

# Birds sacrifice oxidative protection for reproduction

Popko Wiersma<sup>1\*</sup>, Colin Selman<sup>2</sup>,  
John R. Speakman<sup>2,3</sup> and Simon Verhulst<sup>1</sup>

<sup>1</sup>Zoological Laboratory of the University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

<sup>2</sup>Aberdeen Centre for Energy Regulation and Obesity, Department of Zoology, Tillydrone Avenue, University of Aberdeen, Aberdeen AB24 2TZ, UK

<sup>3</sup>Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK

\* Author for correspondence (p.wiersma@biol.rug.nl).

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**Oxidative metabolism has reactive oxygen species (ROS) as unavoidable by-products, and the damage ROS inflicts on DNA, proteins and lipids is considered to be a major agent of senescence. Increasing reproductive effort accelerates senescence, but whether reproductive effort is increased at the expense of protection against oxidative damage has not yet been tested. We manipulated reproductive effort in zebra finches through brood size manipulation and measured the activity of two major antioxidant enzymes (superoxide dismutase (SOD) and glutathione peroxidase (GPx)) in the pectoral muscle after 19–20 days of brood rearing. Oxidative stress is reflected by the balance between oxidative protection and ROS exposure, and we therefore scaled SOD and GPx activity to daily energy expenditure (DEE) as an index of ROS production. SOD and GPx activity decreased with increasing brood size by 28% and 24%, respectively. This effect was identical in the two sexes, but arose in different ways: males did not change their DEE, but had lower absolute enzyme activity, and females increased their DEE, but did not change absolute enzyme activity. This result suggests that senescence acceleration by increased reproductive effort is at least in part mediated by oxidative stress.**

**Keywords:** antioxidants; superoxide dismutase; glutathione peroxidase; oxidative stress; costs of reproduction; *Taeniopygia guttata*

## 1. INTRODUCTION

The rate of senescence, defined as a decrease in residual reproductive value with advancing age (Hamilton 1966; Partridge & Barton 1996), varies sharply, even between closely related species of similar size (Finch 1994). Such variation is, theoretically, at least partly understood: the rate of extrinsic mortality determines optimal resource allocation between reproduction and somatic maintenance (Kirkwood & Rose 1991; Kirkwood & Austad 2000). An experimentally induced increase in resource allocation to reproduction decreases the residual reproductive value (Dijkstra *et al.* 1990), and this 'cost of reproduction' is conceptually similar to an acceleration of senescence. This provides a link between comparative studies on lifespan variation and intraspecific studies on reproductive rate.

However, it is an open question to what extent senescence and the costs of reproduction are caused by the same mechanisms.

Metabolic processes that consume oxygen continuously produce a wide variety of reactive oxygen species (ROS), which damage DNA, proteins and lipids (Cadenas 1995; von Schantz *et al.* 1999). Accumulating oxidative damage is considered a major agent of cellular senescence and death (Finch 1994; Beckman & Ames 1998), but whether reproductive effort increases oxidative stress has not yet been studied. Oxidative damage can be prevented through a suite of antioxidants (Felton 1995), such as carotenoids, vitamin C and a number of endogenous antioxidant enzymes that convert ROS into less reactive molecules. Higher resource allocation to somatic maintenance and repair in long-lived species could result in better defences against ROS, but surprisingly antioxidant enzyme activity is lower in long-lived species (Pérez-Campo *et al.* 1998; Barja 2002). Species also differ in ROS production per O<sub>2</sub> volume that is metabolized (Holmes *et al.* 2001). Since variation in antioxidant enzyme activity can be interpreted only in relation to ROS production (Barja 2002), this complicates the comparative approach. We used the alternative of phenotypic manipulation, which ensures an identical genetic and physiological background of groups differing in residual reproductive value. We manipulated reproductive effort in birds and measured the activity of antioxidant enzymes at the end of the nestling phase. To our knowledge, this is the first experimental test of a trade-off between reproduction and oxidative protection.

We manipulated reproductive effort in captive zebra finches, *Taeniopygia guttata*, by manipulating brood size to either two or six young. Zebra finches (in particular females) adjust their metabolic rate to the altered brood size (Deerenberg 1996) and suffer a cost of reproduction when brood size is increased (Deerenberg *et al.* 1996). To test whether parents sacrifice oxidative protection for parental care, we measured the activity of two antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx). Both enzymes play an important role in preventing oxidative stress (Sohal *et al.* 1995; Parkes *et al.* 1998; de Haan *et al.* 1998). SOD transforms superoxide ( $\cdot\text{O}_2$ ) to hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), which can be metabolized to water and molecular oxygen by GPx and catalase (Finkel & Holbrook 2000; catalase was not measured for practical reasons). Oxidative damage is determined by the balance between oxidative protection and ROS exposure, and we therefore scaled enzyme activity to daily energy expenditure (DEE) measured in zebra finches (Deerenberg 1996). Because ROS production does not depend on enzymatic reactions, oxygen consumption provides an index of ROS production when comparing individuals with identical physiology (Loft *et al.* 1995; Finkel & Holbrook 2000).

