**Methods**

*Study System and Field Data Collection*

LBBG from the Canadian High Arctic flyway overwinter almost exclusively around the coast of Ireland before staging for approximately one month on the south-western coast of Iceland, and then migrating to breeding grounds in the Queen Elizabeth Islands, Arctic Canada. Birds were captured from the greater Dublin Bay area (53.33N, 6.13W) in Ireland from February – April, and from various sites in south-western Iceland (centred around the Alftanes peninsula 64.10N, 22.01W) in May for 3 consecutive years (2014-16). LBBG were captured under license using cannon nets in various terrestrial and intertidal feeding sites. Birds were extracted from nets and held individually in hessian sacks prior to processing.

Individual birds were ringed and had standard morphometrics (wing length (cm ± 1), headbill length (mm ± 0.5), and body mass (g ± 25)) taken along with a blood sample from the tarsal vein (~0.5 ml). This sample was immediately transferred to an eppendorf tube and separated by centrifugation (12000 *g*  for 3 mins) into plasma and red blood cells before samples were flash frozen in liquid nitrogen and held within a cryogenic shipper (Taylor-Wharton CX100, Jencons, UK) while in the field. All samples were flash frozen within 6 min of collection, stored at -80°C in the laboratory, and analysed within 6 months of collection.

*Scaled Body Mass*

Mass in a migratory bird is necessarily highly variable depending on the period of the year in which it is measured, and is strongly non-linear in capital breeding species, such as LBBG, where rapid gains in fat stores occur prior to migratory flights. LBBG typically gain an additional one third of their body mass from wintering ground weights to staging ground departure, and this mass gain corresponds well with abdominal fat deposition in this and other waterfowl species (Madsen & Klaassen 2006, Harrison et al 2013). As such we followed the methods of Harrison *et al* (2013) to calculate a seasonally corrected estimate of mass that is independent of individual body size. In brief, we calculated the scaled mass index of all captured individuals in 2014 - 16 from the ratio of a least-squares regression of log(mass) on log(headbill length) to the correlation coefficient between these variables (Peig & Green 2010). We then corrected these scaled mass estimates for temporal variation by fitting a third order polynomial term for day of capture to fit this known seasonal mass trajectory (comparison to linear fit: F3,1059 = 258.96, P<0.0001; comparison to second order polynomial: F1,1061 = 515.68, P<0.0001; adjusted R2 = 0.35), and used the residuals from this model as corrected mass estimates, referred to hereafter as ‘scaled mass’.

*Sexing of Individuals*

LBBG are size-dimorphic with males being structurally larger than females. As such, a discriminant function based on morphological differences was used to classify birds as male, female or unknown (if in a small overlapping zone). We verified this classification by comparing the results of the discriminant function to sex determined manually through examination of breast down on LBBG captured in Iceland (n = 295). Here females are already growing distinctive dark grey down that is used to line the nest, which is absent in males. This comparison produced 0 misclassifications between morphology and down, with 4% (n = 9) classified as unknown by the discriminant function. All birds that were morphologically indeterminate (mostly second year birds that are still structurally smaller and are not necessarily entering breeding condition in the year following hatching) plus ten additional individuals on the boundary of the discriminant function (for confirmatory purposes) were molecularly sexed by a commercial company (Animal Genetics, St Austell, UK). This revealed only one misclassification based on the discriminant function, and added 23 additional birds of previously undetermined sex to our overall sample.

*Measures of Oxidative Stress*

In all cases, laboratory assays were conducted within 6 months of sample collection to ensure high quality results without sample degredation, as conducted by other multi-year assessments of oxidative stress in wild animals (Christensen et al 2016, Vitikainen et al 2017), thus samples from each year were analysed separately rather than being stored until all could be analysed simultaneously.

*Oxidative Damage to Lipids*

Plasma concentrations of malondialdehyde (MDA), a product of lipid peroxidation, were determined using high performance liquid chromatography (HPLC) following the method of Nussey *et al* 2009, adapted from Agarwal & Chase (2002). In brief, a 10 μl plasma aliquot was vortexed with 10 μl of butylated hydroxytoluene, 80 μl phosphoric acid (0.44 M) and 20 μl 2-thiobarbituric acid solution (42 mM). This mixture was heated at 100 °C for 1 hr, cooled on ice for 5 mins, then vortexed with 100 μl n-butanol for 20 s before 40 μl of the upper phase was drawn off for HPLC (Dionex Corporation, USA). The HPLC system was fitted with a Hewlett-Packard Hypersil 5μ ODS 100 x 4.6 mm column and a 5μ ODS guard column and used a methanol-buffer (40:60 v/v) as the mobile phase, with the buffer comprising an anhydrous solution of potassium monobasic phosphate (50 mM) at pH 6.8. Fluoresence detection was performed at 515 and 553 nm (RF2000, Dionex Corporation, USA). A standard curve was generated in a parallel assay, using serial dilutions of 5 μM 1,1,3,3-tetraethoxypropane (which hydrolyses to produce MDA) in 40% ethanol. Sample results 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 samples from 68 individuals). All repeatabilities of oxidative stress biomarkers were calculated using MCMCglmm (Hadfield 2010).

