**Opinion article for Trends in Ecology and Evolution:**

# *Does reproduction cause oxidative stress? An open question*

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**Abstract: There has been substantial recent interest in the possible role of oxidative stress as a mechanism underlying life history trade-offs, particularly with regard to reproductive costs. Several recent papers have found no evidence that reproduction increases oxidative damage, and so have questioned the basis of the hypothesis that oxidative damage mediates the reproduction-lifespan trade-off. However, we suggest here that the absence of the predicted relationships could be due to a fundamental problem in the design of all of the published empirical studies, namely a failure to manipulate reproductive effort. We conclude by suggesting experimental approaches that might provide a more conclusive test of the hypothesis.**

## The hypothesised role of oxidative stress in mediating life-history trade-offs

The basic concept of a life-history trade-off is that resource acquisition is limited and so increased resource allocation to one trait is at the expense of other traits requiring the same resource. In the context of reproductive costs, greater investment in current reproduction can only be achieved at a cost to future reproduction, self-maintenance and/or growth [1]. Such trade-offs have been documented for some time, and the recent focus has been in identifying the physiological mechanisms that underlie them [2]. One such putative mechanism that has received considerable recent attention is the role of Reactive Oxygen Species (ROS), created primarily by the mitochondria as a by-product of ATP production. While ROS have an important signalling role [3], they can also cause oxidative damage to biomolecules such as lipids, proteins and DNA [4]. Oxidative stress is defined as a shift in the delicate balance between the production of ROS and their neutralization via the antioxidant defence or oxidative damage repair systems, such that there is an increase in the level of oxidative damage [3,4]. This damage contributes to the gradual

deterioration of bodily function over time, and is thought to be a major factor underlying senescence [4], although the link is not as straightforward as once presumed [5]. This has led to the hypothesis that oxidative stress could be a key mechanism underlying the trade-off between reproductive effort and lifespan: greater investment in reproduction might result in faster somatic deterioration (and hence reduced life expectancy) since increased allocation to reproduction means that the body can no longer invest so heavily in defence against oxidative stress [6-8]. This hypothesis therefore predicts both that increased reproductive effort is associated with increased oxidative damage to the soma, and that the damage shortens lifespan.

**An apparent lack of evidence for the hypothesis**

Much of the early work (including our own) purporting to investigate the links between life history strategies and oxidative stress was inconclusive since there was too much of a focus on antioxidant defences rather than oxidative damage or repair. A reduction in antioxidant defences in breeding individuals is hard to interpret in the absence of concurrent measurements of damage, since it could indicate either that the defences are depleted by a high rate of ROS production (or a need to shift resources away from this defence system), or that a *reduced* production of ROS means that defences have been down-regulated due to their not being needed [6-8]. We need to know the extent to which ROS production levels are overwhelming defence capability and generating damage, and so measuring antioxidant defences is not sufficient. In order to look for evidence of oxidative stress it is therefore better to measure as many components of the system as possible (i.e. levels of damage and repair as well as antioxidants [6,8]).

A flurry of studies over the last 2-3 years has redressed the balance by measuring markers of oxidative damage to lipids, proteins and/or DNA in breeding animals. While domesticated livestock can show increases in maternal oxidative damage in mothers at the time of parturition

[9,10], such animals have been selected for extreme reproductive output and so cannot be considered representative; moreover, these studies invariably fail to include data on non-breeding controls (Table 1), so making it hard to rule out seasonal or ontogenetic causes of changes in oxidative stress. However, studies of non-domesticated species have largely come to the somewhat unexpected conclusion that reproduction causes little or no increase in parental levels of oxidative damage [11-19]; this has led several authors to question the whole basis for the hypothesis that oxidative stress is a mechanism underlying the cost of reproduction [14,17,18]).

## Weaknesses in experimental design

### *The need to manipulate reproductive effort*

Several explanations have been put forward to explain this discrepancy between life history theory and the empirical findings; these include a pre-emptive upregulation of antioxidant defences in breeding individuals to avoid incurring damage, the failure to undertake measurements in natural conditions and the failure to use the appropriate range of assays of damage [8,17,18]. However, we think that the most important factor has not yet been adequately recognised. It is important to remember that we expect evolution to have equipped animals with the capacity to manage their reproductive effort so as to achieve the optimal balance between current and residual reproductive effort. For iteroparous species, we expect that the effort put into reproduction by individuals will be tailored to optimise long term damage i.e. to maximise expected lifetime reproductive output. As far as we are aware, all studies published to date that have measured oxidative damage in relation to reproduction have not manipulated reproductive effort (Table 1). Instead they have used correlational data, comparing levels of oxidative damage in individuals naturally breeding at different rates, or an experimental approach that has simply manipulated the opportunity for animals to breed, rather than the effort that they exert when breeding.

