Meta-analysis reveals that reproductive strategies are associated with sexual differences in oxidative balance across vertebrates

David Costantini

UMR 7221, Muséum National d’Histoire Naturelle, 7 rue Cuvier 75231 Paris Cedex 05, France, Department of Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, Berlin 10315, Germany, and Behavioural Ecology & Ecophysiology Group, Department of Biology, University of Antwerp, Universiteitsplein 1, Wilrijk 2610, Belgium

*Address correspondence to David Costantini. E-mail: davidcostantini@libero.it.

Received on 16 December 2016; accepted on 16 January 2017

Abstract

Oxidative stress is a key physiological mechanism underlying life-history tradeoffs. Here, I use meta-analytic techniques to test whether sexual differences in oxidative balance are common in vertebrates and to identify which factors are associated with such differences. The dataset included 732 effect size estimates from 100 articles (82 species). Larger unsigned effect size (meaning larger sexual differences in a given marker) occurred in: reptiles and fish; those species that do not provide parental care; and oviparous species. Estimates of signed effect size (positive values meaning higher oxidative stress in males) indicated that females were less resistant to oxidative stress than males in: reptiles while males and females were similar in fish, birds, and mammals; those species that do not provide parental care; and oviparous species. There was no evidence for a significant sexual differentiation in oxidative balance in fish, birds, and mammals. Effect size was not associated with: the number of offspring; whether the experimental animals were reproducing or not; biomarker (oxidative damage, non-enzymatic, or enzymatic antioxidant), the species body mass; the strain (wild vs. domestic); or the study environment (wild vs. captivity). Oxidative stress tended to be higher in females than males across most of the tissues analyzed. Levels of residual heterogeneity were high in all models tested. The findings of this meta-analysis indicate that diversification of reproductive strategies might be associated with sexual differences in oxidative balance. This explorative meta-analysis offers a starting platform for future research to investigate the relationship between sex and oxidative balance further.

Key words: antioxidants, oviparity, oxidative damage, parental care, vertebrates, viviparity.

Males and females do not simply differ in how they look like, but differences greatly extend far beyond those of morphological traits. Sexually antagonistic selection has promoted different trait optima in males and females in many traits. For example, in many vertebrate species, the 2 sexes have conflicting reproductive strategies, particularly over the mode and frequency of mating (Parker 2006). Also the amount of parental care invested may greatly differ between males and females across species or even within species (Alonso-Alvarez and Velando 2012; Balshine 2012). Because the expression of many of these traits is linked to physiological mechanisms, it might be expected that selection acting on the physiological traits would also differ between males and females, leading to different physiological phenotypes. Sexual differences are actually evident at physiological level, steroid hormones being a renowned example (Norris and Lopez 2010). Males and females may also differ in other traits, for example in their immunological responses to foreign and...
self-antigens, and show distinctions in innate and adaptive immune responses (Klein and Flanagan 2016). Variation between sexes in basal metabolic rate has also been found and suggested to be due to sex linked nuclear genes (Boratyński et al. 2016).

In recent times, there has been growing interest in the role of oxidative stress as a mediator of life history evolution. Oxidative stress is the rate at which biomolecular oxidative damage is generated, which results from a complex interaction between compounds that oxidize (e.g., free radicals) and compounds that protect against oxidation (antioxidants) (Costantini and Verhulst 2009; Halliwell and Gutteridge 2015). Much recent work has shown that oxidative stress may be connected with life history traits like reproduction or growth (Costantini 2014). One common aspect of this recent work is that males and females have frequently been shown to differ in some aspects of the oxidative balance, be it generation of oxidative damage or up/down regulation of antioxidants (see references in the Supplementary Materials). The reasons for such sexual differences in oxidative balance are currently unknown. A reason might lie with the way sexes respond to selective pressures. For example, variation in the extent to which each sex contributes to parental care may influence the regulation of oxidative balance because of the metabolic demands required by parental investment. It might for example be expected that (i) differences in oxidative balance between males and females are attenuated in those species where both sexes contribute to parental care, (ii) females suffer more oxidative stress than males in those species where most of the parental work is on the female, or (iii) females of species that generate many offspring (e.g., number of eggs or pups) would suffer more oxidative stress than females of species that produce less offspring.

The aim of this study was to use meta-analytic techniques to test whether sexual differentiation in resistance to oxidative stress is ubiquitous across vertebrates and to review evidence for which factors might explain any differences between sexes in oxidative balance. A meta-analytical approach was used because it enables to estimate the size of a given difference. A diverse range of 4 taxonomic classes of vertebrates were considered in order to assess whether differences in oxidative balance between sexes are consistent across taxa with a different evolutionary history. Invertebrates were not considered in this meta-analysis given that they differ dramatically from vertebrates for many biological traits. The contribution of several factors that might be associated with sexual variation in oxidative balance was tested: if the species provides or does not provide parental care; if the species lays eggs (oviparous) or gives birth to fully formed offspring (viviparous); number of pups or eggs generated. The contribution of each factor was tested while taking into account some confounding factors that vary across studies, such as which markers of oxidative stress were measured and in what tissue. Sexual differences in tissue oxidative stress were also analyzed in order to test whether males and females differ in how they prioritize antioxidant protection of tissues.

Materials and Methods

Data collection

A comprehensive review of the literature was performed on the Web of Science using the combinations of the keywords “Fish”, “Amphibians”, “Reptiles”, “Birds”, or “Mammals” with “Oxidative stress”, “Oxidative damage”, or “Antioxidants”. I then searched for additional studies via cross-referencing from hits from this search. The authors of 56 articles were contacted to provide data missing in the selected papers; data were obtained by 40 of them.

