

1 Do bar-headed geese train for high altitude flights?

2

3 Running title: Do geese train for high-altitude flight?

4

5 Hawkes LA^{1*}, Batbayar N², Butler PJ³, Chua B⁴, Frappell PB⁵, Meir JU⁶, Milsom WK⁴, Natsagdorj T², Parr N¹,
6 Scott GR⁷, Takekawa JY⁸, Wikelski M^{9,10}, Witt MJ¹¹ & Bishop CM^{12*}

7

8 ¹ University of Exeter, Centre for Ecology and Conservation, College of Life and Environmental Sciences,
9 Penryn Campus, Cornwall, TR10 9FE, UK

10 ² Wildlife Science and Conservation Center, Ulan Bataar, Mongolia

11 ³ University of Birmingham, School of Biosciences, Birmingham B15 2TT, UK

12 ⁴ Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T
13 1Z4, Canada

14 ⁵ Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania 7001, Australia.

15 ⁶ NASA Johnson Space Centre, Houston, Texas, USA

16 ⁷ Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON L8S 3K1, Canada

17 ⁸ Audubon California, Richardson Bay Audubon Center & Sanctuary, Tiburon, California, 94920 USA

18 ⁹ Max Planck Institute for Ornithology, D-82319 Seewiesen, Germany

19 ¹⁰ University of Konstanz, Department of Biology, D-78457 Konstanz, Germany

20 ¹¹ University of Exeter, Environment and Sustainability Institute, College of Life and Environmental Sciences,
21 Penryn Campus, Cornwall, TR10 9FE, UK

22 ¹² Bangor University, School of Biological Sciences, Bangor, Gwynedd, LL57 2UW, UK

23

24 * Corresponding authors

25

26 Lucy Hawkes

27 University of Exeter

28 Centre for Ecology and Conservation

29 College of Life and Environmental Sciences

30 Penryn Campus

31 Cornwall, TR10 9FE, UK

32 Tel: (01326) 259399
33 Email: l.hawkes@exeter.ac.uk
34
35 Charles Bishop
36 Bangor University
37 School of Biological Sciences
38 Bangor
39 Gwynedd, LL57 2UW, UK
40 Tel: (01248) 382 315
41 Email: c.bishop@bangor.ac.uk

42 **ABSTRACT**

43 Exercise at high altitude is extremely challenging, largely due to hypobaric hypoxia (low oxygen levels
44 brought about by low air pressure). In humans, the maximal rate of oxygen consumption decreases with
45 increasing altitude, supporting progressively poorer performance. Bar-headed geese (*Anser indicus*) are
46 renowned high altitude migrants and, although they appear to minimise altitude during migration where
47 possible, they must fly over the Tibetan Plateau (mean altitude 4,800 metres) for much of their annual
48 migration. This requires considerable cardiovascular effort, but no study has assessed the extent to which
49 bar-headed geese may train prior to migration for long distances, or for high altitudes. Using implanted
50 loggers that recorded heart rate, acceleration, pressure and temperature: we found no evidence of training
51 for migration in bar-headed geese. Geese showed no significant change in summed activity per day or
52 maximal activity per day. There was also no significant change in maximum heart rate per day or minimum
53 resting heart rate, which may be evidence of an increase in cardiac stroke volume if all other variables were
54 to remain the same. We discuss the strategies used by bar-headed geese in the context of training
55 undertaken by human mountaineers when preparing for high altitude, noting the differences between their
56 respective cardiovascular physiology.

