1   **Measures of oxidative state are primarily driven by extrinsic factors in a long-distance**

2   **migrant**

3

4   **Thomas W Bodey[1,2*], Ian R Cleasby[1,3], Jonathan D Blount[1], Freydis Vigfusdottir[1,4], Kerry**

5   **Mackie[1], Stuart Bearhop[1*]**

6

7   [1]Centre for Ecology & Conservation, University of Exeter Penryn Campus, Penryn, Cornwall, TR10 9FE, UK

8   [2]School of Biological Sciences, University of Auckland, Auckland, NZ

9   [3] RSPB Centre for Conservation Science, Inverness, UK

10   [4]Faculty of Life and Environmental Sciences and School of Social Sciences, University of Iceland, Reykjavik,

11   Iceland

12   [*]Corresponding Author

13

14

15   **Abstract**

16   Oxidative stress is a likely consequence of hard physical exertion, and thus a potential

17   mediator of life-history tradeoffs in migratory animals. However, little is known about the

18   relative importance of intrinsic and extrinsic stressors on the oxidative state of individuals in

19   wild populations. We quantified the relationships between air temperature, sex, body

20   condition, and three markers of oxidative state (malondialdehyde, superoxide dismutase

21   and total antioxidant capacity), across hundreds of individuals of a long-distance migrant

22   (the brent goose *Branta bernicla hrota*) during wintering and spring staging. We found that

23   air temperature and migratory stage were the strongest predictors of oxidative state. This

24   emphasizes the importance of extrinsic factors in regulating the oxidative state of migrating

25   birds, with differential effects across the migration. The significance of abiotic effects

26 demonstrates an additional mechanism by which changing climates may affect migratory

27 costs.

28

29 **Key Words**

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

31

32 **Introduction**

33

34 A migratory life style places huge physiological demands on individuals [1]. While there is

35 some understanding of the costs of such behaviour, large gaps remain. One such case is

36 oxidative stress, a measure of the oxidative damage caused to important biomolecules,

37 which is an important physiological stressor [2–4]. Metabolic processes naturally result in

38 the formation of reactive oxygen species (ROS) and conditions that increase metabolic rate

39 have the potential to increase oxidative damage [2]. This means that upregulation of

40 antioxidant defences or repair mechanisms could occur to counter the oxidative cost of

41 migration, yet antioxidants are also required for reproductive investment [2,5]. Therefore,

42 the antioxidant system should play a key role in mitigating some of the costs of migration

43 [2,6,7], with differences in oxidative state affecting variation in survival and reproductive

44 performance, [2,3,5,8,9].

45

46 Although studies of captive animals have highlighted the potential impacts of oxidative

47 stress [2–4], such benign environments may not reveal the true costs faced by wild

48 organisms, where a range of factors interact to affect their oxidative state [2,4,10].

49 Migratory animals face significant oxidative burdens resulting from annual movements, and

50   must also face locations that differ in both biotic and abiotic conditions. Yet there is limited

51   information on the impacts of migration or tradeoffs between internal and external states,

52   although studies suggest active responses occur within the redox system [6,7].

53

54   Here we examine the relative importance of intrinsic (biotic) and extrinsic (abiotic and

55   biotic) factors in affecting the oxidative state of light-bellied brent geese *Branta bernicla*

56   *hrota* (LBBG) using data collected across three consecutive years on >350 individuals on the

57   wintering and staging grounds. This species is a capital breeder that gains >50% of its

58   bodyweight to fuel extremely long migrations (> 12 000 km annually) and reproductive

59   attempts, fueled through the catabolism of fats that are highly sensitive to oxidative

60   damage [2,3,11]. In addition, climatic conditions significantly affect the survival and

61   breeding success of individuals [11,12], making this an excellent system to quantify the

62   relationship between individual body condition, weather and three frequently used markers

63   of oxidative state.

64

65

66

67

68

69

70   **Methods**

71   *Study System and Data Collection*

72   LBBG from the study flyway overwinter around coastal Ireland, migrating to Arctic Canada

73   via staging grounds in Iceland. We captured birds using cannon nets around Dublin Bay,

74   Ireland (53.33N, 6.13W) from February – April, and from Iceland (Alftanes peninsula,

75   64.10N, 22.01W) in May, in 3 consecutive years. LBBG were held individually in sacks then

76   ringed, had standard morphometrics taken (see electronic supplementary material [ESM])

77   and blood drawn from the tarsal vein. This sample was separated into plasma and red blood

78   cells by centrifugation (12000 g for 3 mins), flash frozen in liquid nitrogen within 6 min of

79   collection, stored at -80 °C, and analysed within 6 months.