An article was selected if it contained a comparison of oxidative stress markers between adult males and females. The following exclusion criteria were applied: i) studies that measured expression of antioxidant genes because I was interested in the biochemical differences between sexes; ii) studies that used metrics of free radical generation as an index of oxidative stress, since they do not provide direct evidence of oxidative stress (reactive species might be mopped up before oxidative damage is generated); iii) studies where necessary information for calculating effect size was unavailable. Overall, the final dataset included 732 effect sizes from 100 articles (82 species: 7 fish, 5 reptiles, 44 birds, and 26 mammals) (Almroth et al. 2008; Alonso-Alvarez et al. 2004a, 2004b; Barrera-García et al. 2012; Beamonte-Barrientos and Verhulst 2013; Beaulieu and Schaefer 2014, Beaulieu et al. 2010, 2011, 2014; Bertrand et al. 2006; Bilham et al. 2013; Bize et al. 2008; Bonisoli-Alquati et al. 2010; Canovas et al. 2014; Casagrande et al. 2011, 2012a, 2012b; Cecere et al. 2016; Christensen et al. 2015; Christie et al. 2012; Cohen et al. 2008; Costantini and Bonadonna 2010; Costantini and Dell’omo 2015; Costantini et al. 2007, 2008, 2010, 2012a, 2012b, 2013, 2014, 2014a, 2014b; Costantini 2010; Cram et al. 2015a, 2015b; Depboylu et al. 2013; Ehrenbrink et al. 2006; Enares et al. 2016; Figueiredo-Fernandes et al. 2006b; Georgiev et al. 2015; Gomes et al. 2013; Grunst et al. 2014; Heiss and Schoech 2012; Herrera-Dueñas et al. 2014; Isaksson et al. 2009, 2011, 2013; Isaksson 2013; Jolly et al. 2012; Kamper et al. 2009; Kanerva et al. 2012; Kayali et al. 2007; Kurhalyuk et al. 2009; Langley-Evans and Sculley 2005; Leclaire et al. 2015; Lilley et al. 2014; Lopes et al. 2002; Lopez-Arrabé et al. 2014; Lopez-Cruz et al. 2010; Lodsat et al. 2013; Lozano et al. 2013; Lucas and French 2012; Marasco et al. 2012; Mielnik et al. 2011; Montgomery et al. 2011, 2012; Norte et al. 2009; Ojeda et al. 2012; O’Keeffe 2013; Orupesa et al. 2013; Ouyang et al. 2016; Pap et al. 2014, 2015; Pike et al. 2007; Raja-Aho et al. 2012; Reichert et al. 2014; Romero-Haro et al. 2015, 2016; Rubolini et al. 2012; Schneerberger et al. 2013, 2014; Shao et al. 2012; Sharick et al. 2015; Stier et al. 2014a, 2014b; Tobler et al. 2013; van de Crommenacker et al. 2011; Vaugoyeau et al. 2015; Vazquez-Medina et al. 2007; Vitousek et al. 2016; Wegmann et al. 2015a, 2015b; and Wiersma et al. 2004).

Oxidative status metrics were categorized into the following groups: i) oxidative damage biomarkers including DNA damage (e.g., 8-oxo-dg), protein damage (e.g., protein carbonyls), lipid damage (e.g., lipid hydroperoxides, malondialdehyde-MDA, isoprostanes), and general damage (e.g., reactive oxygen metabolites-ROMs, total oxidant status-TOS, thiobarbituric acid reactive substances-TBARS); ii) non-enzymatic antioxidants including thiols (e.g., total thiols, glutathione) and non-enzymatic antioxidant capacity (e.g., KRL, OXY, ABTS); and iii) antioxidant enzymes (e.g., catalase, glutathione-S-transferase, glutathione peroxidase, superoxide dismutase). Oxidative status metrics were further categorized by assay (e.g., TBARS, MDA, Protein carbonyls, d-ROMs, KRL, GSH) and by tissue (e.g., blood, brain, liver, muscle).

Data on body mass were collected from online databases like http://genomics.senescence.info/species/, http://animaldiversity.umich.edu/ and http://www.fishbase.org/search.php.

Effect size calculation

The compute.es package (Del Re 2013) in R (R Core Team 2013) was used to calculate the standardized effect size Hedges’ g from test statistics (e.g., t-values or F-ratios) or descriptive statistics (e.g., means,
standard deviations) and sample sizes that were reported in papers. For Hedges effect size estimate, the type I and II error rates can increase if the number of studies is very low (< 15) but the precision of the estimate increases with increasing number of studies (unlike other effect size measures; e.g., log response ratio) (Lajeunesse and Forbes 2003). Thus, given the large sample size of the current meta-analyses, Hedges was deemed an appropriate effect size estimate.

Moderators included and categorization
As the relationship between sex and oxidative balance might be explained by various factors, several explanatory variables (termed moderators in meta-analysis) were considered to be included in the analyses: taxonomic class; parental care (no parental care, female parental care, biparental care); mode of reproduction (oviparous and viviparous); family size (number of either eggs or pups); reproductive status (whether the experimental animals were reproducing or not when the biomarkers were measured); species body mass; strain (wild vs. domestic individuals); biomarker (oxidative damage, non-enzymatic antioxidant, enzymatic antioxidant); study environment (wild vs. captivity). Further moderators were included as random effects: biological matrix where a given marker was analyzed (to accounting for variation in matrices analyzed across studies); laboratory assay (to accounting for variation in assays performed across studies); article (to accounting for the non-independence of effect sizes from the same study); species (to accounting for the non-independence of effect sizes from the same species); taxonomic class (to partly control for phylogeny, which is difficult to do as the dataset was rather unevenly distributed across 4 taxonomic classes).

Meta-analytic techniques
Meta-analytic multilevel mixed-effects models were implemented using the rma.mv function in the metafor package (Viechtbauer 2010) in R (R Core Team 2013). The extracted Hedges’ g values were the response variables in the statistical models. Estimates were weighted according to the sampling variance to account for different sample sizes across studies. Each model output included the QE-test for residual heterogeneity, indicating whether the unexplained variance is greater than expected by chance. All the analyses were done using either unsigned or signed estimates of effect size. Unsigned values indicate the magnitude of the difference in a given marker between males and...
females. Signed values indicate which sex suffered more oxidative stress: a positive effect size indicates that either oxidative damage is higher or a given antioxidant is lower in males than females, implying higher oxidative stress in males. Effect size estimates were considered significant only when they did not overlap zero. Between group comparisons for specific moderators were run only when effect size estimates of the 2 groups did not overlap zero. Between group comparisons are significant when there is no overlap in effect size estimates.

Publication bias
Publication bias was assessed by examining funnel plots of effect size against the log of sample size for each dataset (Møller and Jennions 2001). The plot should be in the shape of a “funnel” with larger variance in effect sizes at small sample sizes and a decreasing variance with increasing sample size. If only significant findings were published, one might expect there to be a “gap” in the lower left of the graph, where for small samples effect sizes must be relatively large to be statistically significant. The funnel plots in the present study indicate there was no publication bias. This is confirmed by the fact that sample size was not significantly associated with Hedges’ g values ($Q_M = 1.77$, df = 1, $P = 0.18$ with article as random factor; $Q_M = 0.18$, df = 1, $P = 0.67$ with article and species as random factors).