57

58 Key words: Bar-headed geese; Biologging; Heart rate; High-altitude; Migration; Training.

59 INTRODUCTION

60 Migratory birds undertake some of the most challenging feats of exercise of any vertebrate animals on the
61 planet. For example, arctic terns (*Sterna paradisaea*) have been recorded making annual migration round
62 trips of more than 80,000 km (Egevang et al. 2010), bar-tailed godwits (*Limosa lapponica*) have been
63 recorded making non-stop flights of 11,000 km in a little more than a week (Gill et al. 2009), and great snipe
64 (*Gallinago media*) can maintain flight speeds of 80 km h⁻¹ for several days (Klaassen et al. 2011). Flight is
65 one of the cheapest forms of locomotion per unit of distance, but flapping flight is extremely costly per unit of
66 time, requiring ten-times or more the resting basal metabolic rate (Butler 2016, Bishop 1999). Some large
67 birds (such as raptors) have therefore evolved behaviours such as soaring, gliding and thermalling to reduce
68 flight costs, requiring them to flap very little to remain aloft (Sherub et al. 2016, Duriez et al. 2014; Nourani
69 and Yamaguchi 2017). Avian flight muscle is consequently highly adapted, and has two- to five-times greater
70 mitochondrial density than most mammalian skeletal muscle, leading to a much higher mass specific ATP
71 turnover rate (Kuzmiak-Glancy and Willis 2014). Avian flight muscle also generates the highest mass specific
72 power outputs (up to 433 W kg⁻¹) of any cyclically contracting vertebrate muscle (Askew and Marsh 2002).

73
74 Some species of birds not only migrate long distances but also travel to high altitudes en-route to their
75 breeding or wintering grounds (Prins & Namgail 2017). As altitude increases, barometric pressure
76 decreases, leading to reduced partial pressure of oxygen and consequently to hypoxic conditions. For
77 example, at the mean altitude of the Tibetan Plateau (mean altitude ~4,800 metres), there is approximately
78 50% of the oxygen at sea level (Cerretelli 1976). The challenge of supplying sufficient oxygen for aerobic
79 metabolism in flight is therefore magnified. Bar-headed geese (*Anser indicus*) migrate across the Tibetan
80 Plateau, and while flying northwards in spring make the fastest recorded sustained rates of climb over the
81 Himalayas, flying from approximately sea-level to 4,500 metres altitude in under eight hours, with no
82 opportunity to acclimatise (Hawkes et al. 2011). By contrast, human athletes show reduced metabolic rates
83 and increased lactate production at altitude (Smith et al. 2015, Chapman et al. 2015), with $\dot{V}O_{2\text{MAX}}$ declining
84 by about 6 to 8% per 1,000 metres of altitude (Wehrlin et al. 2016). One might therefore imagine, if
85 considering the context of human climbers, that bar-headed geese would undergo considerable preparation
86 for their annual migration not only in training for distance but also for altitude exposure (but see Tucker et al.
87 1968).

88
89 (i) Training in humans

90 In humans, athletic training usually involves either endurance exercise (e.g. long distance running or
91 cycling), exercise for varying durations close to $\dot{V}O_{2\text{ MAX}}$ (the maximal sustainable rate of oxygen
92 consumption) or short, intense 'explosive strength training' sessions. Such training exercise produces a
93 variety of physiological responses that enhance capacity for efficient work output and use of oxygen
94 (Hellsten and Nyberg 2011; Hoffman 2014; Jones and Carter 2000a, b, Joyner and Coyle 2008). For
95 example, training can increase the volume and thickness of the left ventricle of the heart, leading to an
96 increase in stroke volume (the amount of blood ejected per heart beat) (Weiner et al. 2015). Training can
97 increase blood haemoglobin content, red cell count, and plasma volume (Mairbäurl 2013) and can decrease
98 lactate production during exercise and increase the rate of lactate removal when it is produced (Jones and
99 Carter 2000a).

100

101 Training can also alter the relative proportions of muscle fibre types, increase the storage of intra-muscular
102 lipids, and increase their relative use during exercise (Dubé et al. 2016) as well as increase muscle capillarity
103 and myoglobin concentration (Jones and Carter 2000a). Finally, it can increase the size, number, and
104 function of mitochondria (Bishop et al. 2014). Human training for high altitude usually involves repeated
105 exposure to hypobaria (either simulated or at moderate altitudes), which produces a range of physiological
106 adaptations for enhancing oxygen supply to the tissues (e.g. erythropoiesis, increase in ventilation and heart
107 rate, Bärtsh and Saltin 2008; increase in mitochondrial volume density, Jacobs et al. 2016). Most
108 mountaineering expeditions to very high altitude acclimatise over a series of staged ascents over several
109 weeks, recovering at a base camp in between. Training and acclimatisation therefore has a significant
110 impact on the physiological capacity for exercise and high altitude tolerance in humans.

