Integrating oxidative ecology into conservation physiology

Michaël Beaulieu¹,*, Anne-Mathilde Thierry², Daniel González-Acuña³ and Michael J. Polito⁴

¹Faculty of Biology, Department of Evolutionary Biology and Animal Ecology, University of Freiburg, Hauptstraße 1, 79104 Freiburg, Germany
²Université de Strasbourg, IPHC, 23 rue Becquerel, 67087 Strasbourg, France
³Facultad de Ciencias Veterinarias, Universidad de Concepción, Casilla 537, Chillán, Chile
⁴Woods Hole Oceanographic Institution, 266 Woods Hole Road MS 50, Woods Hole, MA 02543, USA;
*Corresponding author: Faculty of Biology, Department of Evolutionary Biology and Animal Ecology, University of Freiburg, Hauptstraße 1, 79104 Freiburg, Germany. Email: miklvet@hotmail.fr

Ecologists have recently shown great interest in using physiological markers as indicators of the health of animal populations. In this context, the measurement of markers of oxidative balance, such as antioxidant defences and oxidative damage, may be a valuable tool. Indeed, at the individual level, antioxidant defences are positively associated with fertility and survival probability, while elevated oxidative damage during reproduction or growth may negatively affect recruitment and survival. Therefore, variation in oxidative balance is likely to influence demographic processes. This suggests that conservationists may be able to use oxidative markers to monitor population health. Yet, the connection between these markers and demographic parameters first needs to be established. We present here preliminary results obtained in colonies of breeding Gentoo (Pygoscelis papua) and Adélie penguins (Pygoscelis adeliae), showing that antioxidant defences strongly reflect population trends. However, population trend was not related to oxidative damage. This suggests that in the context of the emerging field of conservation physiology, antioxidant defences may represent a key parameter to monitor population health. We therefore exhort other research teams to assess the generality of this finding in other biological models, especially in species of conservation concern.

Key words: Antarctica, demography, oxidative balance, penguins, population decline, seabirds

Editor: Steven Cooke
Received 1 March 2013; Accepted 8 March 2013

Conserv. Physiol. (2013) 1: doi: 10.1093/conphys/cot004

Why may markers of oxidative balance be worth examining in the context of conservation physiology?

Conservationists need accurate tools to evaluate the health of animal populations in their natural habitat. Towards this end, they have recently started to use physiological markers as indicators of population health (Wikelski and Cooke, 2006). So far, most research in conservation physiology has concentrated on endocrine and immunological parameters in response to environmental perturbations (Stevenson et al., 2005). In comparison to endocrine and immunological ecology, oxidative ecology is a relatively recent research field, and has not yet been considered with respect to conservation issues. Yet, the measurement of oxidative markers may be valuable in view of the ubiquity of oxidative processes in biological systems and of their effects on individuals’ fitness.

Excesses of reactive oxygen species (ROS) relative to antioxidant defences result in the production of oxidative damage [because reactive nitrogen species are thought not to be as damaging as ROS (Monaghan et al., 2009), we will not consider them in the present manuscript]. For instance, this may happen...
when organisms experience acute or prolonged physical activity (Costantini et al., 2008; Fletcher et al., 2012), when they reproduce (Alonso-Alvarez et al., 2010; Christe et al., 2012; Van de Crommenacker et al., 2012), or when they are exposed to anthropogenic pro-oxidative agents (Bonisoli-Alquati et al., 2010; Koivula and Eeva, 2010). High levels of oxidative damage can lead to accelerated cell ageing, thereby decreasing fertility and survival probability (Haenold et al., 2005; Monaghan et al., 2009). Accordingly, individuals with enhanced antioxidant defences or reduced oxidative damage have better fertility and survival (Bize et al., 2008; Freeman-Gallant et al., 2011; Saino et al., 2011; Losdat et al., 2012; Noguera et al., 2012). Moreover, the intrinsic oxidative balance of organisms interacts with the oxidative constraints (e.g. radiation, temperature, and prey availability) imposed by environmental conditions (Beaulieu et al., 2010). Such interaction may lead populations with low antioxidant defences to decrease when faced with pro-oxidative environmental conditions (Møller and Mousseau, 2007). This raises the hypothesis that oxidative balance and demographic processes may be interrelated, and that oxidative markers, reflecting demographic trends, may be used by conservationists as indicators of population health. Yet, the connection between oxidative balance and demographic parameters still needs to be established.