Results
Preliminary analyses showed that the moderators biomarker (unsigned effect size: $Q_M = 2.75$, df = 2, $P = 0.25$; signed effect size: $Q_M = 0.77$, df = 2, $P = 0.68$), strain (signed effect size: $Q_M = 0.23$, df = 1, $P = 0.63$), study environment (signed effect size: $Q_M = 0.27$, df = 1, $P = 0.61$), reproductive status (unsigned effect size: $Q_M = 0.04$, df = 1, $P = 0.84$; signed effect size: $Q_M = 0.62$, df = 1, $P = 0.44$), or species body mass (unsigned effect size: $Q_M = 0.22$, df = 1, $P = 0.64$; signed effect size: $Q_M = 2.61$, df = 1, $P = 0.11$) were not significantly associated with estimates of effect size. Thus, these moderators were not further considered in the next analyses. Strain ($Q_M = 10.71$, df = 1, $P = 0.001$; mean, 95% lower and higher confidence interval: domestic, 0.84, 0.49, 1.19; wild, 0.56, 0.24, 0.89) and study environment ($Q_M = 8.57$, df = 1, $P = 0.003$; mean, 95% lower and higher confidence interval: captivity, 0.75, 0.43, 1.08; wild, 0.55, 0.24, 0.87) were, however, significantly associated only with unsigned effect size estimates. The inclusion of these 2 moderators in the following models for unsigned effect size did not affect substantially the outcomes, so they were not included in the final models (unless otherwise noted).

There was a significant association between unsigned effect size and taxonomic class ($Q_M = 29.46$, df = 3, $P < 0.001$; effect size
estimates were significantly larger in fish than birds and mammals (both P < 0.001) and in reptiles than birds (P = 0.01), while the difference between mammals and reptiles was close to significance (P = 0.07; P = 0.04 when both strain and study environment are included as moderators) (Figure 1A). There was also a significant association between unsigned effect size and parental care (Q_M = 14.30, df = 2, P = 0.0008), with only species that do not provide parental care producing effect size estimates that did not overlap zero (Figure 2A). The association between unsigned effect size and mode of reproduction was also significant (Q_M = 9.09, df = 1, P = 0.0026). The unsigned effect size was significantly larger than zero in oviparous species, while effect size estimates of viviparous species overlapped zero (Figure 3A). Family size was positively associated with unsigned effect size (Q_M = 15.98, df = 1, P < 0.001), but the association was no longer significant when 1 outlier Salmo trutta was removed from the model (Q_M = 1.29, df = 1, P = 0.26). All other moderators were not significantly associated with unsigned effect size. In a further model, species were categorized by mode of reproduction and parental behavior (5 categories in total, Figure 4A). This new predictor was significantly associated with unsigned effect size (Q_M = 25.1, df = 4, P < 0.0001). The confidence interval did not overlap zero only for oviparous species with biparental care or with female parental care, which did not differ from each other (Figure 4A).

There was a significant association between signed effect size and taxonomic class (Q_M = 8.83, df = 3, P = 0.032), with only reptiles producing effect size estimates that did not overlap zero (Figure 1B). There was also a significant association between signed effect size and parental care (Q_M = 9.04, df = 2, P = 0.011), with only species that do not provide parental care producing effect size estimates that did not overlap zero (Figure 2B). The association between signed effect size and mode of reproduction was significant (Q_M = 4.38, df = 1, P = 0.036), however, the confidence interval overlapped zero for both viviparous and oviparous species (Figure 3B). Finally, there was a significant association between signed effect size and the tissue in which a given biomarker was measured (Q_M = 16.06, df = 7, P = 0.025) while controlling for article, species, assay, taxonomic class, and biomarker. The confidence interval of each analyzed tissue overlapped zero (Figure 5). All other tested moderators were not significant. As with unsigned effect size, in a further model, species were categorized by mode of reproduction and parental behavior (5 categories in total). This new predictor was significantly associated with signed effect size (Q_M = 11.8, df = 4, P = 0.02). The confidence interval did not overlap zero only for oviparous species (i.e., fish and reptiles) that do not provide any parental care (Figure 4B).

The QE-test revealed significant levels of residual heterogeneity in all models tested (P < 0.0001), implying that the variance not accounted for by the moderators was significantly greater than expected.
reproductive event is not associated with sexual differences in oxidative balance.

Although sexual differences in oxidative balance were particularly more pronounced in fish and reptiles, estimates of signed effect size showed that only in reptiles there was also a significant difference between males and females in terms of oxidative stress. The higher oxidative stress experienced by female reptiles should be taken cautiously because only 5 species were included in this meta-analysis, thus this result might be influenced by the nature of the selected papers. For example, in the Crocodylus moreletii, females provide parental care (Dzul-Caamal et al. 2016), which is widespread in crocodilians, with the females guarding nests and young (Ferguson 1985). In the Ctenolophus subcristatus the higher oxidative stress observed in females might have been due to the sampling that was mostly carried out during the reproductive season when females experience high metabolic costs for egg production and for nest excavation (Costantini et al. 2009). In the Ctenophorus pictus, Olsson et al. (2012) found that males have significantly higher antioxidant enzyme activity than females throughout the mating season, agreeing with a selection history for higher male activity levels due to long hours of patrolling territories at high temperatures in desert Australia and competing for mating opportunities. On the other hand, females had higher damage to DNA than males. The higher oxidative stress in female than in male reptiles might be explained by a high investment of female reptiles in the generation of offspring. A central paradigm of life history theory is that a high investment of resources into reproduction (e.g., number of offspring generated) would result in less resources available for self-maintenance (e.g., antioxidant protection). Fish species included in this meta-analysis invest massively in egg production, generating from approximately 14–1,285 eggs/offspring per reproductive event, while the number of either eggs or pups generated from the other classes of vertebrates range from 1 to 30. It is, therefore, unclear why female fish did not have more oxidative stress than male fish as was the case for reptiles.