111

112 *(ii) Training in non-human animals*

113 Undoubtedly, selection pressures on wild animals to be optimally physically fit in their respective
114 environments are very different from those acting on modern, largely sedentary humans (Caldwell 2016).
115 Wild animals must constantly be able to forage efficiently and evade predation, and for many species the
116 ability to move between seasonally changing habitats is key to survival (Chapman et al. 2014). For the
117 purposes of the present study, therefore, the context of human training is unrealistic, but there is no alternate
118 framework for how to improve physical fitness without exercise training. It would appear that there is no
119 existing evidence to suggest that any wild animals undertake voluntary activity to 'keep fit' (Halsey 2016;
120 Portugal et al. 2011), but there are observations of wild mice (and even a wild frog) running freely on
121 exercise wheels when they are available to them (Meijer and Robbers 2014). Captive mice and rats,

122 subjected to training, have shown similar improvements in exercise capacity to those detailed above for
123 humans (Goutianos et al. 2015; Kemi et al. 2004, Waters et al. 2004). Butler & Turner (1988) trained captive
124 tufted ducks (*Aythya fuligula*) to swim on a water flume in captivity and showed that training enhanced
125 muscle capillarity and reduced heart rate for a given rate of oxygen consumption, which suggests an
126 accompanying increase in cardiac stroke volume. Training, therefore, clearly produces an effect in non-
127 human animals. There is also evidence that wild animals can respond to high altitude exposure. For
128 example, lowland resident deer mice (*Peromyscus maniculatus*), exposed to hypoxia equivalent to 4,300
129 metres for six to ten weeks increased their tidal volume (Ivy and Scott 2015), increased mitochondrial
130 respiratory capacity and decreased the production of ROS (reactive oxygen species; Mahalingam et al.
131 2017). Given that migratory animals probably need to increase their physical fitness, from sprint-burst type
132 fitness sufficient for chasing prey and evading predators, to endurance type fitness required to support long-
133 distance migration, it may seem surprising, at least from an anthropomorphic point of view, that there is not
134 more evidence for training in wild animals.

135

136 (iii) *Training for migration in birds*

137 The phenomenon of '*zugunruhe*' (migratory restlessness) has been well described in captive birds (Gwinner
138 1968). At times of the year when birds in captivity would otherwise be migrating, they can exhibit agitated
139 movements in specific directions that correspond to their natural migratory pathways, providing an
140 opportunity for study (for a review and new directions, see (Muheim et al. 2014)). However, in the wild
141 songbirds apparently switch into migratory mode without previous locomotory preparations (Zúñiga et al.
142 2016). At the same time as this restlessness, avian muscle also undergoes significant biochemical changes,
143 for example, increase in fatty acid binding proteins (FABP; improving transport and delivery of fatty acids to
144 flight muscles) and citrate synthase activity (which is indicative of muscle oxidative capacity) (Guglielmo et
145 al. 2002; Saunders and Klemm 1994). Several studies have provided evidence to suggest that such changes
146 are likely endogenous and not brought about by training (Bauchinger and Biebach 2006; Bishop et al. 1998;
147 Dietz et al. 1999; McFarlan et al. 2009; Portugal et al. 2009; Price et al. 2011; Vézina et al. 2007). It has also
148 been suggested, however, that flight muscle hypertrophy in wild birds may be brought about as a
149 consequence of pre-migratory mass gain which would increase wing loading and therefore the relative
150 workload in flight (so called 'power training', (Marsh and Storer 1981)). There is evidence for flight muscle
151 hypertrophy from a number of studies on captive birds (Marsh 1984; Marsh and Storer 1981; Lindstrom et
152 al. 2000; Butler and Turner 1988).