It may be initially assumed that increased levels of physiological markers classically associated with deteriorated fitness parameters at the individual level would reflect poor population health. However, such an assumption does not necessarily hold true. For instance, even if increased corticosterone levels predict decreased reproductive performance in common murres (Uria lomvia), corticosterone levels do not differ significantly between increasing and declining colonies, even when population trends differ drastically (Kitaysky et al., 2007). Consequently, even if corticosterone levels may act on fitness components, their measurement appears inappropriate to estimate the health of populations of common murres. This example emphasizes the necessity to assess the relationship between physiological markers and demographic indices directly, before being able to use them as indicators of population health. The examination of oxidative balance in populations of known contrasting trends may therefore be a useful first step to assess the validity of the relationship between oxidative balance and population health.

**Examination of oxidative balance in populations of Pygoscelis penguins with contrasting population trends**

According to the International Union for Conservation of Nature (IUCN), two of the three currently existing Pygoscelis species, namely the Gentoo penguin (Pygoscelis papua) and the Adélie penguin (Pygoscelis adeliae), are near threatened (IUCN, 2012). However, their status varies greatly between populations, which show contrasting demographic trends depending both on species and region. Indeed, over the few last decades, populations of Gentoo penguins have been stable or increasing around the Antarctic Peninsula, while populations of Adélie penguins have been decreasing (Hinke et al., 2007). Conversely, in other regions of Antarctica, populations of Adélie penguins have been increasing (Table 1). The high dependence of Adélie penguins on decreasing stocks of Antarctic krill (Euphausia superba) around the Antarctic Peninsula is likely to be responsible for their decline in this region (Trivelpiece et al., 1987; Hinke et al., 2007). Compared with other populations, Adélie penguins from the Antarctic Peninsula may therefore have to intensify their foraging effort to feed. This may result in lower antioxidant defences and higher oxidative damage, as observed in birds increasing their flying effort (Costantini et al., 2008). Moreover, they may be limited in their ability to invest in antioxidant defences, because their feeding requirements may not be entirely satisfied, and their endogenous resources may be limited. Finally, low consumption of krill, rich in antioxidants (Tou et al., 2007), may result in low antioxidant defences and high oxidative damage (Beaulieu et al., 2010). These potential changes in oxidative balance may contribute to explaining the negative impact of krill depletion on demographic parameters in Adélie penguins (Nicol et al., 2008; Tierney et al., 2009; Trivelpiece et al., 2011).

As Adélie penguins from the Antarctic Peninsula exhibit decreasing population trends, they are expected to have lower antioxidant capacity and higher oxidative damage than (i) Gentoo penguins from the same region, and (ii) Adélie penguins from other Antarctic regions. In order to assess this prediction, we examined the oxidative balance of breeding Gentoo and Adélie penguins from three different regions of the Antarctic Peninsula (Ardley Island, Gabriel González Videla, and Admiralty Bay) and from Adélie Land (Dumont d’Urville). For each colony, the published results of long-term population-monitoring studies provided us with mean annual population changes (Table 1). Oxidative balance was measured in plasma samples collected during the chick-rearing period of the austral summer 2010–2011, by using the OXY-adsorbent tests (Diacron International, Grosseto, Italy), which measures total antioxidant capacity, and the d-ROM test (Diacron International, Grosseto, Italy), which measures hydroperoxide, resulting from the attack of ROS on organic substrates (and therefore reflecting oxidative damage; for details on the procedure, see Beaulieu et al., 2010).

