ABSTRACT

Oxidative stress has long been associated with life history traits such as pace of life, longevity and ageing, to reproduction, fecundity and survival. Reactive oxygen species (ROS) are highly reactive molecules, the majority of which are created as a natural by-product of aerobic respiration. ROS can serve as biologically functional molecules, for example in cell signalling. Owing to their highly reactive nature, however, if their production exceeds an organisms ability to quench and regulate them they may cause damage to macromolecules such as DNA, proteins, and lipids. Traditional models of oxidative stress predict higher levels of production of reactive oxygen species (ROS), with increased levels of metabolic output. The ideas envisioned in these models often involve trade-offs occurring in the form of increased oxidative damage due to heightened energy requirements, ideas under increasing scrutiny.

Presented in this thesis are four studies using free-living birds, great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) overwintering and breeding in a nest box colony. The studies have aimed to investigate this relationship between overall energy use and the buildup of damage created by the production of ROS. Utilising naturally occurring differences in the metabolic outputs of these animals, i.e., sampling while at rest during the night and during daytime activity, markers of oxidative status were measured: in plasma (oxidative damage: d-ROM's, non-enzymatic antioxidant capacity: OXY, circulating antioxidant: Uric acid) and in erythrocytes (antioxidant enzymes: SOD,CAT, and GPx, and the antioxidant thiol glutathione in its reduced and oxidised forms: GSH and GSSG).

Two studies provide direct comparisons between oxidative stress markers at these time points during the period of maximum energy requirements (during the summer reproductive event while food provisioning for young) and owing to the ecology of these species it was possible to collect repeat samples from individual females. A third study also provides a direct comparison between active and resting oxidative status in birds sampled during the non-

reproductive season in mid-winter. Fourthly, another natural occurring difference in energy requirements was examined, namely, between reproducing and non-reproducing birds, the data being acquired during the previous three studies.

Previous research has shown that the metabolic rates between these resting and active states to be different by several fold. In addition, it has often been assumed that the reproductive period represents the highest energetic requirements of a bird in its annual cycle, a theory with mixed results in relation to oxidative stress. Under the assumption that periods of heightened energy use will lead to an increase in oxidative damage, the repeated sampling allowed for a direct comparison between opposite and "extreme" points of metabolic requirements during the diurnal cycle of the birds. While all data combined compared reproductive events during the summer and non-reproductive self-maintenance during the winter.

Contrary to expectations, the predicted increase in plasma oxidative damage was not seen during any of the periods of heightened energy requirements, with several examinations providing opposing results. Neither erythrocyte antioxidant enzymes nor plasma non-enzymatic capacity could explain the results found in the measures of oxidative damage. Uric acid, the final product of protein breakdown and a potent antioxidant, was found to be higher in all samples taken during periods of heightened activity, which may perhaps explain why oxidative damage was not found to be higher at higher energy output.

In conclusion, the results of the study presented in this thesis challenge the hypothesis that oxidative stress is elevated during periods of high energy use. It thus adds to the growing body of evidence that the models of oxidative trade-offs are over simplified in the best of cases, and completely wrong in the worst. Included are discussions on the potential non-metabolic drivers of oxidative stress such as circadian/diurnal hormonal cycles and patterns of oxidative stress for example, and a call for a more profound understanding of how these may also impact the results seen in studies throughout the years.