## 2. MATERIAL AND METHODS

### (a) Animals and housing

Pairs of wild-type zebra finches in cages of dimensions 40 cm × 80 cm × 40 cm (h × w × d) were given nesting material 16–20 days after pair formation. Room temperature was 25 °C (range of 23–27 °C) and light conditions were 14 L : 10 D. Food (tropical seed mixture) and water were present *ad libitum*. A teaspoon of egg food was given three times per week until completion of the clutch.

### (b) Manipulation

Clutches were checked regularly to record the hatching date, and broods were manipulated to contain two ( $n = 10$ ) or six ( $n = 9$ )

nestlings when the chicks were 1–3 days old. Brood size after manipulation was not correlated with original brood size ( $r = -0.086$ ,  $p = 0.73$ ).

#### (c) Antioxidant enzyme assays

Birds were killed by cervical dislocation when their nestlings were 19–20 days old. Pectoral muscle samples were immediately immersed in liquid nitrogen within 80 s after death and stored at  $-70^{\circ}\text{C}$ . Total activity of SOD and of the Se-dependent isozyme of GPx were measured as described by Selman *et al.* (2000). One unit of SOD activity is the amount of enzyme that caused 50% inhibition of pyrogallol autoxidation. One unit of GPx is the amount of enzyme that oxidizes  $1\ \mu\text{M}$  of NADPH per minute. Blanks were run to correct for spontaneous reactions in the absence of the enzyme (López-Torres *et al.* 1991). Enzyme activities are expressed per milligram of protein, which was measured using the Bradford method (Bradford 1976).

#### (d) Energy expenditure

Parental DEE was measured when the chicks were 14–16 days old using doubly labelled water (Speakman 1997) by Deerenberg (1996) in the same experimental design as in the present study. Based on these data we estimated that male DEE was  $53.3\ \text{kJ d}^{-1}$ , independent of brood size, and female DEE was  $48.8$  and  $67.5\ \text{kJ d}^{-1}$  for broods of two and six young, respectively.

#### (e) Statistical analyses

Nestling measurements were compared using general linear mixed models (GLMM) to avoid pseudoreplication (Rabasch *et al.* 2000). Generalized linear models (GLM) and  $t$ -tests were applied in other cases. All tests were performed using SPSS (v. 11.0; SPSS Inc.). Protein and antioxidant enzyme measurements in males and females of pairs were uncorrelated (protein:  $r = 0.14$ ,  $n = 17$ ,  $p = 0.58$ ; SOD:  $r = 0.36$ ,  $n = 17$ ,  $p = 0.15$ ; GPx:  $r = 0.14$ ,  $n = 17$ ,  $p = 0.60$ ), and were therefore treated as independent samples. Averages and parameter estimates are shown with their standard error.

### 3. RESULTS

Independent of sex, parents rearing large broods lost  $1.06 \pm 0.49\ \text{g}$  more mass from the onset of breeding to the day that the chicks were 17 days old than birds rearing small broods (GLM:  $t_{34} = 2.17$ ,  $p = 0.037$ ). Nestlings in large broods were  $1.68 \pm 0.31\ \text{g}$  lighter than nestlings in small broods (GLMM:  $\chi^2_1 = 13.49$ ,  $p < 0.0005$ ). Thus parents rearing large broods were constrained in their chick-feeding abilities, in agreement with earlier experiments (Deerenberg *et al.* 1996).

Independent of sex, relative GPx activity (enzyme activity scaled to daily energy expenditure;  $\text{U ml}^{-1}\ \text{O}_2\ \text{d}^{-1}$ ) was 24% lower, and relative SOD activity was 28% lower in birds rearing large broods (figure 1). Absolute SOD activity decreased significantly with increasing brood size from  $1080.1 \pm 88.9$  (two chicks) to  $888.1 \pm 80.7$  (six chicks;  $t$ -test:  $t_{33} = 2.06$ ,  $p = 0.037$ ). Absolute GPx activity decreased from  $3581 \pm 303$  (two chicks) to  $3213 \pm 273$  (six chicks), but this difference was not significant ( $t$ -test:  $t_{32} = 0.88$ ,  $p = 0.39$ ). Apparently, the decrease in relative GPx activity was largely caused by the increase in  $\text{O}_2$  consumption rate in the birds rearing large broods, while the decrease in relative SOD activity was caused by both an increase in  $\text{O}_2$  consumption rate and a decrease in SOD activity (figure 2).