In agreement with our initial hypothesis, penguins from increasing populations had higher antioxidant capacity than penguins from decreasing populations (Fig. 1). This was true when considering each penguin colony independently (Table 2, Model 1; Fig. 2) or irrespective of species, location (Table 2, Model 2), and levels of oxidative damage (Table 2, Model 3). This positive relationship between antioxidant capacity and population trend may be related to the adaptive advantages conferred by higher antioxidant defences, such as enhanced fertility and survival, as already observed in other bird species (Bize et al., 2008; Saino et al., 2011; Losdat et al., 2012). Moreover, penguins from declining populations may be unable to keep antioxidant defences as high as...
penguins from increasing colonies, because they are energetically limited and cannot invest effectively in antioxidant defences (Fletcher et al., in press).

Given that low oxidative damage may confer the same advantages in terms of fertility and survival as high antioxidant capacity (Helfenstein et al., 2010; Freeman-Gallant et al., 2011; Noguera et al., 2012), lower levels of oxidative damage were also expected in increasing populations. However, even though oxidative damage also differed between colonies (Table 2, Model 1; Fig. 1), there was no general trend between oxidative damage and population trend (Fig. 2). This absence of relationship was confirmed when the species, the location, or the antioxidant capacity were taken into account in statistical analyses (Table 2, Models 2 and 3). The absence of relationship between oxidative damage and population trend may come from the fact...
that we sampled breeding individuals. Indeed, low levels of oxidative damage, expected in increasing populations, may be counterbalanced by elevated oxidative damage related to high investment in reproduction (Fletcher et al., in press; Stier et al., 2012), which is also expected in increasing populations, hence hiding any visible relationship between oxidative damage and population trend. In this context, it would be theoretically interesting to examine whether a negative relationship between oxidative damage and population trend is observed in penguins outside of the breeding season [which may be practically difficult because (i) birds are generally not present on the colony at that time, and (ii) population trend is generally calculated during breeding].

Future directions and potential applications to conservation

The aim of this article is to stimulate conservation ecologists to integrate markers of oxidative balance into the array of physiological parameters used to monitor the health of animal populations in their natural habitat (Wikelski and Cooke, 2006). Recently published articles have provided solid evidence that variation in oxidative balance of free-ranging animals affects fitness components (Bize et al., 2008; Freeman-Gallant et al., 2011; Saino et al., 2011; Losdat et al., 2012; Noguera et al., 2012). Here, by measuring the oxidative balance of Pygoscelis penguins from colonies with contrasting demographic trends, we show that variation in oxidative balance may be related to demographic processes, because we found a strong association between plasma antioxidant defences and population trends. These results suggest that antioxidant defences could be used by conservationists as an indicator to estimate the health of populations of unknown demographic trend. For instance, Gentoo penguins from O’Higgins Base (Antarctic Peninsula, 69°19’S, 57°53’W) have an antioxidant capacity of 253.1 ± 8.0 mmol−1 HOCl neutralized (n = 17, M. Beaulieu, personal data), but their population trend is unknown. Based on the results of our study (Fig. 2), this population is likely to be stable or slightly increasing.

As oxidative balance potentially shapes life-history trade-offs (Monaghan et al., 2009), animal species are likely to modulate their oxidative balance with respect to their own life-history traits. For instance, experimentally increased breeding constraints result in opposite effects on the antioxidant defences of Adélie penguins and zebra finches (Taeniopygia guttata), which show increased and decreased antioxidant capacity, respectively (Alonso-Alvarez et al., 2004; Wiersma et al., 2004; Beaulieu et al., 2011). This discrepancy may be explained by the fact that Adélie penguins are long lived and favour self-maintenance, while zebra finches are short lived and favour current reproduction at the expense of self-maintenance (Stearns, 1989). It is therefore possible that a study examining the relationship between oxidative balance and population trend in a short-lived species would show opposite results to those obtained in long-lived Pygoscelis penguins. In that case, increasing populations of zebra finches would have lower antioxidant defences during reproduction than declining populations. This example also emphasizes the importance of considering the life stage when measuring oxidative balance to assess population health. Indeed, because the regulation of oxidative balance must be critical in terms of fitness only during oxidatively challenging conditions, oxidative markers may reflect population health only during life stages associated with higher vulnerability to oxidative damage (e.g. growth, reproduction, and ageing) or in oxidatively challenging environmental conditions (e.g. radioactive, hot environment). It is therefore possible that the relationship between population trend and antioxidant capacity that we observed in breeding Pygoscelis penguins would disappear in non-breeding penguins. In contrast, a negative relationship between oxidative damage and population trend may be apparent only outside the breeding season (see above). These examples emphasize the necessity to conduct further studies examining the relationship between oxidative balance and population trends in various conditions, for conservationists to use oxidative markers as indicators of population health. We therefore urge other research teams to explore this relationship further (i) in biological systems with different life-history traits, (ii) during different life stages, and (iii) in variable environmental conditions.

