Measures of oxidative state are primarily driven by extrinsic factors in a long-distance migrant.

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Abstract

Oxidative stress is a likely consequence of hard physical exertion, and thus a potential mediator of life-history tradeoffs in migratory animals. However, little is known about the relative importance of intrinsic and extrinsic stressors on the oxidative state of individuals in wild populations. We quantified the relationships between air temperature, sex, body condition, and three markers of oxidative state (malondialdehyde, superoxide dismutase and total antioxidant capacity), across hundreds of individuals of a long-distance migrant (the brent goose Branta bernicla hrota) during wintering and spring staging. We found that air temperature and migratory stage were the strongest predictors of oxidative state. This emphasizes the importance of extrinsic factors in regulating the oxidative state of migrating birds, with differential effects across the migration. The significance of abiotic effects
demonstrates an additional mechanism by which changing climates may affect migratory costs.

Key Words

Antioxidants; Avian; Body Mass; Carry-over Effect; Reproduction

Introduction

A migratory life style places huge physiological demands on individuals [1]. While there is some understanding of the costs of such behaviour, large gaps remain. One such case is oxidative stress, a measure of the oxidative damage caused to important biomolecules, which is an important physiological stressor [2–4]. Metabolic processes naturally result in the formation of reactive oxygen species (ROS) and conditions that increase metabolic rate have the potential to increase oxidative damage [2]. This means that upregulation of antioxidant defences or repair mechanisms could occur to counter the oxidative cost of migration, yet antioxidants are also required for reproductive investment [2,5]. Therefore, the antioxidant system should play a key role in mitigating some of the costs of migration [2,6,7], with differences in oxidative state affecting variation in survival and reproductive performance, [2,3,5,8,9].

Although studies of captive animals have highlighted the potential impacts of oxidative stress [2–4], such benign environments may not reveal the true costs faced by wild organisms, where a range of factors interact to affect their oxidative state [2,4,10]. Migratory animals face significant oxidative burdens resulting from annual movements, and
must also face locations that differ in both biotic and abiotic conditions. Yet there is limited information on the impacts of migration or tradeoffs between internal and external states, although studies suggest active responses occur within the redox system [6,7].

Here we examine the relative importance of intrinsic (biotic) and extrinsic (abiotic and biotic) factors in affecting the oxidative state of light-bellied brent geese *Branta bernicla hrota* (LBBG) using data collected across three consecutive years on >350 individuals on the wintering and staging grounds. This species is a capital breeder that gains >50% of its bodyweight to fuel extremely long migrations (> 12 000 km annually) and reproductive attempts, fueled through the catabolism of fats that are highly sensitive to oxidative damage [2,3,11]. In addition, climatic conditions significantly affect the survival and breeding success of individuals [11,12], making this an excellent system to quantify the relationship between individual body condition, weather and three frequently used markers of oxidative state.
Methods

Study System and Data Collection

LBBG from the study flyway overwinter around coastal Ireland, migrating to Arctic Canada via staging grounds in Iceland. We captured birds using cannon nets around Dublin Bay, Ireland (53.33N, 6.13W) from February – April, and from Iceland (Alftanes peninsula, 64.10N, 22.01W) in May, in 3 consecutive years. LBBG were held individually in sacks then ringed, had standard morphometrics taken (see electronic supplementary material [ESM]) and blood drawn from the tarsal vein. This sample was separated into plasma and red blood cells by centrifugation (12000 g for 3 mins), flash frozen in liquid nitrogen within 6 min of collection, stored at -80 °C, and analysed within 6 months.

Explanatory Variables: Biotic and Abiotic Factors

Scaled Body Mass

Mass in migratory birds is highly variable and strongly non-linear. As such we followed the methods of Harrison et al [11] to calculate a seasonally corrected estimate of mass that is independent of individual body size (see ESM), referred to hereafter as ‘scaled mass’.

Sexing of Individuals

A discriminant function based on morphological differences was used to classify birds as male, female or undetermined (if in a small overlapping zone). All ‘undetermined’ birds were molecularly sexed by a commercial company (Animal Genetics, St Austell, UK).

Weather
Mean daily temperature for Feb – Apr in all study years were obtained from MET Éireann, with similar data for May obtained from Icelandic Met Office stations. We examined a range of temperature metrics but present here results using a two day average of mean temperature (hereafter ‘temperature’) as the different metrics did not produce qualitatively differing results (see ESM Tables S1, S2).

