of oxidative damage in order to assess whether the effects of reproduction and contaminants on oxidative balance are systemic or are limited to particular molecular classes or body tissues.

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Table 1. Loadings (expressed as correlation coefficient of each single contaminant with PC1 or PC2) of variables onto the first two principal components.

	Oxidative damage		Haptoglobin	
	PC1	PC2	PC1	PC2
HCB	0.509	**0.658**	0.480	**0.685**
γ-HCH	0.308	0.485	0.274	0.476
PCB-52	**0.704**	-0.474	**0.719**	-0.458
2,4-DDE	**0.761**	-0.066	**0.765**	-0.093
PCB-101	**0.715**	-0.610	**0.724**	-0.602
Cis-chlordane	**0.788**	0.314	**0.798**	0.317
Trans-nonachlor	**0.653**	0.518	**0.656**	0.502
PCB-118	**0.710**	-0.640	**0.724**	-0.618
4,4-DDD	**0.622**	0.524	**0.634**	0.502
PCB-153	**0.800**	-0.210	**0.795**	-0.195
PCB-138	**0.798**	-0.411	**0.802**	-0.382
PCB-180	0.576	0.040	0.562	0.067
Mirex	0.541	**0.647**	0.528	**0.657**

The first two principal components explained 67% of the total variance of contaminants for the oxidative damage database (PC1 = 44%, PC2 = 23%) and for the haptoglobin database (PC1 = 45%, PC2 = 22%). Positive and negative loadings indicate a positive or a negative correlation between the contaminant and the PC1 or the PC2; contaminants with the same loading sign, therefore, go in the same direction. DDD = dichlorodiphenyldichloroethane, DDE = dichlorodiphenyldichloroethylene, HCB = hexachlorobenzene, γ-HCH = lindane, PCB = polychlorinated biphenyl.

Table 2. Generalized linear models explaining the factors that influenced the plasma oxidative damage and plasma haptoglobin variation in wandering albatrosses.

	Inital Model			Final Model				Inital Model			Final Model		
Oxidative damage	df	Wald	p-value	df	Wald	p-value	Haptoglobin	df	Wald	p-value	df	Wald	p-value
reprod status	1	6.01	0.014	1	11.43	0.001		1	1.94	0.164			
sex	1	1.79	0.180	1	4.62	0.032		1	1.09	0.296			
age	1	1.88	0.170					1	0.04	0.844			
mercury	1	0.15	0.702					1	2.51	0.113			
reprod status × age	1	5.39	0.020	1	4.19	0.041		1	0.07	0.794			
reprod status × mercury	1	0.67	0.415					1	3.63	0.057			
reprod status × sex	1	1.29	0.257					1	5.31	0.021	1	6.95	0.008
sex × age	1	0.03	0.859					1	2.65	0.103			
sex × mercury	1	5.24	0.022	1	5.68	0.017		1	0.02	0.896			
reprod status	1	7.585	0.006	1	6.63	0.010		1	0.206	0.650			
sex	1	0.519	0.471					1	2.186	0.139			
age	1	1.250	0.264					1	0.129	0.719			
PC1	1	0.869	0.351	1	4.27	0.039		1	0.233	0.629			
reprod status × age	1	2.891	0.089					1	0.053	0.818			
reprod status × PC1	1	0.018	0.894					1	0.096	0.756			
reprod status × sex	1	0.085	0.771					1	4.299	0.038	1	7.90	0.005
sex × age	1	0.472	0.492					1	2.622	0.105			
sex × PC1	1	0.798	0.372					1	0.778	0.378			
reprod status	1	10.98	0.001	1	11.67	0.001		1	0.56	0.456			
sex	1	0.44	0.507					1	2.67	0.103			
age	1	1.15	0.283					1	0.39	0.531			
PC2	1	0.02	0.902					1	0.13	0.718			
reprod status × age	1	2.63	0.105					1	0.01	0.925			
reprod status × PC2	1	0.00	0.975					1	0.39	0.532			
reprod status × sex	1	0.00	0.950					1	2.04	0.153	1	7.90	0.005
sex × age	1	0.44	0.509					1	2.23	0.136			
sex × PC2	1	0.21	0.648					1	0.01	0.923			