153

154 For wild birds, the picture is less clear. In eared grebes (*Podiceps nigricollis*), an increase in body mass does
155 not appear to be correlated with flight muscle hypertrophy, and “conspicuous flapping exercise” has been
156 noted during recovery from wing moult, which might represent training (Gaunt et al. 1990). Bishop et al.
157 (1998) demonstrated that citrate synthase activity in the pectoral muscles of barnacle geese (*Branta*
158 *leucopsis*) was higher in wild than captive barnacle geese, and Pelters et al. (1999) demonstrated that FABP
159 was higher in wild than captive gosling and adult barnacle geese, suggesting that some element of activity in
160 the wild might be important in muscle hypertrophy, aerobic capacity and fuelling. The advent of biologging
161 technology (Kays et al. 2015) has enabled researchers to record movement and aspects of animal biology
162 and that can be used to address the question of training for migration in birds. Using implanted heart rate
163 loggers, Portugal et al. (2011) showed that wild barnacle geese at their summer breeding area in
164 Spitsbergen did not appear to increase the daily amount of time (22 minutes) spent flying in the weeks
165 leading up to migration and therefore were likely not training for migration. Their work remains the only study
166 to date on wild migratory birds.

167

168 In the present study, we set out to investigate whether there was any evidence of training for migration in a
169 population of bar-headed geese deployed from nesting grounds (elevation 2,050 m) in Mongolia. Bar-headed
170 geese make one of the most demanding migrations on earth, crossing the Himalayan mountains and the
171 Tibetan Plateau (mean elevation 4,800 metres) as they make their migration between India, China and
172 Mongolia (Bishop et al. 2015; Hawkes et al. 2012; Hawkes et al. 2011). We used implanted loggers to look
173 for patterns of changes in heart rate that might suggest increases in physiological fitness and, for the first
174 time, acceleration loggers to look for patterns in activity per day leading up to migration.

175

176 **METHODS**

177 Bar-headed geese (n=4, 2.4 kg mean body mass, range 2.1 to 2.6 kg) were captured on a breeding lake in
178 western Mongolia (Terkhiin Tsagaan lake, 48.148 °N, 99.577 °E) where they annually moult their primary
179 flight feathers and are unable to fly for two to three weeks of the summer. Geese were captured in July 2011
180 during moult using 'drive trapping', herding the geese with inflatable kayaks on their breeding lakes into
181 shoreline corral nets. In order to investigate whether the geese trained prior to their autumn (southward)
182 migration, we implanted the geese with loggers to record heart rate, acceleration, temperature and pressure
183 (Figure 1; (Spivey and Bishop 2014)). Geese were always captured, implanted, and released on the same
184 day, and the loggers were surgically implanted into the abdominal cavity of the geese by a veterinary
185 surgeon, under isoflurane anaesthesia and using sterile instruments. Each logger was sterilised using
186 ethylene oxide in the UK prior to the fieldwork. All work was carried out with ethical permission from Bangor
187 University and following ethical regulations set out by the UK Home Office Animal in Scientific Procedures
188 Act (ASPA 1986). Birds implanted with loggers were released with green neck collars bearing alpha-numeric
189 identifying codes (P35, P37, P41 and P43; Figure 1b). The following year, geese were re-sighted during
190 surveys of the same breeding lakes and recaptured in order to remove the loggers and download the data.

191
192 The logger recorded internal body temperature and pressure every 30 seconds, acceleration at 180 Hz in 18
193 second bursts every two minutes and ECG via titanium end cap electrodes in the same 18-second burst
194 every two minutes (Spivey & Bishop 2014). The logger was 7 cm long and 2 cm diameter, weighing a total of
195 32 g. Acceleration data were used to calculate VeDBA (vectoral dynamic body acceleration (Qasem et al.
196 2012)) which describes dynamic movement by the geese in three dimensions (heave, surge, and sway).
197 Heart rate was derived from the raw ECG data using a custom beat detection script in R. Because data were
198 collected at intervals (to maximise logger battery life), data were summarised into two-minute averaged bins
199 for analysis. The onset of migration, defined here as the first southerly movement away from the breeding
200 grounds, was derived using plots of daily mean VeDBA and altitude (Figure S1).