The results of the meta-analysis also showed that sexual differences in oxidative balance were larger in those species that provide parental care when compared with those that do not provide parental care. Empirical research has shown that providing care benefits parents by increasing offspring survival and increasing their reproductive success (Alonso-Alvarez and Velando 2012; Balshine 2012). However, parental care also has potential costs, such as decreased survival and reproductive perspectives (Alonso-Alvarez and Velando 2012; Balshine 2012). Although sexual differences in oxidative balance were larger in those species that provide parental care, females suffered more oxidative stress than males only in those species that do not provide parental care. In those species that do not provide parental care, most of the reproductive cost is on the female, which has to invest in embryo development or in the production of multiple eggs. Thus, this result might indicate that generation of offspring is costly in terms of oxidative stress. It is, however, unclear why this oxidative cost for females did not also emerge in those species where it is only the female that provides parental care, rather the effect size was similar to that of species with biparental care. It might be that in these species mothers may be adapted to resist oxidative stress in order to not compromise their capability of providing parental care. This result raises the exciting hypothesis that evolution of parental care would have been associated with that of mechanisms governing the oxidative balance and that this coevolution might have differed between species with uniparental care. Another reason for this result might lie with males of species.
with biparental care experiencing high costs for male–male competition. In many vertebrate species, males typically compete intensely for mates (Alonso-Alvarez and Velando 2012; Balshine 2012). Thus, the oxidative costs of reproduction for males in species with intense male–male competition might be similar to those that females experience for care provisioning.

The reason for the lack of difference in oxidative stress between sexes in those species with biparental care might also lie with a high intra-species variation between mates in the amount of parental effort. Studies on passerine birds have shown that there is not a fixed amount of investment that a given sex puts into reproduction. For example, a member of the pair may increase its effort in order to compensate for a lower breeding effort of its mate who had previously stressful experiences (Spencer et al. 2010). Thus, these results suggest that the larger sexual differences in oxidative balance in species with biparental care as indicated by unsigned but not by signed effect size would indicate that only 1 of the 2 sexes is experiencing high oxidative stress.

In oviparous species, there was stronger evidence for sexual differences in oxidative balance, with a tendency for oviparous females to suffer more oxidative stress than viviparous females. One major problem in interspecific comparisons about variation in physiological costs between reproductive modes is that a number of physiological differences may complicate the ability to attribute differences in costs to reproductive mode only. There are a few species that can reproduce by either viviparity or oviparity that can provide excellent study models to test further the association between oxidative balance and mode of reproduction. For example, Foucart et al. (2014) compared oxygen consumption, as a reflection of energy costs, during reproduction between oviparous and viviparous females of the reproductively bimodal lizard Zootoca vivipara. Female oxygen consumption progressively increased over the course of reproduction, peaking just prior to laying/delivery when it was 46% (oviparous form) and 82% (viviparous form) higher than it was at the pre-reproductive stage. Conversely, post-ovulation total increase in oxygen consumption was more than 3 times higher in viviparous females, reflecting a dramatic increase in embryonic metabolism as well as maternal metabolic costs of pregnancy. It has therefore been suggested that selection for transition from oviparity to viviparity should have provided benefits that outweigh the substantial energy costs that are incurred (Foucart et al. 2014). Given the results of this meta-analysis, it is tempting to speculate that selection for higher resistance to oxidative stress might have contributed to favor evolution of viviparity. For example, in viviparous species, a higher resistance of females to oxidative stress might protect offspring from the pathological consequences associated with accumulation of oxidative damage during embryogenesis (Vitikainen et al. 2016).

The results of this meta-analysis provided little support for sexual differences in tissue sensitivity to oxidative stress. Although confidence intervals overlapped zero for each of the tissues analyzed, in 7 out of 8 tissues the predicted effect size was negative (indicating higher oxidative stress in females). A previous meta-analysis suggested that females were more susceptible to oxidative stress when being exposed to an experimental increase of stress hormones (Costantini et al. 2011). What are the exact mechanisms via which females might be less resistant to oxidative stress, at least in some tissues, remains an open question. However, the results of the meta-analysis also showed that sexual differences in oxidative balance were not necessarily due to 1 of the 2 sexes always suffering more oxidative stress than the other. Although it cannot be excluded that regulation of the oxidative balance might differ to some extent between males and females, this differential regulation does not appear to translate in different oxidative statuses. The evolution of a given trait can be influenced by correlations between the effects of genes on male and female characters, and selection acting on 1 sex may produce a correlated response in the other sex (Lande and Arnold 1983). Many genes that regulate the resistance to oxidative stress have been identified (Allen and Tresini 2000; Rotblat et al. 2013), thus although selection on specific genes might differ between males and females, the overall selective effect on oxidative stress on 1 sex might produce a correlated response in the other.

In conclusion, this meta-analysis showed that phylogeny (class effect), parental behavior, and mode of reproduction contribute to explain sexual differences in either oxidative balance or resistance to oxidative stress. This work showed that males and females were generally similar in resistance to oxidative stress. Moreover, this work...

\[\text{Figure 5. The meta-analysis showed a significant association between tissue in which a given marker of oxidative stress was measured and signed Hedges' g (positive values indicating higher oxidative stress in males than in females). The predicted effect sizes (mean and 95% confidence interval at right) of each analyzed tissue included zero, indicating that they were not statistically significant.}\]
did not provide strong support for role of reproductive investment in terms of the number of offspring generated in explaining sexual differences in oxidative balance. Because of the gaps in current literature, it was not always possible to disentangle the relative contributions of moderators. For example, females had higher oxidative stress than males in oviparous species that do not provide any parental care, which included only fish and reptile species in this dataset. In all the tested models, there was significant residual heterogeneity, implying that there are additional moderators not considered here that might be responsible for the residual variation. For example, previous work showed that hormonal differences between sexes may be associated with those in immunological traits and parasite burden (Klein 2000). Also sexual differences in the probability of extrinsic mortality (e.g., due to predation) might be important because investment in a phenotype resistant to oxidative stress is expected to decrease when chances of survival are low.

Overall, the results of this work emphasize that the need to manage oxidative stress in an optimal way may have contributed significantly to drive the evolution of reproductive strategies. The findings of this meta-analysis offer a starting platform for future research to investigate the reasons of and mechanisms driving sexual differences in oxidative balance further.