Acknowledgements

This work was supported by the Antarctic Science Bursary, Instituto Antártico Chileno (INACH), American Ornithologist Union, and Sigma Xi. We thank the French Polar Institute Paul-Emile Victor (IPEV), US Antarctic Marine Living Resources program, Raytheon Polar Services, G. Watters, and W. Trivelpiece for providing logistical support. Animal use was conducted under approved protocols from IPEV (Prg. 137), INACH (Project T-27-10), the University of North Carolina Wilmington (A0910-20), and a US National Science Foundation Antarctic Conservation Act permit provided to G. Watters (2011-005). We are grateful to Dr. S. Cooke as well as three anonymous referees for constructive comments on the manuscript.
References

Alonso-Álvarez C, Bertrand S, Devevey G, Prost J, Faivre B, Sorci G (2004) Increased susceptibility to oxidative stress as a proximate cost of reproduction. Ecol Lett 7: 363–368.

Alonso-Álvarez C, Pérez-Rodriguez L, Garcia JT, Vinuela J, Mateo R (2010) Age and breeding effort as sources of individual variability in oxidative stress markers in a bird species. Physiol Biochem Zool 83: 110–118.

Antarctic Treaty Consultative Meeting (2010) Management Plan for Antarctic Specially Protected Area No. 150 Ardley Island, Maxwell Bay, King George Island (25 de Mayo).

Beaulieu M, Ropert-Coudert Y, Le Maho Y, Ancel A, Criscuolo F (2010) Foraging in an oxidative environment: relationship between δ13C values and oxidative status in Adélie penguins. Proc R Soc B 277: 1087–1092.

Beaulieu M, Reichert S, Le Maho Y, Ancel A, Criscuolo F (2011) Oxidative stress and telomere length in a long-lived bird facing a costly reproductive event. Funct Ecol 25: 577–585.

Bize PB, Deveye 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.

Bonisoli-Alquati A, Mousseau TA, Møller AP, Caprioli M, Saino N (2010) Increased oxidative stress in barn swallows from the Chernobyl region. Comp Biochem Physiol A 155: 205–210.

Christe P, Glaizot 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 B 279: 1142–1149.

Cobley ND, Shears JR (1999) Breeding performance of gentoo penguins (Pygoscelis papua) at a colony exposed to high levels of human disturbance. Polar Biol 21: 355–360.

Costantini D, Dell’Ariccia G, Lipp H-P (2008) Long flights and age affect oxidative status of homing pigeons (Columba livia). J Exp Biol 4: 377–381.

Fletcher QE, Selman C, Boutin S, McAdam AG, Woods SB, Sea AY, Leeuwenburgh C, Speakman JR, Humphries MM (in press) Oxidative damage increases with reproductive energy expenditure and is reduced by food-supplementation. Evolution doi:10.1111/evo.12014.

Freeman-Gallant CR, Amidon J, Berdy B, Wein S, Taff CC, Haussmann MF (2011) Oxidative damage to DNA related to survivorship and carotenoid-based sexual ornamentation in the common yellowthroat. Biol Lett 7: 429–432.

Haenold R, Wassaf DM, Heinemann SH, Hoshi T (2005) Oxidative damage, aging and anti-aging strategies. Age 27: 183–199.

Helfenstein F, Losdat S, Møller AP, Blount JD, Richner H (2010) Sperm of colourful males are better protected against oxidative stress. Ecol Lett 13: 213–222.