201
202 In order to investigate whether geese trained for migration, we looked for significant changes in five key
203 variables leading up to the onset of migration for each bird. (i) Whether the total amount of exercise
204 undertaken increased leading up to migration. We used summed VeDBA per day to estimate total exercise
205 because it encompassed all dynamic movement by the bird while the logger was recording. (ii) Whether the
206 magnitude of the maximal activity undertaken each day increased prior to migration. We hypothesised that
207 maximal activity could encompass strenuous take-off manoeuvres, climbing flights, or other demanding

208 activities. We used the maximum VeDBA recorded in any two-minute block per day to estimate this. (iii)
209 Whether intense, probable 'ground based wing flapping events' increased in frequency prior to migration. We
210 inspected one-hour sections of data per goose to develop a thresholding approach: First we considered any
211 section where VeDBA was four G or more for over six minutes as 'flight', and therefore excluded it from the
212 analysis. For the remaining data, we extracted all instances where VeDBA exceeded 6 G, assigned it as
213 probable ground based wing flapping, or a putative take-off event and counted the number of these 'spikes'
214 happening per day. There are no other activities undertaken by these birds that should lead to such a high
215 dynamic acceleration value. (iv) Whether maximal heart rates increased leading up to migration. We
216 hypothesised that maximal heart rate could encompass strenuous take off manoeuvres, climbing flights or
217 other demanding activities that might encompass training. We used the maximum heart rate recorded in any
218 two-minute block per day to estimate maximal heart rate. (v) Finally, in order to investigate whether there
219 was any evidence of the geese becoming physiologically fitter (not implying any evolutionary sense), we
220 looked to see if the overnight resting heart rate decreased, which may be evidence of an increase in cardiac
221 stroke volume if all other variables were to remain the same. We used minimum heart rate recorded in any
222 two-minute block per day to estimate this parameter, which always occurred overnight.

223
224 Because heart rate and acceleration data from the loggers are serially auto-correlated (i.e. within each
225 animal's dataset, subsequent data points are not independent), data were analysed using generalised least
226 squares models (Zuur et al. 2009) using the *gls* function in the R package 'nlme' (Pinheiro et al. 2011). We
227 applied a first order auto-regression (AR1) autocorrelation structure, which allows the user to account for
228 serial autocorrelation within each bird's dataset, and compared models with (*gls*) and without (*lm*) auto-
229 correlation structure using corrected Akaike values (AICc) using the R package 'MuMIn' (Barton 2014). For
230 each statistic, we report the model that had the lower AIC value, indicating better fit. The model took the form
231 *gls*(parameter ~ days leading up to migration) where the parameters of interest were sum VeDBA, max
232 VeDBA, maximum, minimum daily heart rate and sum of daily spikes in VeDBA.

233

234 RESULTS

235 The four loggers recorded data for a median of 171 days in total (including the full migration and some time
236 wintering, range 158 to 222 days; example in Figure 2). The four geese departed for migration 35, 42, 46
237 and 51 days after logger deployment in late August and early September (Figure S1).

238

239 (i) *Does the total amount of exercise increase prior to migration?*

240 There was no evidence that there was an increase in the total amount of exercise carried out each day
241 leading up to migration for any of the four geese (sum VeDBA did not change significantly; P37 *g/s* $t = 0.91$,
242 $AIC = 451.6$, $p > 0.05$; P41 *g/s* $t = 0.08$, $AIC = 612.9$, $p > 0.05$; P43 *g/s* $t = 1.95$, $AIC = 437.3$, $p > 0.05$; P35 *g/s* $t =$
243 2.00 , $AIC = 463.5$, $p = 0.05$; Figure 3), suggesting they did not undertake more physical activity as they got
244 closer to migratory departure. Three of the four geese exhibited a spike in activity, approximately 33 days
245 prior to migration (Figure 3A-C). The geese were not being directly observed during this time, so it is not
246 clear what the stressor might have been, although we suggest predator presence might be a likely
247 explanation.

248

249 (ii) *Does the magnitude of the maximal activity undertaken each day increase prior to migration?*