Response Variables: Measures of Oxidative State

**Oxidative Damage to Lipids**

Plasma concentrations of malondialdehyde (MDA) were determined using high performance liquid chromatography following Nussey et al [7] (see ESM), and are expressed in μM. A subset of samples run in duplicate showed high repeatability (R = 0.91 (95% CRI 0.85 - 0.94), n = 136).

**Superoxide Dismutase Activity**

Total SOD activity in plasma was determined using a colorimetric assay kit (Cayman Chemicals, Michigan, USA). Repeatability of the assay was high (R = 0.82 (95% CRI 0.77 - 0.85), n = 584), and results are expressed in units/ml.

**Residual Total Antioxidant Capacity**

Total non-enzymatic antioxidant capacity of plasma samples was measured using a Cayman Chemicals colorimetric assay kit and spectrophotometer, with high repeatability (R = 0.81 (95% CRI 0.75 - 0.85), n = 798). Results are expressed as Trolox-equivalent antioxidant concentrations (TEAC, mM). As TEAC levels have been reported to be strongly dependent on uric acid concentration [13], plasma concentrations of uric acid were determined using a
third Cayman Chemicals assay kit and spectrophotometer. Assay repeatability was good, and comparable with other studies \( R = 0.73 \) (95% CRI 0.66 - 0.79), \( n = 234 \). Residuals from a linear model using uric acid as the predictor and TEAC as the response variable were used as a measure of plasma antioxidants excluding the effects of uric acid [13], hereafter ‘RTEAC’.

**Statistical Analysis**

To test for the combined effects of biotic and abiotic factors on LBBG oxidative stress measures, we fitted separate general linear mixed models with each oxidative state measure in turn as the response variable. The full model included all three-way interactions between the fixed effects of migration stage (the country of capture), scaled mass, sex and temperature, and the random effect of year. Model simplification was conducted using an information theoretic approach. Goodness of fit estimates were calculated using a likelihood-ratio based pseudo-R-squared [14].
Results

Samples were collected from 356 adults across three years (2014, n = 104; 2015, n = 181; 2016, n = 71) on the wintering (n = 195) and staging (n = 161) grounds. Candidate model sets provided good explanatory power for MDA and SOD but not RTEAC (Table 1, Table S3). For MDA the clear top model revealed opposing relationships between migratory stages in relation to temperature, with warmer temperatures resulting in elevated damage levels on wintering grounds, but with the opposite relationship on the staging grounds (Table 1, Fig 1). While similar factors featured additively in competing candidate models for antioxidant metrics, the null model was the most appropriate in both cases. There was negligible support for intrinsic factors as explanatory variables for any oxidative state measures.

<table>
<thead>
<tr>
<th>Oxidative Status Measure</th>
<th>Log likelihood</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>ω</th>
<th>Random Effect Variance Year / Residual</th>
<th>Adj R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Migration stage *</td>
<td>-312.00</td>
<td>636.2</td>
<td>0.00</td>
<td>0.87</td>
<td>0.378 / 0.314</td>
<td>0.533</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Migration stage *</td>
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<td>640.3</td>
<td>4.04</td>
<td>0.12</td>
<td>0.376 / 0.313</td>
<td>0.536</td>
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<td></td>
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<tr>
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<td>644.1</td>
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<td>0.536</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td><strong>SOD</strong></td>
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<tr>
<td>Null</td>
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<td>719.7</td>
<td>0.00</td>
<td>0.60</td>
<td>1.675 / 0.416</td>
<td>0.670</td>
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<td>1.76</td>
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<td>1.671 / 0.409</td>
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<tr>
<td><strong>RTEAC</strong></td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Null</td>
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<td>690.6</td>
<td>0.00</td>
<td>0.63</td>
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</table>
Table 1. Top candidate models explaining variation in oxidative damage (MDA) and antioxidants (SOD, RTEAC) in LBBG. ΔAICc = difference compared to top model, ω = model weighting, Adj $R^2$ = model fit.