*Superoxide Dismutase Activity*

Total SOD activity in plasma was determined using a colorimetric assay kit (Cayman Chemicals, Michigan, USA). Samples were prepared according to the kit instructions, with samples being diluted 1:250 with buffer solution prior to assaying with a spectrophotometer (Spectramax M2, Molecular Devices, Sunnyvale, CA, USA). SOD activities were calibrated using a serial dilution of bovine SOD run on each plate. Repeatability of the assay was good (R = 0.82 (95% CRI 0.77 - 0.85), n = 584 samples from 292), assessed in a subset of samples run on duplicate plates. One unit is defined as the quantity of enzyme required to provide 50% dismutation of the superoxide radical, and results are expressed in units/ml.

*Total Antioxidant Capacity and Uric Acid Concentration*

Total non-enzymatic antioxidant capacity of plasma samples (diluted 1:5 with buffer solution) was also measured using a Cayman Chemicals colorimetric assay kit and spectrophotometer. This assay reflects levels of a wide range of potentially circulating antioxidants including vitamin E, carotenoids and thiols. Results are expressed as Trolox-equivalent antioxidant concentrations (TEAC, mM). Again, repeatability of a subset of samples was was good (R = 0.81 (95% CRI 0.75 - 0.85), n = 798 samples from 399 individuals).

As TEAC levels have been reported to be strongly dependent on uric acid concentration (an antioxidant of debated importance due to its contrasting role as an avian waste product that can also increase during periods of low food availability (Cohen et al 2007, Alonso-Alvarez & Ferrer 2001), we determined the plasma concentrations of uric acid (1:20 dilution) using a third Cayman Chemicals fluorescence assay kit and the spectrophotometer. Uric acid concentrations were less repeatable than other measures, but still high and comparable with other studies (R = 0.73 (95% CRI 0.66 - 0.79), n = 234 samples from 117 individuals). As we found a positive relationship between TEAC and uric acid levels, residuals from a linear model using uric acid as the predictor and TEAC as the response variable were then taken and used in further analyses as a measure of plasma antioxidants that excludes the effects of uric acid (Cohen et al 2007), hereafter ‘RTEAC’.

*Environmental Variables*

Mean daily temperature (hereafter ‘temperature’) for Feb – Apr in all study years were obtained from MET Éireann (station a maximum of 12 km from capture sites), with similar data obtained from Icelandic Met Office stations (maximum 20 km from capture sites) for May. As the production of pro-oxidants and the deployment of defence and repair mechanisms represent highly dynamic physiological processes, we examined a range of temperature metrics for inclusion in our models. The three considered were: a) the day of capture only, b) a short-term average calculated from the date of capture plus the day prior to this, or c) an average from the seven days leading up to capture for each individual. These different metrics did not produce qualitatively differing results in any analyses (see below).

*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 (GLMMs) with each oxidative stress measure in turn as the response variable. Values for MDA and SOD were natural log-transformed to conform with assumptions of normality. In all cases, 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. During data exploration we initially included ‘time to sample’ (the difference between cannon firing time and blood sampling time) but as this variable provided no explanatory power, we did not include it in the final presented analysis. Model simplification was conducted using an information theoretic approach via the MuMIn package (Burnham & Anderson 2002, Barton 2016), with candidate models considered to be competing with the top model if AICc values were within 2 units. Models that differed by a single additional term that did not improve the AICc score by more than two units were not considered to be competing as such additional terms can be regarded as uninformative (Arnold 2010). Goodness of fit estimates were calculated for GLMMs using a likelihood-ratio based pseudo-R-squared (Nakagawa & Schielzeth 2013). All statistical analyses were conducted in R 3.3.1 (R Core Development Team 2016).

Analyses presented include only adult LBBG (birds identified by plumage to be ≥2 years old) for which we had data on all measures of oxidative state (n = 356 individuals), although repeating analyses on expanded datasets including all birds with data for each specific marker (dataset increased by 4-15% depending on the marker) showed no qualitative differences in the candidate model sets (results not shown).