Acknowledgements
I thank Livia Carello for helping with the data collection; Shona Smith for advice on statistical analyses; José Aguirre, Carlos Alonso-Alvarez, Rene Beament-Barrientos, Michael Beaulieu, Pierre Bize, Stefania Casagrande, Marie Charpentier, Philippe Christe, Alan Cohen, Janske van de Crommenacker, Tapio Eeva, Susannah French, Ismael Galván, Andrea Gronst, Mark Hausmann, Fabrice Helfenstein, Amparo Herrera-Durías, Caroline Isakssson, Mirella Kanerva, Ana-Lourdes Oropesa Jiménez, Ádám Zoltán Leventi, Thomas Lilley, Jimena López-Arrábé, Sylvain Losdat, George Lozano, Neil Metcalfe, Magdalene Vitousek, and Christian Voigt for providing comments or helping with their work; Sylvain Losdat and 1 anonymous reviewer for providing comments that helped me to improve the presentation of the work.

Funding
This work has been founded by a FWO postdoctoral fellowship and by a von Humboldt research fellowship for experienced researchers.

Supplementary Material
Supplementary material can be found at http://www.cz.oxfordjournals.org/.

References
Allen RG, Tresmi S, 2000. Oxidative stress and gene regulation. Free Radic Biol Med 28:463–499.
Almroth BC, Sturve J, Stephensen E, Holth TF, Förlin L, 2008. Protein carbonyls and antioxidant defenses in cokring wrasse Symphodus melops from a heavy metal polluted and a PAH polluted site. Mar Environ Res 66:271–277.
Alonso-Alvarez C, Bertrand S, Devevey G, Guillard M, Prost J et al., 2004a. An experimental test of the dose dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. Am Nat 164:651–659.
Alonso-Alvarez C, Bertrand S, Devevey G, Prost J, Fairev B et al., 2004b. Increased susceptibility to oxidative stress as a proximate cost of reproduction. Ecol Lett 7:363–368.
Alonso-Alvarez C, Velando A, 2012. Benefits and costs of parental care. In: Roye RJ, Smiseth PT, Kollerik M, editors. The Evolution of Parental Care. Oxford: Oxford University Press, 40–61.
Balshine S, 2012. Patterns of parental care in vertebrates. In: Roye NJ, Smiseth PT, Kollerik M, editors. The Evolution of Parental Care. Oxford: Oxford University Press, 62–80.
Barrera-Garcia A, O’hara T, Galván-Magaña F, Ménuez-Rodriíguez LC, Castellini JM et al., 2012. Oxidative stress indicators and trace elements in the blue shark Prionace glauca off the east coast of the Mexican Pacific Ocean. Comp Biochem Physiol Part C 156:59–66.
Beament-Barrientos R, Verhulst S, 2013. Plasma reactive oxygen metabolites and nonenzymatic antioxidant capacity are not affected by an acute increase of metabolic rate in zebra finches. J Comp Physiol B 183:675–683.
Beaulieu M, Ropert-Coudert Y, Le Maho Y, Ancel A, Criscuolo F, 2010. Foraging in an oxidative environment: relationship between d13C values and oxidative status in Adélie penguins. Proc R Soc Lond B 277:1087–1092.
Beaulieu M, Reichert S, Le Maho Y, Ancel A, Criscuolo F, 2011. Oxidative status and telomere length in a long-lived bird facing a costly reproductive event. Funct Ecol 25:577–585.
Beaulieu M, Schaefer HM, 2014. The proper time for antioxidant consumption. Physiol Behav 128:54–59.
Beaulieu M, Mbouamba S, Willaume E, Kappeler PM, Charpentier MJ, 2014. The oxidative cost of unstable social dominance. J Exp Biol 217:2629–2632.
Bertrand S, Criscuolo F, Fairev B, Sorci G, 2006. Immune activation increases susceptibility to oxidative tissue damage in zebra finches. Funct Ecol 20:1022–1027.
Bilham K, Sin YW, Newman C, Buesching CD, Macdonald DW, 2013. An example of life history antecedence in the European badger Meles meles: rapid development of juvenile antioxidant capacity, from plasma vitamin E analogue. Ethol Ecol Evol 25:330–350.
Bize P, Devevey G, Monaghan P, Doligez B, Christie P, 2008. Fecundity and survival in relation to oxidative stress in a free-living bird. Ecology 89:2584–2593.
Bonosioli-Alquati A, Mousseau TA, Moller AP, Capirol M, Saino N, 2010. Increased oxidative stress in barn swallows from the Chernobyl region. Comp Biochem Physiol Part A 155:205–210.
Boratyński Z, Ketola T, Koskela E, Mappes T, 2016. The sex specific genetic variation of energetics in bank voles, consequences of introgression? Evol Biol 43:37–47.
Canovas M, Mentaberre GG, Tvarijonaviciute A, Casas-Díaz E, Navarro N et al., 2014. Fluctuating asymmetry could be reliable proxy for oxidative stress in vertebrates. PeerJ 2:e616v1.
Casagrande S, Dell’omo G, Costantini D, Tagliavini J, Groothuis T, 2011. Variation of a carotenoid-based trait in relation to oxidative stress and endocrine status during the breeding season in the Eurasian kestrel: a multifactorial study. Comp Biochem Physiol Part A 160:16–26.
Casagrande S, Costantini D, Groothuis T, 2012a. Interaction between sexual steroids and immune response in affecting oxidative status of birds. Comp Biochem Physiol Part A 163:296–301.
Casagrande S, Costantini D, Dell’omo G, Tagliavini J, Groothuis T, 2012b. Differential effects of testosterone metabolites oestradiol and dihydrotestosterone on oxidative stress and carotenoid-dependent colour expression in a bird. Behav Ecol Sociobiol 66:1319–1331.
Cecere J, Capirol M, Carnevali C, Colombo G, Dalle-Donne I et al., 2016. Dietary flavonoids advance timing of moult but do not affect redox status of juvenile blackbirds Tardus merula. J Exp Biol doi:10.1242/jeb.141424.
Christensen LNL, Selman C, Blount JD, Pilkington JG, Watt KA et al., 2015. Plasma markers of oxidative stress are uncorrelated in a wild mammal. Ecol Evol 5:5096–5108.
Christie P, Glazot O, Strepparava N, Devevey G, Fumagalli L, 2012. Twofold cost of reproduction: an increase in parental effort leads to higher malarial
parasitaemia and to a decrease in resistance to oxidative stress. Proc R Soc Lond B 279:1142–1149.

Cohen AA, McGraw KJ, Wiersma P, Williams JB, Robinson WD et al., 2008. Interspecific associations between circulating antioxidant levels and life history variation in birds. Am Nat 172:178–193.