80

81   *Explanatory Variables: Biotic and Abiotic Factors*

82   *Scaled Body Mass*

83   Mass in migratory birds is highly variable and strongly non-linear. As such we followed the

84   methods of Harrison *et al* [11] to calculate a seasonally corrected estimate of mass that is

85   independent of individual body size (see ESM), referred to hereafter as 'scaled mass'.

86

87   *Sexing of Individuals*

88   A discriminant function based on morphological differences was used to classify birds as

89   male, female or undetermined (if in a small overlapping zone). All 'undetermined' birds

90   were molecularly sexed by a commercial company (Animal Genetics, St Austell, UK).

91

92   *Weather*

93   Mean daily temperature for Feb – Apr in all study years were obtained from MET Éireann,

94   with similar data for May obtained from Icelandic Met Office stations. We examined a range

95   of temperature metrics but present here results using a two day average of mean

96   temperature (hereafter 'temperature') as the different metrics did not produce qualitatively

97   differing results (see ESM Tables S1, S2).

98

99   *Response Variables: Measures of Oxidative State*

100   *Oxidative Damage to Lipids*

101   Plasma concentrations of malondialdehyde (MDA) were determined using high performance

102   liquid chromatography following Nussey *et al* [7] (see ESM), and are expressed in μM. A

103   subset of samples run in duplicate showed high repeatability (R = 0.91 (95% CRI 0.85 - 0.94)

104   n = 136).

105

106   *Superoxide Dismutase Activity*

107   Total SOD activity in plasma was determined using a colorimetric assay kit (Cayman

108   Chemicals, Michigan, USA). Repeatability of the assay was high (R = 0.82 (95% CRI 0.77 -

109   0.85), n = 584), and results are expressed in units/ml.

110

111   *Residual Total Antioxidant Capacity*

112   Total non-enzymatic antioxidant capacity of plasma samples was measured using a Cayman

113   Chemicals colorimetric assay kit and spectrophotometer, with high repeatability (R = 0.81

114   (95% CRI 0.75 - 0.85), n = 798). Results are expressed as Trolox-equivalent antioxidant

115   concentrations (TEAC, mM). As TEAC levels have been reported to be strongly dependent on

116   uric acid concentration [13], plasma concentrations of uric acid were determined using a

117   third Cayman Chemicals assay kit and spectrophotometer. Assay repeatability was good,

118   and comparable with other studies (R = 0.73 (95% CRI 0.66 - 0.79), n = 234). Residuals from

119   a linear model using uric acid as the predictor and TEAC as the response variable were used

120   as a measure of plasma antioxidants excluding the effects of uric acid [13], hereafter

121   'RTEAC'.

122

123

124   *Statistical Analysis*

125   To test for the combined effects of biotic and abiotic factors on LBBG oxidative stress

126   measures, we fitted separate general linear mixed models with each oxidative state

127   measure in turn as the response variable. The full model included all three-way interactions

128   between the fixed effects of migration stage (the country of capture), scaled mass, sex and

129   temperature, and the random effect of year. Model simplification was conducted using an

130   information theoretic approach. Goodness of fit estimates were calculated using a

131   likelihood-ratio based pseudo-R-squared [14].

132

133 **Results**

134

135 Samples were collected from 356 adults across three years (2014, n = 104; 2015, n = 181;

136 2016, n = 71) on the wintering (n = 195) and staging (n = 161) grounds. Candidate model sets

137 provided good explanatory power for MDA and SOD but not RTEAC (Table 1, Table S3). For

138 MDA the clear top model revealed opposing relationships between migratory stages in

139 relation to temperature, with warmer temperatures resulting in elevated damage levels on

140 wintering grounds, but with the opposite relationship on the staging grounds (Table 1, Fig

141 1). While similar factors featured additively in competing candidate models for antioxidant

142 metrics, the null model was the most appropriate in both cases. There was negligible

143 support for intrinsic factors as explanatory variables for any oxidative state measures.