**Table S1**. Top candidate model sets using an average of mean daily temperature from the previous 7 days alongside scaled mass, sex, migration stage and the random effect of year explaining variation in oxidative damage (MDA) and antioxidants (SOD, RTEAC) in light-bellied brent geese together with the variance explained (Adj R2). ΔAICc is the difference in model fit, ω is the model weighting.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Oxidative Status Measure** | **Log likelihood** | **AICc** | **ΔAICc** | **ω** | **Adj R2** |
| **MDA** |  |  |  |  |  |
| Migration stage \* Temperature | -320.25 | 652.7 | 0.00 | 0.76 | 0.502 |
|  |  |  |  |  |  |
| Migration stage \* Temperature + Sex | -320.56 | 655.4 | 2.70 | 0.20 | 0.507 |
|  |  |  |  |  |  |
| Migration stage \* Temperature + Migration stage \* Sex | -321.10 | 658.6 | 5.87 | 0.04 | 0.509 |
|  |  |  |  |  |  |
| **SOD** |  |  |  |  |  |
| Null | -356.81 | 719.7 | 0.00 | 0.86 | 0.670 |
| Migration stage  | -356.16 | 724.4 | 4.75 | 0.09 | 0.671 |
| Sex | -358.47 | 725.0 | 5.36 | 0.06 | 0.670 |
|  |  |  |  |  |  |
| **RTEAC** |  |  |  |  |  |
| Null | -342.27 | 690.6 | 0.00 | 0.63 | 0.002 |
| Migration stage | -341.94 | 692.0 | 1.37 | 0.32 | 0.017 |
| Sex + Mass | -342.61 | 695.4 | 4.78 | 0.06 | 0.065 |

**Table S2**. Top candidate model sets using the mean daily temperature from the previous 24 hours alongside scaled mass, sex, migration stage and the random effect of year explaining variation in oxidative damage (MDA) and antioxidants (SOD, RTEAC) in light-bellied brent geese together with the variance explained (Adj R2). ΔAICc is the difference in model fit, ω is the model weighting.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Oxidative Status Measure** | **Log likelihood** | **AICc** | **ΔAICc** | **ω** | **Adj R2** |
| **MDA** |  |  |  |  |  |
| Migration stage  | -329.10 | 666.3 | 0.00 | 0.77 | 0.454 |
|  |  |  |  |  |  |
| Migration stage \* Temperature  | -328.93 | 670.1 | 3.77 | 0.12 | 0.475 |
|  |  |  |  |  |  |
| Migration stage + Sex | -330.01 | 670.2 | 3.87 | 0.12 | 0.457 |
|  |  |  |  |  |  |
| **SOD** |  |  |  |  |  |
| Null | -356.81 | 719.7 | 0.00 | 0.82 | 0.670 |
| Temperature | -357.88 | 723.9 | 4.19 | 0.10 | 0.674 |
| Migration stage  | -3568.16 | 724.4 | 4.75 | 0.08 | 0.671 |
| **RTEAC** |  |  |  |  |  |
| Null | -342.27 | 690.6 | 0.00 | 0.63 | 0.002 |
| Migration stage | -341.94 | 692.0 | 1.37 | 0.32 | 0.017 |
| Mass + Sex | -342.61 | 695.4 | 4.78 | 0.06 | 0.065 |