Costantini D, Cardinale M, Carere C, 2007a. Oxidative damage and antioxidant capacity in two migratory bird species at a stop-over site. Comp Biochem Physiol Part C 144:363–371.

Costantini D, Coluzzu C, Fanfani A, Dell’omo G, 2007b. Effects of carotenoid supplementation on colour expression, oxidative stress and body mass in rehabilitated captive adult kestrels Falco tinnunculus. J Comp Physiol B 177:723–731.

Costantini D, Fanfani A, Dell’omo G, 2008. Effects of corticosteroids on oxidative damage and circulating carotenoids in captive adult kestrels Falco tinnunculus. J Comp Physiol B 178:829–835.

Costantini D, Verhulst S, 2009. Does high antioxidant capacity indicate low oxidative stress?. Funct Ecol 23:506–509.

Costantini D, Dell’omo G, Dell’ippo PS, Marquez C, Snell H et al., 2009. Temporal and spatial covariation of gender and oxidative stress in the Galápagos land iguana Conolophus subcristatus. Physiol Biochem Zool 82:430–437.

Costantini D, 2010. Effects of diet quality on serum oxidative status and body mass in male and female pigeons during reproduction. Comp Biochem Physiol Part A 156:294–299.

Costantini D, Bonadonna F, 2010. Patterns of variation of serum oxidative stress markers in two seabird species. Pol Resp 29:30–35.

Costantini D, Carello L, Fanfani A, 2010. Relationships among oxidative status, breeding conditions and life-history traits in free-living great tits Parus major and common starlings Sturnus vulgaris. Ibis 152:793–802.

Costantini D, Marasco V, Moller AP, 2011. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. Journal of Comparative Physiology B 181:447–456.

Costantini D, Monaghan P, Metcalfe N, 2012a. Early life experience primes resistance to oxidative stress. J Exp Biol 215:2820–2826.

Costantini D, Ferrari C, Pasquaretta C, Cavallone E, Carere C et al., 2012b. Interplay between plasma oxidative status, cortisol and coping styles in wild alpine marmots, Marmota marmota. J Exp Biol 215:374–383.

Costantini D, Monaghan P, Metcalfe N, 2013. Loss of integration is associated with reduced resistance to oxidative stress. J Exp Biol 216:2213–2220.

Costantini D, 2014. Oxidative Stress and Hormones in Evolutionary Ecology and Physiology. Berlin, Heidelberg: Springer-Verlag.

Costantini D, Casasole G, Enn M, 2014. Does reproduction protect against oxidative stress?. J Exp Biol 217:4237–4243.

Costantini D, Bonsoi-Aliqua A, Rubolini D, Caprioli M, Ambrosini R et al., 2014a. Nestling rearing is antioxidant demanding in female barn swallows Hirundo rustica. Veterinarska Medicina 59:541–548.

Costantini D, Meille¨re A, Carriere C, Lecomte V, Sorci G et al., 2014b. Oxidative stress in relation to reproduction, contaminants, gender and age in a long lived seabird. Oecologia 175:1107–1116.

Costantini D, Dell’omo G, 2015. Oxidative stress predicts long-term resight probability and reproductive success in Scopoli’s shearwater Calonectris diomedea. Consers Physiol 3:0024.

Cram DL, Blount JD, Young AJ, 2015a. Oxidative status and social dominance in a wild cooperative breeder. Funct Ecol 29:229–238.

Cram DL, Blount JD, Young AJ, 2015b. The oxidative costs of reproduction are group-size dependent in a wild cooperative breeder. Proc R Soc Lond B 282:20152031.

Del Re AC, 2013. Compute.es: compute effect sizes. R package version 0.2–2. Available from: http://cran.r-project.org/web/packages/compute.es.

Dębicki B, Giri M, Olgac C, Dogra-I Abbasoglu S, Uysal M, 2013. Response of liver to lipopolysaccharide treatment in male and female rats. Exp Toxicol Pathol 65:643–650.

Duzi-Caama R, Hernández-López A, Gonzalez-Jauregui M, Padilla SE, Giron-Pérez MI et al., 2016. Usefulness of oxidative stress biomarkers evaluated in the snout scraping, serum and peripheral blood cells of Crocodylus moreletii from Southeast Campeche for assessment of the toxic impact of PAHs, metals and total phenols. Comp Biochem Physiol Part A 200:35–46.

Ehrenbrink G, Hakenhaas FS, Salomons TB, Petrucci AP, Sandri MR et al., 2006. Antioxidant enzymes activities and protein damage in rat brain of both sexes. Exp Gerontol 41:368–371.

Emaresi G, Henry I, Gonzalez E, Roulin A, Bize P, 2016. Sex- and melanism-specific variations in the oxidative status of adult tawny owls in response to manipulated reproductive effort. J Exp Biol 219:73–79.

Ferguson MWJ, 1985. The reproductive biology and embryology of the crocodilians. In: Gans C, Billet FS, Maderson PFA, editors. Biology of the Reptilia. New York: JohnWiley, 330–491.

Figueiredo-Fernandes A, Fontainhas-Fernandes A, Peixoto F, Rocha E, Reis Henriquez MA, 2006b. Effect of paraquat on oxidative stress enzymes in tilapia Oreochromis niloticus at two levels of temperature. Pest Biochem Physiol 85:97–103.

Foucart T, Lourdas O, DeNardo DF, Heulin B, 2014. Influence of reproductive mode on metabolic costs of reproduction: insight from the bimodal lizard Zootoca vivipara. J Exp Biol 217:4049–4056.

Gueguen A, Muchemenipin MP, Prall SP, Emery-Thompson M, Maestriperi D, 2015. Male quality, dominance rank, and mating success in free-ranging rhesus macaques. Behav Ecol 26:763–772.

Gomes ALS, Gonçalves AFG, Vieira JLF, Marceliano MLV, da Silva JMC, 2014. Natural gaps associated with oxidative stress in Willisornis poeciloglossus (Aves: Thamnophilidae) in a tropical forest. Acta Amaz 44:207–212.

Grunst AS, Salgado-Ortiz J, Rotenberg JT, Grunst ML, 2014. Phaeomelanin- and carotenoid based pigmentation reflect oxidative status in two populations of the yellow warbler Setophaga petechia. Behav Ecol Sociobiol 68:669–680.