Hinke JT, Salwicka K, Trivelpiece SG, Watters GM, Trivelpiece WZ (2007) Divergent responses of Pygoscelis penguins reveal a common environmental driver. Oecologia 153: 845–855.

IUCN (2012) The IUCN Red List of Threatened Species. Version 2012.2. http://www.iucnredlist.org (last accessed 4 February 2013)

Jenouvrier S, Barbraud C, Weimerskirch H (2005) Sea ice affects the population dynamics of Adélie penguins in Terre Adélie. Polar Biol 29: 413–423.

Kitaysky A, Piatt J, Wingfield J (2007) Stress hormones link food availability and population processes in seabirds. Mar Ecol Prog Ser 352: 245–258.

Koivula MJ, Eeva T (2010) Metal-related oxidative stress in birds. Environ Pollut 158: 2359–2370.

Losdat S, Helfenstein F, Blount JD, Marri V, Maronde L, Richner H (2012) Nestling erythrocyte resistance to oxidative stress predicts fledging success but not local recruitment in a wild bird. Biol Lett 9: 20120888.

Møller AP, Mousseau TA (2007) Determinants of interspecific variation in population declines of birds after exposure to radiation at Chernobyl. J Appl Ecol 44: 909–919.

Monaghan P, Metcalfe NB, Torres R (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol Lett 12: 75–92.

Nicol S, Clarke J, Romaine SJ, Kawaguchi S, Williams G, Hosie GW (2008) Krill (Euphausia superba) abundance and Adélie penguin (Pygoscelis adeliae) breeding performance in the waters off the Béchervaise Island colony, East Antarctica in 2 years with contrasting ecological conditions. Deep Sea Res Pt II 55: 540–557.

Noguera JC, Kim S-Y, Velando A (2012) Pre-fledgling oxidative damage predicts recruitment in a long-lived bird. Biol Lett 8: 61–63.

Saino N, Caprioli M, Romano M, Boncoraglio G, Rubolini D, Ambrosini R, Alquati AB, Romano A (2011) Antioxidant defenses predict long-term survival in a passerine bird. PLoS One 6: e19693. doi:10.1371/journal.pone.0019593

Steams SC (1989) Trade-offs in life-history evolution. Funct Ecol 3: 259–268.

Stevenson RD, Tuberty SR, DeFur PL, Wingfield JC (2005) EcoPhysiology and Conservation: the contribution of endocrinology and immunology. Integr Comp Biol 45: 1–3.

Stier A, Reichert S, Massemin S, Bize P, Criscuolo F (2012) Constraint and cost of oxidative stress on reproduction: correlative evidence in laboratory mice and review of the literature. Front Zool 9: 37.

Tierney M, Emmerson L, Hindell M (2009) Temporal variation in Adélie penguin diet at Béchervaise Island, east Antarctica and its relationship to reproductive performance. Mar Biol 156: 1633–1645.

Tou JC, Jaczynski J, Chen YC (2007) Krill for human consumption: nutritional value and potential health benefits. Nutr Rev 65: 63–77.

Trivelpiece WZ, Trivelpiece SG, Volkman NJ (1987) Ecological segregation of Adélie, gentoo, and chinstrap penguins at King George Island, Antarctica. Ecology 68: 351–361.
Trivelpiece WZ, Hinke JT, Miller AK, Reiss C, Trivelpiece SG, Watters GM (2011) Variability in krill biomass links harvesting and climate warming to penguin population changes in Antarctica. Proc Natl Acad Sci USA 108: 7625–7628.

Van de Crommenacker J, Richardson DS, Koltz AM, Hutchings K, Komdeur J (2012) Parasitic infection and oxidative status are associated and vary with breeding activity in the Seychelles warbler. Proc R Soc B 279: 1466–1476.

Wiersma P, Selman C, Speakman JR, Verhulst S (2004) Birds sacrifice oxidative protection for reproduction. Proc R Soc B 271(Suppl): 360–363.

Wikelski M, Cooke SJ (2006) Conservation physiology. Trends Ecol Evol 21: 38–46.