Relative enzyme activity was lower when brood size was large in both sexes, but these effects arose in different ways (figure 2). In females, absolute enzyme activity was only marginally lower when brood size was large, and relative enzyme activity decreased due to an increase in DEE. In males, however, there was no significant effect of brood size on DEE, but a significant decrease in enzyme activity, and consequently also a decrease in relative enzyme activity.

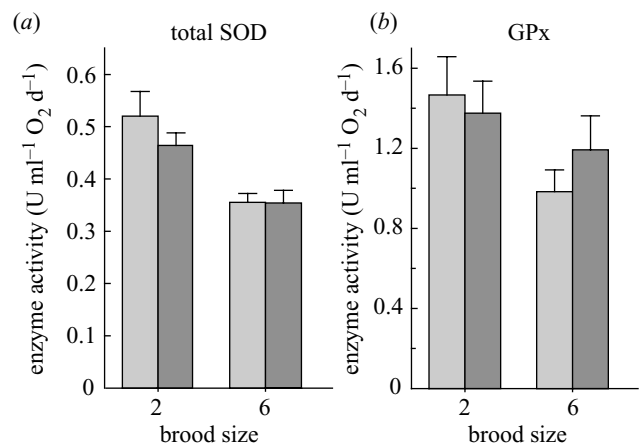


Figure 1. (a) Average total superoxide dismutase (SOD) and (b) glutathione peroxidase (GPx) activity (+ s.e.) in pectoral muscles expressed per whole body  $\text{O}_2$  consumption rate per day of males (dark-grey bars) and females (light-grey bars) rearing broods of two or six young. Independent of sex (SOD: interaction sex and brood size:  $F_{1,31} = 0.78$ ,  $p = 0.38$ ; sex:  $F_{1,31} = 0.84$ ,  $p = 0.37$ ; GPx: interaction sex and brood size:  $F_{1,30} = 0.82$ ,  $p = 0.37$ ; sex:  $F_{1,30} = 0.13$ ,  $p = 0.72$ ), relative activity of both enzymes decreased with increasing brood size (mean  $\text{U ml}^{-1}\ \text{O}_2\ \text{d}^{-1}$ , sexes combined, SOD: two young:  $0.490 \pm 0.026$ ,  $n = 19$ ; six young:  $0.355 \pm 0.014$ ,  $n = 16$ ;  $F_{1,33} = 19.3$ ,  $p < 0.001$ ; GPx: two young:  $1.42 \pm 0.12$ ,  $n = 19$ ; six young:  $1.08 \pm 0.10$ ,  $n = 15$ ;  $F_{1,32} = 4.35$ ,  $p < 0.05$ ). U stands for international Bergmeyer units.

### 4. DISCUSSION

Individuals invest in reproduction at the expense of their future reproductive output (see the review in Dijkstra *et al.* 1990), and, on a different level, species can be characterized by their place in the lifespan/reproduction spectrum, ranging from short lived with high reproductive rates to very long lived with low annual investment in reproduction (Ricklefs & Wikelski 2002). Oxidative stress resulting from resource allocation to reproduction has been suggested as an important mechanism mediating this trade-off on both levels. For example, results from invertebrate studies (*Drosophila melanogaster* and *Caenorhabditis elegans*) indicate that protection against oxidative damage plays an important role in determining lifespan (Golden *et al.* 2002). Our finding that one of the main defences against ROS decreased with increasing brood size supports this suggestion, although further work is required to demonstrate that reduced oxidative protection results in increased oxidative damage and fitness costs. To our knowledge, this is the first experimental demonstration of a trade-off between reproductive effort and oxidative protection.

In both sexes oxidative protection was reduced when brood size was large, but this effect arose in different ways. In males this was owing to a decrease in enzyme activity, whereas in females this was owing to an increase in energy expenditure (figure 2). This suggests that the two sexes had different strategies to cope with an increase in workload. Sexual differences in adjustment of energy expenditure to manipulated brood size have previously been found in other bird species (Moreno *et al.* 1995; Verhulst & Tinbergen 1997). Unfortunately, we were not able to measure energy expenditure directly in our experiment, but such