Halliwell BH, Gutteridge JMC, 2015. Free Radicals in Biology and Medicine. 5th edn. Oxford: Oxford University Press.

Heins R, Schoch SJ, 2012. Oxidative cost of reproduction is sex specific and correlated with reproductive effort in a cooperatively breeding bird, the Florida scrub jay. Physiol Biochem Zool 85:499–503.

Herrera-Dueñas A, Pineda J, Antonio MT, Aguirre JJ, 2014. Oxidative stress of house sparrow as bioindicator of urban pollution. Ecol Indic 42:6–9.

Isaksson C, Sturve J, Almrot BC, Andersson S, 2009. The impact of urban environment on oxidative damage (TBARS) and enzymatic and non-enzymatic defence system in lungs and liver of great tits Parus major. Environ Res 109:46–50.

Isaksson C, While GM, McEvoy J, van de Crommenacker J, Olsson M et al., 2011. Aggression, but not testosterone, is associated to oxidative status in a free-living vertebrate. Behaviour 148:713–731.

Isaksson C, 2013. Opposing effects on glutathione and reactive oxygen metabolites of sex, habitat, and spring date, but no effect of increased breeding density in great tits Parus major. Ecol Evol 3:2730–2738.

Isaksson C, Sepil I, Baramidze V, Sheldon BC, 2013. Explaining variance of avian malaria infection in the wild: the importance of host density, habitat, individual life-history and oxidative stress. BMC Ecol 13:15.

Jolly S, Bado-Nilles A, Lamand F, Turies C, Chadili E et al., 2012. Multi-biomarker approach in wild European bullhead, Cottus gobio, sp., exposed to agricultural and urban environmental pressures: practical recommendations for experimental design. Chemosphere 87:675–683.

Kamper EF, Chatzigeorgiou A, Tsmpoukidi O, Kamper M, Dalla C et al., 2009. Sex differences in oxidant/antioxidant balance under a chronic mild stress regime. Physiol Behav 98:215–222.

Kanerva M, Routti H, Tamuz Y, Nymann M, Bäckman C et al., 2012. Antioxidative defense and oxidative stress in ringed seals Pusa hispida from differently polluted areas. Aquat Toxicol 114–115:67–72.

Kayali R, C¸akatay U, Tekeli F, 2007. Male rats exhibit higher oxidative production than females. J Comp Physiol B 23:499–503.

Klein SL, Flanagan KL, 2016. Sex differences in immune function among vertebrates. Behav Brain Res 31:149–166.

Klein SL, Flanagan KL, 2016. Sex differences in immune responses. Nat Rev Immunol 16:626–638.

Kuralayuk N, Tkachenko H, Palczyńska K, 2009. Antioxidant enzymes profile in the brown trout Salmo trutta trutta with ulcerative dermal necrosis. Bull Vet Inst Pulawy 53:813–818.

Lande R, Arnold SJ, 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.
Lajeunesse MJ, Forbes MR. 2003. Variable reporting and quantitative reviews: a comparison of three metaanalytical techniques. Ecol Lett 6:448–454.

Langley-Evans SC, Sculley DV. 2005. Programming of hepatic antioxidant capacity and oxidative injury in the ageing rat. Mech Ageing Dev 126:804–812.

Leclaire S, Bouret V, Blanchard P, Defrancechi C, Merkling T et al., 2015. Carotenoids increase immunity and sex-specifically affect color and redox homeostasis in a monochromatic seabird. Behav Ecol Sociobiol 69:1097–1111.

Liley TM, Stauffer J, Kanerva M, Eeva T, 2014. Interspecific variation in redox status regulation and immune defence in five bat species: the role of ectoparasitoids. Oecologia 175:811–823.

Lopes PA, Viegas-Crep AM, Nunes AC, Pinheiro T, Marques C et al., 2002. Influence of age, sex, and sexual activity on trace element levels and antioxidant enzyme activities in field mice (Apodemus sylvaticus and Mus spretus). Biol Trace Elem Res 85:227–239.

López-Arrabé J, Cantarero A, Pérez-Rodríguez L, Palma A, Moreno J, 2014. Plumage ornaments and reproductive investment in relation to oxidative status in the pied flycatcher Ficedula hypoleuca sibirica. Can J Zool 92:1019–1027.

López-Cruz RI, Zenteno-Savin T, Galván-Magaña F, 2010. Superoxide production, oxidative damage and enzymatic antioxidant defenses in shark skeletal muscle. Comp Biochem Physiol Part A 156:30–36.

Losdat S, Helfenstein F, Blount JD, Marri V, Maronde L et al., 2013. Nestling development, oxidative stress and sex in four classes of ruffs Philomachus pugnax with different reproductive strategies. Can J Zool 91:212–218.

Lucas LD, French SS. 2012. Stress-induced tradeoffs in a free-living lizard across a variable landscape: consequences for individuals and populations. PLoS ONE 7:e49895.

Marasco V, Spencer KA, Robinson J, Herzyk P, Costantini D, 2013. Developmental post-natal stress can alter the effects of pre-natal stress on the adult redox balance. Gen Comp Endocrinol 191:239–246.

Mielnik MB, Rzeszutek A, Triumf EC, Egelandsdal B, 2011. Antioxidant and enzymatic antioxidant defenses in the red blood cell (RBC) of a high altitude bird, the white-throated dipper Cinclus cinclus. J Exp Biol 214:333–345.

Mortensen KA, Robinson J, Herzyk P, Costantini D. 2013. Developmental post-natal stress can alter the effects of pre-natal stress on the adult redox balance. Gen Comp Endocrinol 191:239–246.

Müller AP, Jennions MD, 2001. Testing and adjusting for publication bias. Trends Ecol Evol 16:580–586.

Montgomery MK, Hulbert AJ, Buttemer WA, 2011. The long life of birds: the role of oxidative stress response genes: bioinformatic analysis of their expression and regulation in birds. J Exp Biol 214:333–345.

Nobre AC, Ramos JA, Sousa JP, Sheldon BC, 2009. Variation of adult great tit Parus major body condition and blood parameters in relation to sex, age, year and season. J Ornithol 150:651–660.

Ojeda NB, Hennington BS, Williamson DT, Hill ML, Betson NE et al., 2012. Oxidative stress contributes to sex differences in blood pressure in adult growth-restricted offspring. Hypertension 60:114–122.

O’Keeffe JL. 2013. Species size predicts oxidative stress in five migratory North American Raptors. [Theses and Dissertations]. Boise State University.