144
145

Oxidative Status Measure	Log likelihood	AICc	ΔAICc	ω	Random Effect Variance Year / Residual	Adj R^2
MDA						
Migration stage * Temperature	-312.00	636.2	0.00	0.87	0.378 / 0.314	0.533
Migration stage * Temperature + Sex	-312.98	640.3	4.04	0.12	0.376 / 0.313	0.536
Migration stage * Temperature + Migration stage * Sex	-313.86	644.1	7.89	0.02	0.376 / 0.313	0.536
SOD						
Null	-356.81	719.7	0.00	0.60	1.675 / 0.416	0.670
Temperature	-356.67	721.4	1.76	0.25	1.671 / 0.409	0.677
Migration stage + Temperature	-356.50	723.2	3.49	0.10	1.642 / 0.406	0.681
RTEAC						
Null	-342.27	690.6	0.00	0.63	0.006 / 0.394	0.002

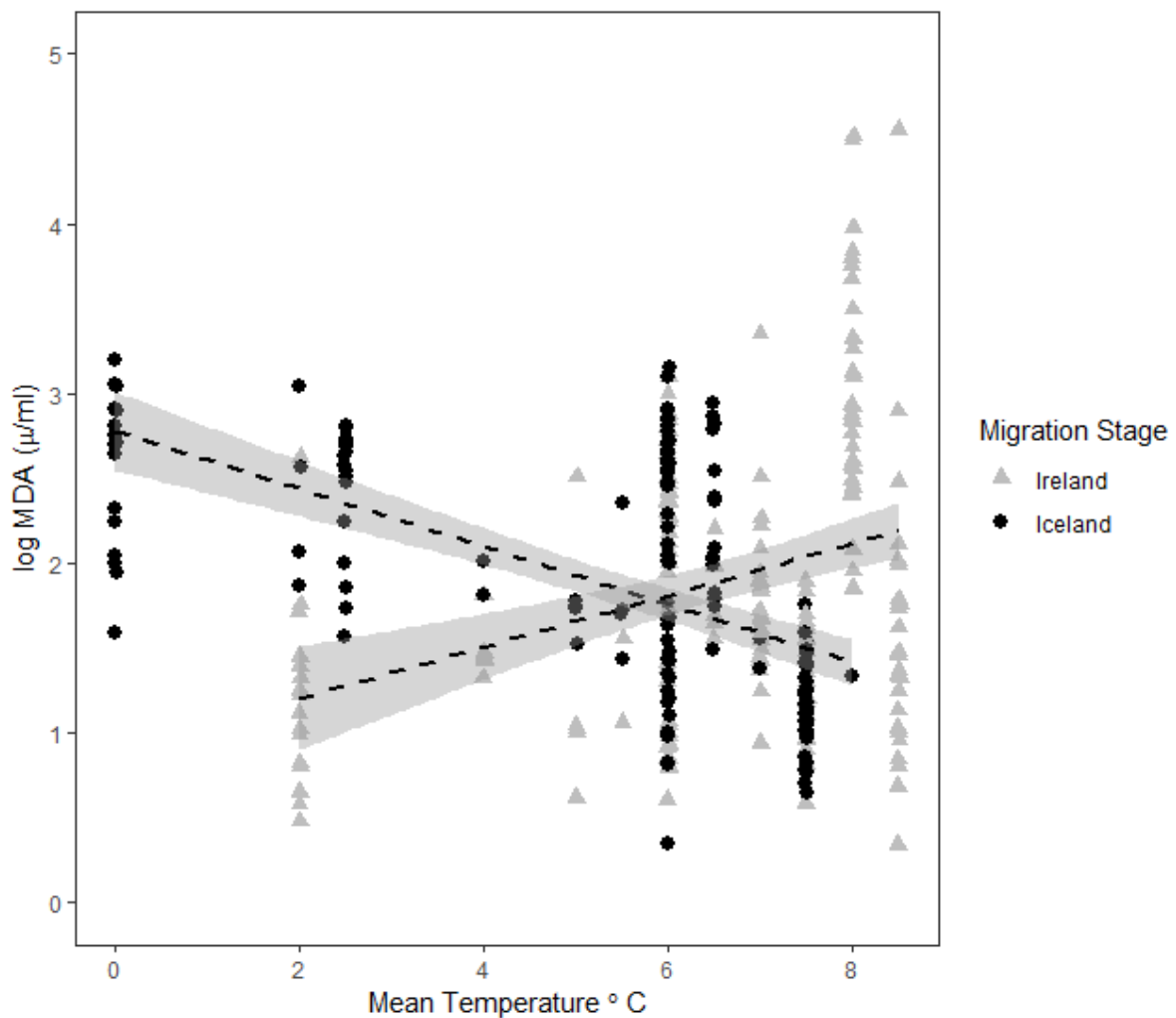|						
Migration stage	-341.94	692.0	1.37	0.32	0.003 / 0.391	0.017
Mass + Sex	-342.61	695.4	4.78	0.06	0.0003 / 0.378	0.065

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.