250 The maximal amount of activity (as indicated by maximum VeDBA), which might have indicated particularly
251 strenuous exercise bouts, did not change leading up to migratory departure for three of the geese (P35 *lm* R^2
252 $= 0.02$, $F_{1,43} = 1.94$, $AIC = 181.7$, $p > 0.05$; P37 *lm* $R^2 = -0.03$, $F_{1,33} = 0.04$, $AIC = 121.5$, $p > 0.05$; P43 *lm* $R^2 =$
253 0.00 , $F_{1,33} = 0.9$, $AIC = 123.2$, $p > 0.05$; Figure 4A, C, D) but did increase significantly for the fourth (P41 *lm* R^2
254 $= 0.22$, $F_{1,48} = 14.46$, $AIC = 193.2$, $p < 0.01$; Figure 4B). There was no correlation between maximum VeDBA
255 and body temperature (Pearson's correlation P35, $r = 55.5$, $p > 0.05$; P37, $r = 38.7$, $p > 0.05$; P41, $r = 69.6$,
256 $p > 0.05$; P43, $r = 52.9$, $p > 0.05$), which suggested that when maximal activity occurred, it was not necessarily
257 sustained long enough to produce a hyperthermic response.

258

259 (iii) *Do probable 'ground based wing flapping events' increase in frequency prior to migration?*

260 The frequency of probable 'ground based wing flapping events' (number of spikes in VeDBA per day) did not
261 increase prior to migration for two geese (P37 *lm* $R^2 = 0.07$, $F_{1,33} = 3.49$, $AIC = 192.6$, $p > 0.05$; P41 *lm* $R^2 =$
262 0.01 , $F_{1,48} = 1.67$, $AIC = 238.7$, $p > 0.05$; Figure 5A, B) but actually decreased prior to migration for the other
263 two geese (P35 *lm* $R^2 = 0.22$, $F_{1,33} = 10.80$, $AIC = 175.8$, $p < 0.01$; P43 *lm* $R^2 = 0.17$, $F_{1,33} = 10.00$, $AIC =$
264 300.2 , $p < 0.01$; Figure 5C, D).

265

266 (iv) *Does the magnitude of the maximal heart rate each day increase prior to migration?*

267 For two of the geese, there was no change in maximum heart rate leading up to migration (P41 *g/s* $t = -0.88$,
268 $AIC = 466.7$, $p > 0.05$; P43 *g/s* $t = 0.80$, $AIC = 439.5$, $p > 0.05$; Figure 6B, C) but two geese had a small but
269 significant decrease in daily maximum heart rate leading up to migration (P35 *g/s* $t = 2.26$, $AIC = 402.2$,
270 $p < 0.01$; P37 *g/s* $t = 2.45$, $AIC = 283.8$, $p < 0.01$; Figure 6A, D), contrary to the prediction of an increase in
271 maximum heart rate.

272

273 (v) *Is there any evidence of physiological improvements prior to migration?*

274 There was no significant change in minimum heart rate for any of the geese over the period leading up to
275 migration (P35 *g/s* $t = -0.93$, $AIC = 330.3$, $p > 0.05$; P37 *g/s* $t = -0.44$, $AIC = 221.8$, $p > 0.05$; P41 *g/s* $t = -1.91$,
276 $AIC = 380.4$, $p > 0.05$; P43 *g/s* $t = -1.32$, $AIC = 217.7$, $p > 0.05$; Figure 5), which might have been indicative of
277 cardiac hypertrophy. There was also no significant change in the range of body temperatures experienced by
278 the four geese (P37 *lm* $R^2 = 0.04$, $F_{1,33} = 2.42$, $AIC = 21.5$, $p > 0.05$; P41 *lm* $R^2 = 0.03$, $F_{1,48} = 2.48$, $AIC =$
279 33.8 , $p > 0.05$; P43 *lm* $R^2 = 0.00$, $F_{1,33} = 1.06$, $AIC = 9.3$, $p > 0.05$; P35 *lm* $R^2 = 0.06$, $F_{1,43} = 3.94$, $AIC = 40.0$,
280 $p < 0.05$; Figure S2), which might have otherwise reflected the difference between intense periods of exercise
281 and sleep at low metabolic rates.