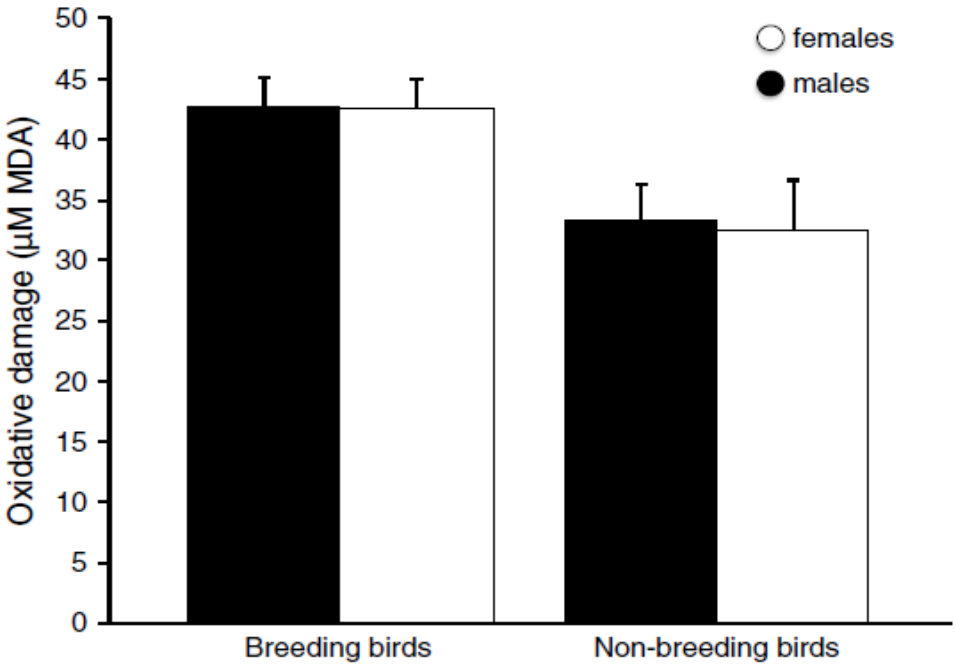

Figure 1. Breeding albatrosses (males, *n* = 40; females, *n* = 31) had higher plasma oxidative damage than non-breeding birds (males, *n* = 33; females, *n* = 11) irrespective of sex. Data are shown as mean plus standard error.

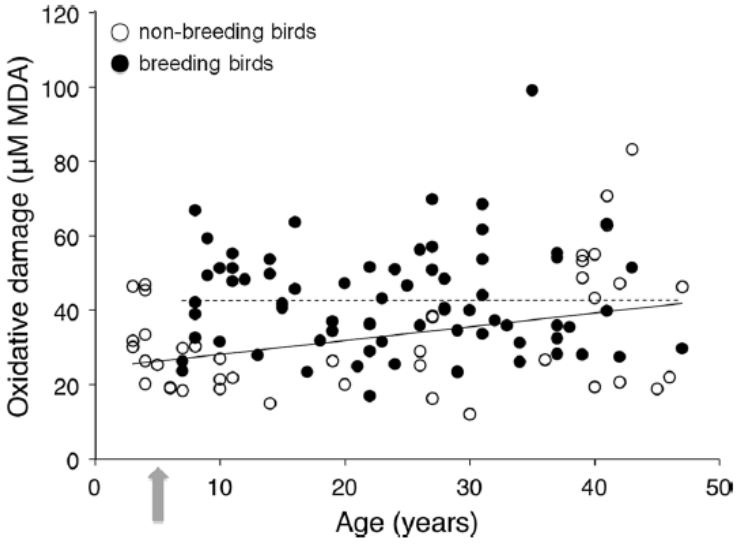

Figure 2. Plasma oxidative damage increased with individual chronological age in non-breeding (n = 44) but not in breeding (n = 71) albatrosses. Dashed line = breeding birds; solid line = non-breeding birds. The arrow indicates the age of first reproduction (6 years; Weimerskirch et al. 1997).

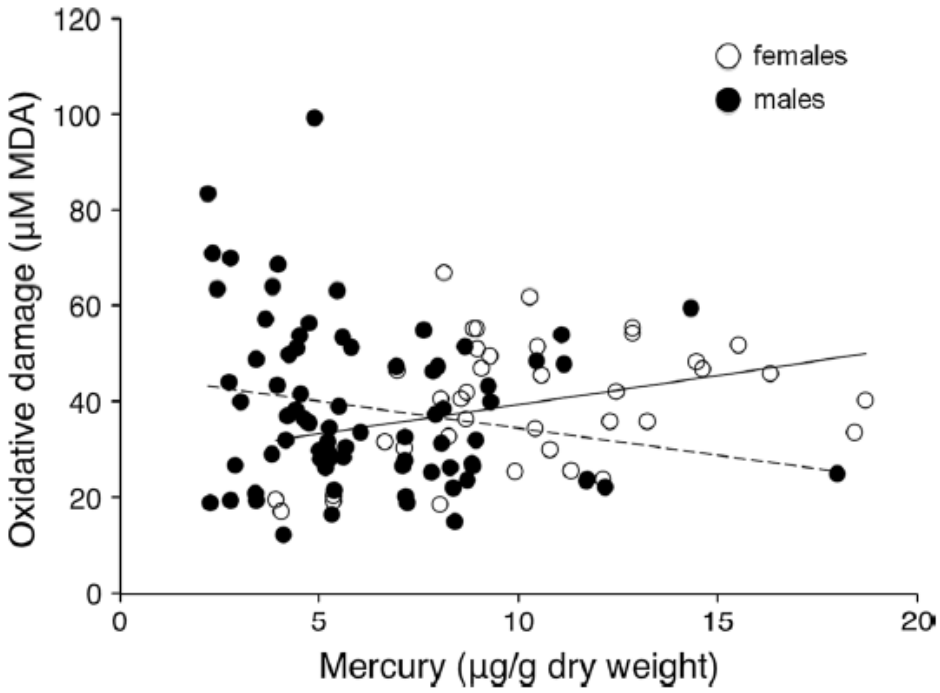

Figure 3. Plasma oxidative damage level increased with red blood cell mercury concentration in females (n = 38) but not in males (n = 77), irrespective of reproductive state. Dashed line = males; solid line = females.