Variation in reproductive effort amongst the breeders in these studies will reflect their individual quality or access to resources. Even when conditions are standardised under laboratory conditions, the number of offspring produced over a fixed time can show huge inter-individual variation (e.g. 7-fold in house mice [14]), presumably reflecting phenotypic differences between parents. The closest to an experimental manipulation of reproductive effort in the studies published so far involves a manipulation of the presence of territorial neighbours in breeding male house mice, which produced treatment-level differences in the investment in scent marking [17]; however, there was no means to alter the amount of scent marking that an individual male actually performed, and so increases in average territorial defence might have been driven by those males in best condition (who could therefore do this while minimising oxidative damage). It should be noted that one additional study [20] did carry out the ideal manipulation of reproductive effort (by altering brood sizes in zebra finches), but measured antioxidant defences rather than oxidative damage.

Protocols that allow animals to breed at their chosen rate ignore the lessons learned from earlier ecological and energetic studies of the cost of reproduction. The earliest of such studies were again correlational and usually failed to show any cost of reproduction; indeed they often found a positive covariation in life history traits (i.e. the individuals with the highest annual reproductive output tended to live longest) [21]. As pointed out in classic papers of the theory of life history trade-offs, this is because both resource allocation and resource *acquisition* can vary, and if the latter is more variable, we will see positive correlations [22]; high quality individuals can both produce more offspring and have a higher survival rate than those of lower quality [22,23]. It was only when reproductive effort was manipulated (e.g. by experimentally increasing or decreasing clutch or family size) that the trade-off between reproduction and future fitness was evident and the true costs of reproduction became apparent [21,24-26].

96 The same approach must now be adopted in studies that measure oxidative stress. The
97 suggestion that experimental manipulation of reproductive effort might be revealing in this
98 context has been mentioned briefly elsewhere [14], but it has not been viewed as a necessary
99 condition for testing the hypothesis that increased reproduction effort generates increased
100 oxidative stress, and no empirical studies have yet embraced it.

101 *The need to ensure that resources are not superabundant*

102 As a second point, it is noteworthy that many of the studies examining the relationship
103 between reproduction and oxidative stress have used conditions of *ad libitum* food. If resources
104 are easily obtained, then animals can potentially increase their intake when breeding to the point
105 where they do not need to reduce investment in somatic maintenance (i.e. there is no resource
106 allocation trade-off - they can invest in reproduction but maintain their level of investment in
107 antioxidant defence and repair mechanisms, so we would not necessarily expect any increase in
108 levels of damage). The male mice mentioned above that were 'encouraged' to invest more in
109 defence of a breeding territory were actually able to increase their body mass over the period of
110 reproduction more than controls [17], presumably because food was provided *ad libitum* and so
111 there was no real trade-off between investment in reproduction and investment in somatic
112 growth. Again this point about the need to take into account the ease of resource acquisition was
113 made many years ago when the distinction between reproductive effort and costs of reproduction
114 were first being debated [27].

115 *The need to establish that there is an effect on lifespan*

116 Even if it can be shown that increased reproductive effort causes an increase in oxidative damage,
117 this is still insufficient to fully test the hypothesis that oxidative stress is part of the mechanism
118 underlying a trade-off between reproduction and adult survival, since the damage might not
119 necessarily lead to a reduction in lifespan. Indeed, it is quite possible that any increase in oxidative

damage might be transient or biologically trivial, and have no long term effect. To examine this question, it is necessary to test for a relationship between level of damage and subsequent survival rate. (It is of course also possible that oxidative damage might affect fitness through effects on future reproductive output, so this also needs to be considered.)

## The way ahead

We agree with Selman et al. [8] that empirical tests of the role of oxidative stress in mediating life histories require appropriate (and preferably multiple) laboratory assays of oxidative damage, based on standardised samples. Ideally these assays should also cover a range of tissues, since oxidative damage might not be equally concentrated in all parts of the body [8]. We also agree that the studies should be carried out under conditions where resources are limiting (rather than supplied *ad libitum*). This does not necessarily mean that they must be based in the field. With an appropriate experimental design and choice of study system it is perfectly possible to demonstrate resource-based trade-offs in laboratory conditions, provided that food is not too easily obtained. In order to avoid the separate confounding complications induced by dietary restriction, the best solution might be to increase the amount of effort required to obtain food (rather than limit its abundance). An experimental protocol in which the animal must work to obtain food has shown that it is possible to replicate the energetic situation faced by animals in the field – but with the advantage that the experimenter has far greater control (see [28,29]). Detailed, individual-based life-history data based on long-term studies of natural populations can provide supporting evidence of reproductive costs [21] but do not enable conclusive tests of the hypothesised trade-offs. This is because the data are correlational, due to individuals selecting their own rate of reproduction: while the phenotypic correlations among life history traits (in this case reproductive effort and measures of oxidative stress or lifespan) might be in the direction that provides