**Table S3**. Candidate mixed model sets with ΔAICc < 10 from the top model for SOD and RTEAC, and ΔAICc < 20 from the top model for MDA (fewer models with good explanatory power), explaining variation in oxidative damage (MDA) and antioxidants (SOD, RTEAC) in light-bellied brent geese. Adj R2 is the variance explained, ΔAICc is the difference in model fit, ω is the model weighting.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Oxidative Status Measure** | **Log likelihood** | **AICc** | **ΔAICc** | **ω** | **Adj R2** |
| **MDA** |  |  |  |  |  |
| Migration stage \* Temperature | -312.003 | 636.2 | 0 | 0.864 | 0.533 |
| Migration stage \* Temperature + Sex | -312.981 | 640.3 | 4.04 | 0.115 | 0.536 |
| Migration stage \* Temperature + Migration stage \* Sex | -313.862 | 644.1 | 7.89 | 0.017 | 0.537 |
| Migration stage \* Temperature + Temperature \* Sex | -315.404 | 647.2 | 10.98 | 0.004 | 0.536 |
| Migration stage \* Temperature + Migration stage \* Sex + Temperature \* Sex | -316.044 | 650.6 | 14.36 | 0.001 | 0.538 |
| Migration stage \* Temperature + Mass | -319.14 | 652.6 | 16.35 | 0 | 0.536 |
| Null | -332.59 | 672.2 | 35.95 | 0 | 0.434 |
|  |  |  |  |  |  |
| **SOD** |  |  |  |  |  |
| Null | -356.81 | 719.7 | 0 | 0.558 | 0.670 |
| Temperature | -356.67 | 721.4 | 1.76 | 0.231 | 0.677 |
| Migration stage + Temperature | -356.50 | 723.2 | 3.49 | 0.098 | 0.681 |
| Migration stage | -358.16 | 724.4 | 4.75 | 0.052 | 0.671 |
| Sex | -358.47 | 725 | 5.36 | 0.038 | 0.670 |
| Temperature + Sex | -358.32 | 726.8 | 7.13 | 0.016 | 0.677 |
| Migration stage + Temperature + Sex | -358.13 | 728.5 | 8.82 | 0.007 | 0.681 |
| **RTEAC** |  |  |  |  |  |
| Null | -342.272 | 690.6 | 0 | 0.554 | 0.002 |
| Migration stage | -341.937 | 692 | 1.37 | 0.279 | 0.017 |
| Mass + Sex | -342.61 | 695.4 | 4.78 | 0.051 | 0.065 |
| Sex | -344.06 | 696.2 | 5.62 | 0.033 | 0.002 |
| Mass | -344.343 | 696.8 | 6.19 | 0.025 | 0.043 |
| Temperature | -344.623 | 697.4 | 6.75 | 0.019 | 0.007 |
| Migration stage + Sex | -343.726 | 697.6 | 7.01 | 0.017 | 0.017 |
| Migration stage + Sex + Mass | -343.158 | 698.6 | 7.94 | 0.01 | 0.073 |
| Migration stage + Mass | -344.705 | 699.6 | 8.97 | 0.006 | 0.052 |
| Migration stage + Temperature | -344.931 | 700 | 9.42 | 0.005 | 0.017 |

**Ethics**

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

**References**

Agarwal R, Chase SD. 2002 Rapid, fluorimetric-liquid chromatographic determination of malondialdehyde in biological samples. *J Chromatogr B Analyt Technol Biomed Life Sci* **775**, 121-126.

Alonso-Alvarez C, Ferrer M. 2001 A biochemical study about fasting, subfeeding and recovery processes in yellow-legged gulls. *Physiol Biochem Zool* **74**, 703-713.

Arnold, TW. 2010 Uninformative Parameters and Model Selection Using Akaike's Information Criterion. *J Wildl Manag* **74,** 1175-1178.

Barton K. 2018 Multi-Model Inference.

Burnham KP, Anderson DR. 2002 Model selection and multimodel inference. A practical information-theoretic approach. Springer-Verlag, New York.

Christensen LL, Selman C, Blount JD, Pilkington JG, Watt KA, Pemberton JM, Reid JM, Nussey DH. 2016 Marker-dependent associations among oxidative stress, growth and survival during early life in a wild mammal. *Proc Roy Soc B* **283** DOI: 10.1098/rspb.2016.1407

Cohen A, Klasing K, Ricklefs R. 2007 Measuring circulating antioxidants in wild birds. *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.* **147**, 110–121.

Hadfield JD. 2010 [MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package](http://www.jstatsoft.org/v33/i02/paper)  *J Stat Soft* **33**, 1-22.

Harrison XA, Hodgson DJ, Inger R, Colhoun K, Gudmundsson GA, McElwaine G, Tregenza T, Bearhop S. 2013 Environmental Conditions during Breeding Modify the Strength of Mass-Dependent Carry-Over Effects in a Migratory Bird. *PLoS One* **8**, 1–9.

Madsen, J. and Klaassen, M. 2006 Assessing body condition and energy budget components by scoring abdominal profiles in free-ranging pink-footed geese Anser brachyrhynchus. *J Av Biol* **37**, 283–287.

Nakagawa S, Schielzeth H. 2013 A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133–142.

Nussey DH, Pemberton JM, Pilkington JG, Blount JD. 2009 Life history correlates of oxidative damage in a free-living mammal population. *Funct. Ecol.* **23**, 809–817.

Peig J, Green AJ. 2010 The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Func Ecol* **24**, 1323-1332.

R Core Team. 2017 R: A language and environment for statistical computing.

Vitikainen EIK, Cant MA, Sanderson JL, Mitchell C, Nichols HJ, Marshall HH, Thompson FJ, Gilchrist JS, Hodge SJ, Johnstone RA, Blount JD 2016 Evidence of Oxidative Shielding of Offspring in a Wild Mammal. *Front Ecol Evol* **4**,58. doi: 10.3389/fevo.2016.00058