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>Δω</th>
<th>Adj $R^2$</th>
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<tr>
<td>Migration stage</td>
<td>-341.94</td>
<td>692.0</td>
<td>1.37</td>
<td>0.32</td>
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<tr>
<td>Mass + Sex</td>
<td>-342.61</td>
<td>695.4</td>
<td>4.78</td>
<td>0.06</td>
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</tbody>
</table>

Figure 1. Lipid damage (MDA) varies significantly with temperature between migration stages. Lines represent linear regressions with 95% confidence intervals.
We examined, for the first time, the oxidative burdens faced by a capital breeder during migration, and found little effect of intrinsic factors. This is surprising since their life history should make LBBG highly susceptible to oxidative stress. Instead, air temperature and migration stage were the strongest influences on measures of oxidative damage, highlighting the role abiotic and extrinsic biotic factors play in regulating the oxidative state of migrating birds.

Weather conditions are known to substantially affect migratory behaviour in many species [1,11], and we found a positive relationship between temperature and oxidative damage on the wintering grounds. As an Arctic breeder, selection is likely to favour an ability to cope with cold spells rather than warm ones, so increasing temperatures as birds prepare to migrate potentially further raises metabolic rate, with consequent increases in lipid damage. Exposure to increased temperatures has been shown to increase MDA levels in domestic chickens Gallus gallus [15]. However, in contrast, the reverse relationship was seen on the staging grounds, where this may be an indirect result of variation in resource availability across stages. On the wintering grounds there is widespread availability and exploitation of maintained grasslands [16]. However, during migration, geese typically overtake the green wave of vegetation growth, so cooler temperatures on the staging grounds will frequently reduce available food via reduced/no plant growth or increased snow cover. Such reductions in food availability have been shown to lead to increases in oxidative damage and decreases in plasma antioxidants in tree swallows Tachycineta bicolor [17].
In contrast to the significant effects seen with for oxidative damage, a null model was the top model for both measures of antioxidant levels. As obligate high-throughput grazers, LBBG have little ability to switch diets, even to seeds or fruits, to replenish antioxidant stores, so increasing circulating antioxidant levels would require upregulation of endogenous reserves, with associated metabolic costs [2]. LBBG may thus tolerate a transient oxidative burden, reserving antioxidants for the longer migration leg and reproductive attempt to come.

The absence of any significant effects of sex or mass on LBBG’s oxidative state may reflect adaptations to an extreme migration that has limited scope for temporal buffering. LBBG breeding success is highly stochastic, so tradeoffs are likely to shift towards adult survival over reproductive investment in this long-lived species [11,12]. However, sampling of individuals on their breeding grounds, or consideration of unmeasured markers, may have revealed allocation differences between the sexes. Although a higher scaled mass is a crucial predictor of reproductive success [11], we found no evidence that LBBG increased circulating levels of antioxidants to protect lipid reserves. The absence of relationships suggests that LBBG, and potentially other long-distance migrants, may be adapted to managing elevated pro-oxidant levels. While long-distance flights can increase oxidative damage [2,7], waterfowl are obligate powered fliers, so are likely to be adapted to this stressor. LBBG also have a short breeding window that necessarily selects for rapid chick growth, a process linked to increased levels of ROS [18]. This early life history may prime LBBG to successfully manage ROS burdens throughout their lifespan [3,10].
These results highlight a further mechanism by which a changing climate may affect Arctic migrants, with increasing temperatures affecting their physiology. Extrinsic factors strongly influence aspects of the annual cycle of migratory species [1,12,19], often through carry-over effects [19]. Changing temperatures may thus create physiological carry-over effects [5,10], a mechanism that warrants further investigation, particularly in relation to how individuals manage oxidative damage during migration.

**Ethics**

All birds were captured and handled under country-specific licenses: Ireland (NPWS 0282016, NPWS 0322014), UK (HO Licence: PPL30/3205), Iceland (IINH 414).

**Data accessibility**

Data are available from the Dryad Digital Repository

https://doi.org/10.5061/dryad.j4k3t6f/1 [20].

**Authors’ contributions**

SB and JB conceived the study. TWB, IRC, FV, KM & SB collected data, with analyses performed by TWB and IRC. TWB, SB & JB drafted the initial manuscript, and all authors contributed to subsequent revisions, approved the final version and agreed to be accountable for all aspects of the work.
Competing interests

We declare we have no competing interests

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Footnotes

Electronic supplementary material is available online at ......

References