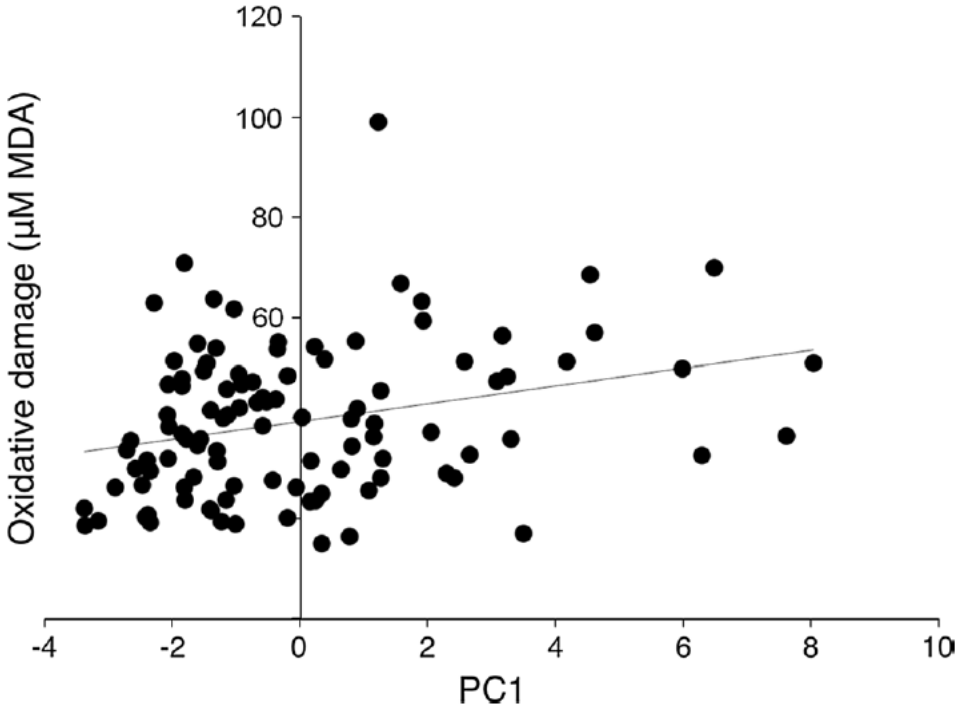

Figure 4. Plasma oxidative damage level was higher in those individuals having higher plasma levels of persistent organic pollutants (r = 0.29, n = 104).

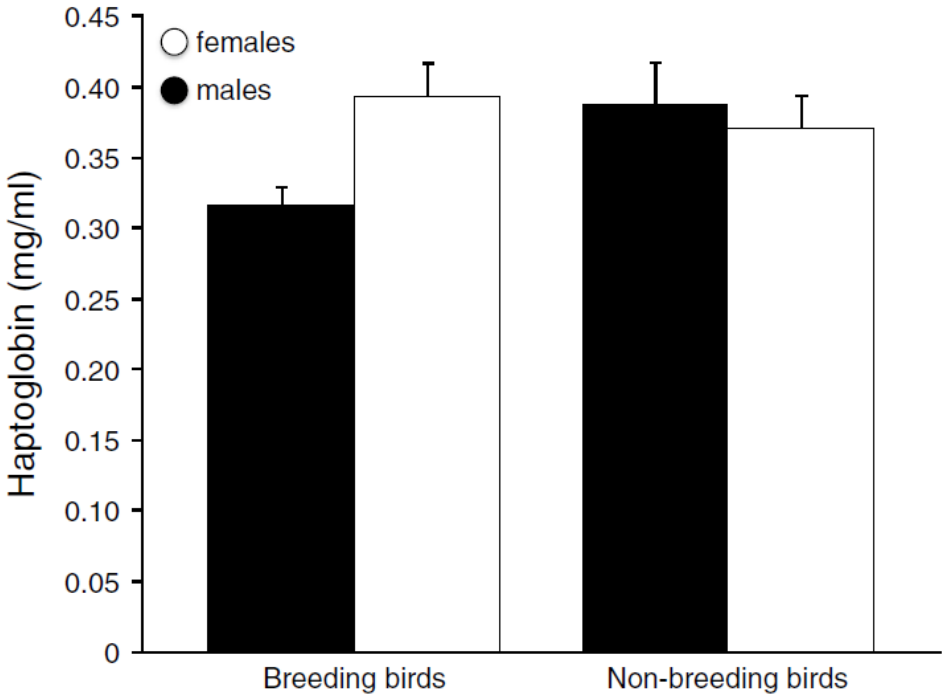

Figure 5. Breeding males (n = 40) had lower plasma concentrations of haptoglobin than breeding females (n = 31; Fisher test P = 0.011) and non-breeding males (n = 32; Fisher test P = 0.017), but not of non-breeding females (n = 11; Fisher test P = 0.197). Data are shown as mean plus standard error.