circumstantial support for the hypothesised relationships, any phenotypic correlations in the opposite direction (e.g. if higher levels of reproduction are associated with lowest levels of damage, or higher survival), or indeed the absence of any relationships, could be an artefact for the reasons given earlier. This makes it impossible to reject the hypotheses unless genetic correlations among life history traits can be examined [21].

Instead an experimental approach should be adopted in which animals (whether in the lab or field) are randomly allocated to treatment groups in which their reproductive investment is manipulated (preferably both upward and downward treatments) away from the 'planned' level, but still within the range seen in the wild. This is perhaps easiest in species exhibiting parental care, if the number of offspring receiving care can be altered [24]. However, physiological approaches that manipulate investment (e.g. by hormonally stimulating the production of extra egg follicles or surgically removing follicles in early stages of development) have also proved highly successful, even in field studies [26,30]. None of these are new techniques - studies of the role of oxidative stress in life history evolution therefore just need to copy the approaches used by ecologists studying the costs of reproduction some decades earlier. Finally, natural or semi-natural conditions may be required for testing the second step in the hypothesis, namely that any oxidative damage incurred through reproduction has an impact on subsequent lifespan.

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247 **Table 1. Summary of empirical studies relating reproduction to levels of oxidative damage.**

248

| Species | Context | Increase in OD? | Manipulate reproduction? | Manipulate RE? | Ref. |
|---|---|---|---|---|---|
| **FISH** | | | | | |
| Smallmouth bass (*Micropterus dolomieu*) | Wild | NS | No† | No | [19] |
| **REPTILES** | | | | | |
| Snow skink (*Niveoscincus ocellatus*) | Wild | NS | No | No | [15] |
| Painted dragon lizard (*Ctenophorus pictus*) | Lab | (+) | No | No | [31] |
| **BIRDS** | | | | | |
| Adélie penguin (*Pygoscelis adeliae*) | Wild | NS | No† | No* | [12] |
| Red-legged partridge (*Alectoris rufa*) | Lab | (+) | No | No | [11] |
| Florida scrub jay (*Aphelocoma coerulescens*) | Wild | + | No† | No | [32] |
| Collared flycatcher (*Ficedula albicollis*) | Wild | NS | No† | No | [16] |
| Seychelles warbler (*Acrocephalus sechellensis*) | Wild | (+) | No | No | [33] |
| **MAMMALS** | | | | | |
| Dairy cow (*Bos taurus*) | Lab | + | No† | No | [9] |
| Dairy cow (*Bos taurus*) | Lab | NS | No† | No | [34] |
| Dairy cow (*Bos taurus*) | Lab | (+) | No† | No | [35] |
| Dairy cow (*Bos taurus*) | Lab | (+) | No† | No | [36] |
| Dairy cow (*Bos taurus*) | Lab | (+) | No† | No | [37] |
| Dairy cow (*Bos taurus*) | Lab | NS | No† | No | [38] |
| Goat (*Capra hircus*) | Lab | (+) | No† | No | [10] |
| Soay sheep (*Ovis aries*) | Wild | NS | No | No | [39] |
| Eastern chipmunk (*Tamias striatus*) | Wild | + | No | No | [13] |
| House mouse (*Mus musculus*) | Lab | +/- | Yes | No | [17] |
| House mouse (*Mus musculus*) | Lab | - | Yes | No | [14] |
| Bank vole (*Myodes glareolus*) | Lab | - | Yes | No | [18] |

249 The table indicates whether the study took place in the wild or in laboratory conditions (in which
250 case food was *ad libitum* in all cases), and whether reproduction was associated with a significant
251 change in levels of parental oxidative damage (OD); + and - indicate a consistent increase and
252 decrease in damage respectively, (+) indicates the increase was inconsistent, +/- that different
253 components showed opposing trends, and NS indicates no significant effects. Also shown is
254 whether or not the study involved manipulation of reproduction (i.e. the opportunity to breed) or
255 of reproductive effort (RE) amongst breeders; † indicates that no data from non-breeding individuals
256 were included (*foraging efficiency of some breeders was handicapped by attachment of devices
257 that impaired locomotion, but this is not necessarily equivalent to manipulating reproductive
258 investment).