Figure 1. Lipid damage (MDA) varies significantly with temperature between migration

stages. Lines represent linear regressions with 95% confidence intervals.

159

160 **Discussion**

161

162 We examined, for the first time, the oxidative burdens faced by a capital breeder during

163 migration, and found little effect of intrinsic factors. This is surprising since their life history

164 should make LBBG highly susceptible to oxidative stress. Instead, air temperature and

165 migration stage were the strongest influences on measures of oxidative damage,

166 highlighting the role abiotic and extrinsic biotic factors play in regulating the oxidative state

167 of migrating birds.

168

169 Weather conditions are known to substantially affect migratory behaviour in many species

170 [1,11], and we found a positive relationship between temperature and oxidative damage on

171 the wintering grounds. As an Arctic breeder, selection is likely to favour an ability to cope

172 with cold spells rather than warm ones, so increasing temperatures as birds prepare to

173 migrate potentially further raises metabolic rate, with consequent increases in lipid damage.

174 Exposure to increased temperatures has been shown to increase MDA levels in domestic

175 chickens *Gallus gallus* [15]. However, in contrast, the reverse relationship was seen on the

176 staging grounds, where this may be an indirect result of variation in resource availability

177 across stages. On the wintering grounds there is widespread availability and exploitation of

178 maintained grasslands [16]. However, during migration, geese typically overtake the green

179 wave of vegetation growth, so cooler temperatures on the staging grounds will frequently

180 reduce available food via reduced/no plant growth or increased snow cover. Such

181 reductions in food availability have been shown to lead to increases in oxidative damage

182 and decreases in plasma antioxidants in tree swallows *Tachycineta bicolor* [17].

183

184 In contrast to the significant effects seen with for oxidative damage, a null model was the

185 top model for both measures of antioxidant levels. As obligate high-throughput grazers,

186 LBBG have little ability to switch diets, even to seeds or fruits, to replenish antioxidant

187 stores, so increasing circulating antioxidant levels would require upregulation of

188 endogenous reserves, with associated metabolic costs [2]. LBBG may thus tolerate a

189 transient oxidative burden, reserving antioxidants for the longer migration leg and

190 reproductive attempt to come.

191

192 The absence of any significant effects of sex or mass on LBBG's oxidative state may reflect

193 adaptations to an extreme migration that has limited scope for temporal buffering. LBBG

194 breeding success is highly stochastic, so tradeoffs are likely to shift towards adult survival

195 over reproductive investment in this long-lived species [11,12]. However, sampling of

196 individuals on their breeding grounds, or consideration of unmeasured markers, may have

197 revealed allocation differences between the sexes. Although a higher scaled mass is a crucial

198 predictor of reproductive success [11], we found no evidence that LBBG increased

199 circulating levels of antioxidants to protect lipid reserves. The absence of relationships

200 suggests that LBBG, and potentially other long-distance migrants, may be adapted to

201 managing elevated pro-oxidant levels. While long-distance flights can increase oxidative

202 damage [2,7], waterfowl are obligate powered fliers, so are likely to be adapted to this

203 stressor. LBBG also have a short breeding window that necessarily selects for rapid chick

204 growth, a process linked to increased levels of ROS [18]. This early life history may prime

205 LBBG to successfully manage ROS burdens throughout their lifespan [3,10].

206

207 These results highlight a further mechanism by which a changing climate may affect Arctic

208 migrants, with increasing temperatures affecting their physiology. Extrinsic factors strongly

209 influence aspects of the annual cycle of migratory species [1,12,19], often through carry-

210 over effects [19]. Changing temperatures may thus create physiological carry-over effects

211 [5,10], a mechanism that warrants further investigation, particularly in relation to how

212 individuals manage oxidative damage during migration.

213

214 **Ethics**

215

216 All birds were captured and handled under country-specific licenses: Ireland (NPWS

217 0282016, NPWS 0322014), UK (HO Licence: PPL30/3205), Iceland (IINH 414).

218

219 **Data accessibility**

220

221 Data are available from the Dryad Digital Repository

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

223

224 **Authors' contributions**

225

226 SB and JB conceived the study. TWB, IRC, FV, KM & SB collected data, with analyses

227 performed by TWB and IRC. TWB, SB & JB drafted the initial manuscript, and all authors

228 contributed to subsequent revisions, approved the final version and agreed to be

229 accountable for all aspects of the work.

230

246   **Footnotes**

247   Electronic supplementary material is available online at …….

248

249

250   **References**

251

252   1.   Newton I. 2007 The Migration Ecology of Birds. Academic Press.