Olsson M, Healey M, Perrin C, Wilson M, Tobler M. 2012. Sex-specific SOD levels and DNA damage in painted dragon lizards Ctenophorus pictus. Oecologia 170:917–924.

Orópesa AL, Gravato C, Guilhermino L, Soler F, 2013. Antioxidant defences and lipid peroxidation in wild white storks Ciconia ciconia from Spain. J Ornithol 154:971–976.

Ouyang JQ, Lendvai AZ, Moore IT, Bonier F, Hausmann MF, 2016. Do hormones, telomere lengths, and oxidative stress form an integrated phenotype? A case study in free-living tree swallows. Integr Comp Biol 56:138–145.

Pap PL, Sesarman A, Vágási CI, Buehler DM, Pátraq L et al., 2014. No evidence for parasitism-linked changes in immune function or oxidative physiology over the annual cycle of an avian species. Physiol Biochem Zool 87:729–739.

Pap PL, Pátraq L, Osváth G, Buehler DM, Versteegh MA et al., 2015. Seasonal patterns and relationships among coccidian infestations, measures of oxidative physiology, and immune function in free-living house sparrows over an annual cycle. Physiol Biochem Zool 88:395–403.

Parker GA, 2006. Sexual conflict over mating and fertilization: an overview. Phil Trans R Soc Lond B 361:235–259.

Pike TW, Blount JD, Bierkeng B, Lindström J, Metzcafé NB, 2007. Carotenoids, oxidative stress and female mating preference for longer lived males. Proc R Soc Lond B 274:1591–1596.

Raja-Aho S, Kanerva M, Eeva T, Lehikoinen E, Suorsa P et al., 2012. Seasonal variation in the regulation of redox state and some biotransformation enzyme activities in the barn swallow (Hirundo rustica L.). Physiol Biochem Zool 85:148–158.

R Core Team et al., 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reichert S, Stier A, Zahn S, Arrive M, Bize P et al., 2014. Increased brood size leads to persistent eroded telomeres. Front Ecol Evol 2:9.

Romero-Haro AA, Canelo T, Alonso-Alvarez C. 2015. Early development conditions and the oxidative cost of social context in adulthood: an experimental study in birds. Front Ecol Evol 3:35.

Romero-Haro AA, Sorci G, Alonso-Alvarez C. 2016. The oxidative cost of reproduction depends on early development oxidative stress and sex in a bird species. Proc R Soc Lond B 283:20160842.

Rotblat B, Grunewald TG, Lepriveur G, Melino G, Knight RA, 2013. Anti-oxidative stress response genes: bioinformatic analysis of their expression and relevance in multiple cancers. Oncotarget 4:2577–2590.

Rubolini D, Colombo G, Ambrosini R, Caprilli M, Clerici M et al., 2012. Sex-related effects of reproduction on biomarkers of oxidative damage in free-living barn swallows Hirundo rustica. PLoS ONE 7:e49855.

Schneebberger K, Czirják GA, Voigt CC, 2013. Inflammatory challenge increases measures of oxidative stress in a free-ranging, long-lived mammal. J Exp Biol 216:4514–4519.

Schneebberger K, Czirják GA, Voigt CC, 2014. Fugivory is associated with low measures of plasma oxidative stress and high antioxidant concentration in free-ranging bats. Naturwissenschaften 101:285–290.

Shao B, Zhu L, Dong M, Wang J, Wang J et al., 2012. DNA damage and oxidative stress induced by endosulfan exposure in zebrafish Danio rerio. Ecotoxicology 21:1533–1540.

Sharick JT, Vazquez-Medina JP, Ortiz RM, Crocker DE, 2015. Oxidative stress is a potential cost of breeding in male and female northern elephant seals. Func Ecol 29:367–376.

Spencer KA, Heidinger BJ, D’alba LB, Evans N, Monaghan P. 2010. Then ver- sus now: effect of developmental and current environmental conditions on incubation effort in birds. Behav Ecol 21:999–1004.

Stier A, Massemin S, Criciucolo F, 2014a. Chronic mitochondrial uncoupling treatment prevents acute cold-induced oxidative stress in birds. J Comp Physiol B 184:1021–1029.

Stier A, Bize P, Rousell D, Schull Q, Massemin S et al., 2014b. Mitochondrial uncoupling as a regulator of life-history trajectories in birds: an experimental study in the zebra finch. J Exp Biol 217:3579–3589.

Tobler M, Sandell MI, Chiriac S, Hasselquist D, 2013. Effects of prenatal testosterone exposure on antidepressant status and bill color in adult zebra finches. Physiol Biochem Zool 86:333–345.

Vaugeois M, Decencière B, Perret S, Karadas F, Meylan S et al., 2015. Is oxido- tative stress influenced by dietary carotenoid and physical activity after moult in the great tit Parus major? J Exp Biol 218:2106–2115.

van de Crommenacker J, Komdeur J, Richardson DS, 2011. Assessing the cost of helping: the roles of body condition and oxidative balance in the Seychelles warbler Acrocephalus seyboldii. PLoS ONE 6:e26423.

Vazquez-Medina JP, Zenteno-Savin T, Elnner B, 2007. Glutathione protection against dose-associated ischemia/reperfusion in ringed seal tissues. J Exp Mar Biol Ecol 345:110–118.
Viechtbauer W, 2010. Conducting meta-analyses in R with the metafor package. *J Stat Softw* 36:1–48.

Vitikainen EI, Cant MA, Sanderson JL, Mitchell C, Nichols HJ et al., 2016. Evidence of oxidative shielding of offspring in a wild mammal. *Front Ecol Evol* 4:58.

Vitousek MN, Tomášek O, Albrecht T, Wilkins MR, Safran RJ, 2016. Signal traits and oxidative stress: a comparative study across populations with divergent signals. *Front Ecol Evol* 4:56.

Wegmann M, Voegeli B, Richner H, 2015a. Oxidative status and reproductive effort of great tits in a handicapping experiment. *Behav Ecol* 26:747–754.

Wegmann M, Voegeli B, Richner H, 2015b. Physiological responses to increased brood size and ectoparasite infestation: adult great tits favour self-maintenance. *Physiol Behav* 141:127–134.

Wiersma P, Selman C, Speakman JR, Verhulst S, 2004. Birds sacrifice oxidative protection for reproduction. *Biol Lett* 271:360–363.