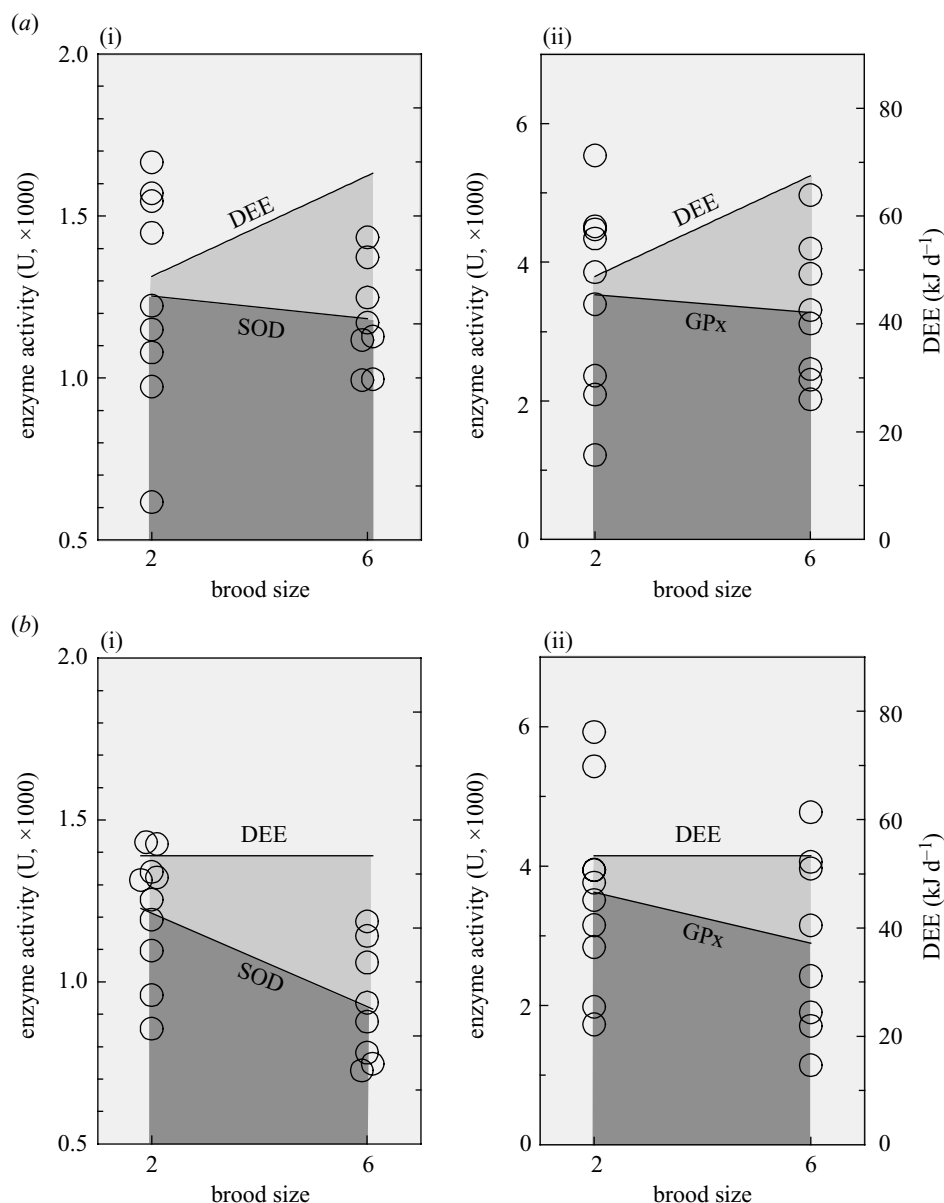


Figure 2. (i) SOD and (ii) GPx activity (U,  $\times 1000$ ; symbols and lines) and daily energy expenditure (DEE; lines only) for (a) females and (b) males rearing experimentally created small or large broods. The lines connect the averages for each sex and brood size. DEE data of males and females are from Deerenberg (1996). Average SOD activity decreased (non-significantly) with brood size from  $1252.2 \pm 113.0$  to  $1182.9 \pm 56.9$  in females ( $t_{15} = 0.53$ ,  $p = 0.60$ ), and from  $1220.8 \pm 62.0$  to  $932.0 \pm 63.7$  in males ( $t_{17} = 3.22$ ,  $p = 0.005$ ). Average GPx activity for females with two and six nestlings was  $3534 \pm 464$  and  $3277 \pm 359$ , respectively ( $t_{15} = 0.43$ ,  $p = 0.67$ ), and for males  $3622 \pm 421$  and  $3140 \pm 445$ , respectively ( $t_{15} = 0.77$ ,  $p = 0.45$ ).

data could potentially reveal further variation in strategies to rear a large brood, for example, in relation to age or sexual attractiveness of the partner.

The decrease in relative antioxidant enzyme activity with increasing brood size can logically be expected to result in an increase in oxidative damage, but this remains to be tested explicitly.

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