253   2.   Skrip MM, McWilliams SR. 2016 Oxidative balance in birds: An atoms-to-organisms-

254   to-ecology primer for ornithologists. *J. F. Ornithol.* **87**, 1–20.

255   3.   Monaghan P, Metcalfe NB, Torres R. 2009 Oxidative stress as a mediator of life

256   history trade-offs: Mechanisms, measurements and interpretation. *Ecol. Lett.* **12**, 75–

257   92.

258   4.   Speakman JR *et al.* 2015 Oxidative stress and life histories: Unresolved issues and

259   current needs. *Ecol. Evol.* **5**, 5745–5757.

260   5.   Blount JD, Vitikainen EIK, Stott I, Cant MA. 2016 Oxidative shielding and the cost of

261   reproduction. *Biol. Rev.* **91**, 483–497.

262   6.   Skrip MM, Bauchinger U, Goymann W, Fusani L, Cardinale M, Alan RR, Mcwilliams SR.

263   2015 Migrating songbirds on stopover prepare for, and recover from, oxidative

264   challenges posed by long-distance flight. *Ecol. Evol.* **5**, 3198–3209.

265   7.   Jenni-Eiermann S, Jenni L, Smith S, Costantini D. 2014 Oxidative stress in endurance

266   flight: An unconsidered factor in bird migration. *PLoS One* **9**, 1–6.

267   8.   Wiersma P, Selman C, Speakman JR, Verhulst S. 2004 Birds sacrifice oxidative

268   protection for reproduction. *Proc. R. Soc. B Biol. Sci.* **271**, S360–S363.

269   9.   Nussey DH, Pemberton JM, Pilkington JG, Blount JD. 2009 Life history correlates of

270   oxidative damage in a free-living mammal population. *Funct. Ecol.* **23**, 809–817.

271   10.   Urvik J, Meitern R, Rattiste K, Saks L, Hõrak P, Sepp T. 2016 Variation in the Markers

272   of Nutritional and Oxidative State in a Long-Lived Seabird: Associations with Age and

273   Longevity. *Physiol. Biochem. Zool.* **89**, 417–440.

274   11.   Harrison XA, Hodgson DJ, Inger R, Colhoun K, Gudmundsson GA, McElwaine G,

275   Tregenza T, Bearhop S. 2013 Environmental Conditions during Breeding Modify the

276   Strength of Mass-Dependent Carry-Over Effects in a Migratory Bird. *PLoS One* **8**, 1–9.

277   12.   Cleasby IR, Bodey TW, Vigfusdottir F, McDonald JL, McElwaine G, Mackie K, Colhoun

278   K, Bearhop S. 2017 Climatic conditions produce contrasting influences on

279   demographic traits in a long-distance Arctic migrant. *J. Anim. Ecol.* **86**, 285–295.

280 13. Cohen A, Klasing K, Ricklefs R. 2007 Measuring circulating antioxidants in wild birds.

281   *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.* **147**, 110–121.

282 14. Nakagawa S, Schielzeth H. 2013 A general and simple method for obtaining R2 from

283   generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133–142.

284 15. Lin H, Decuypere E, Buyse J. 2006 Acute heat stress induces oxidative stress in broiler

285   chickens. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **144**, 11–17.

286 16. Inger R, Harrison XA, Ruxton GD, Newton J, Colhoun K, Gudmundsson GA, McElwaine

287   G, Pickford M, Hodgson DJ, Bearhop S. 2010 Carry-over effects reveal reproductive

288   costs in a long-distance migrant. *J. Anim. Ecol.* **79**, 974-982.

289 17. Stanton R, Clark RG, Morrissey CA. 2017 Intensive agriculture and insect prey

290   availability influence oxidative status and return rates of an aerial insectivore.

291   *Ecosphere* **8**.

292 18. Kim S-Y, Noguera JC, Morales J, Velando A. 2011 Quantitative genetic evidence for

293   trade-off between growth and resistance to oxidative stress in a wild bird. *Evol. Ecol.*

294   **25**, 461–472.

295 19. Harrison XA, Blount JD, Inger R, Norris DR, Bearhop S. 2011 Carry-over effects as

296   drivers of fitness differences in animals. *J. Anim. Ecol.* **80**, 4–18.

297 20. Bodey TW, Cleasby IR, Blount JD, Vigfusdottir F, Mackie K, Bearhop S. Data from:

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

299   migrant. Dryad Digital Repository. https://doi.org/10.5061/dryad.j4k3t6f

300