282

283 **DISCUSSION**

284 Here, we provide evidence that bar-headed geese do not obviously engage in training for migration. Our
285 study is the first to use accelerometry data to study this in wild birds, and suggests that overall (for three out
286 of four birds) there was no increase in the sum or the maximal amount of activity each day leading up to
287 migration. Interestingly, while there is notable variation in the maximal activity undertaken by each bird
288 (doubling or halving from one day to another), the sum of activity is remarkably consistent both within and
289 between individuals at around 600 to 700 G per day. The accelerometry data was also used to identify
290 ground based flapping events, which might have represented 'power training'. These do not appear to
291 increase leading up to migration, and indeed actually decreased for two birds. Likewise, the results showed
292 that maximum daily heart rate decreased for two of the four birds leading up to migration. Perhaps, therefore,
293 study birds might have been conserving energy to lay down greater fat deposits for migration, rather than
294 training, but the cause of this, or prevalence, is not clear and requires further investigation. There may be
295 several reasons for the present observations, which we discuss in turn:

296

297 *(i) Limited data coverage*

298 To prolong battery life, the archival loggers were duty-cycled to record for just 18 seconds of every two
299 minutes throughout the day. Although this represents some of the highest resolution, long-term physiological
300 data ever collected for a migratory bird, data were not collected for 85% of the time. It was not possible to
301 collect complete recordings (covering 100% of the time) to compare with the duty-cycled data. It is possible
302 that episodes of training did not occur during the 15% window when we did collect data. However, it would
303 seem highly unlikely that all training occurred in bouts of less than 102 seconds, all of which we missed for
304 the entire period of several weeks leading up to migration. So while we recognise this shortcoming, we do
305 not believe it is the best explanation for the apparent lack of training. Perhaps also longer recording prior to
306 migration might reveal greater insight into preparation for migration. Increases in maximum VeDBA were
307 observed for the one goose that remained for the longest period prior to departure (see below), and highlight
308 that longer recordings in the future might shed light on whether this was an artefact.

309

310 *(ii) Training at surprisingly small scales*

311 Alternatively, training for migration may occur at a far smaller scale than previously appreciated. Many birds
312 including waterfowl exhibit ground-based wing-flapping behaviour, typically as a preening or social activity.
313 These bouts of flapping usually only last a few seconds but may be metabolically extremely intense (Norberg
314 1996) and thus, it might be that these bouts act as short, intense explosive strength-training sessions.

315 Muscle building in humans is known to respond strongly to such short but high intensity training sessions
316 (Mitchell et al. 2013), so these events could contribute meaningfully to training in birds. In the present study
317 we attempted to identify these from accelerometry data and find that they do not increase prior to migration,
318 suggesting that either these explosive training bouts do not contribute to migratory preparation in bar-headed
319 geese, or that such preparation is continuous from the time of moult.

320

321 *(iii) Endogenous development of flight musculature*

322 It also remains highly possible that increases in flight muscle mass and performance necessary for migratory
323 flight are endogenous, i.e. not brought about as a result of exercise. This has been suggested previously for
324 migratory birds (Bauchinger and Biebach 2006; Bishop et al. 1998; Dietz et al. 1999; Guglielmo et al. 2002;
325 McFarlan et al. 2009; Portugal et al. 2009; Price et al. 2011; Vézina et al. 2007), although other studies
326 suggest that regular flight can be augmented with 'power training' (increasing load to the working muscles)
327 and may produce muscle hypertrophy (Lindstrom et al. 2000; Marsh 1984; Marsh and Storer 1981). It
328 remains to be empirically demonstrated if bar-headed geese can endogenously build flight muscle without
329 power training because we did not measure flight muscle mass in the present study, but barnacle geese in
330 captivity (i.e. deprived of the ability to fly around freely, and therefore exercise their flight muscles) increase
331 flight muscle mass by a third as they regrow their flight feathers after moult (Portugal et al. 2009). In addition,
332 there is no evidence that wild birds 'de-train' (i.e. exhibit a partial or complete anatomical, physiological or
333 performance loss as a consequence of reduction of activity) during long periods without flight, e.g. over the
334 breeding or wintering seasons, although this can be seen in captive birds, such as pigeons (*Columba livia*,
335 Chaplin et al. 1997)). Certainly most wild birds probably experience large fluctuations in flight muscle mass
336 over the course of a year (Jehl 1997) – consuming flight muscle during migratory journeys, rebuilding it on
337 arrival to breeding and wintering grounds, and must regulate this via some process. It is also unclear to what
338 extent power training might be beneficial for endurance exercise.

339

340 The mechanism for such endogenous change appears likely to be hormonally based, brought about by
341 changing photo-period, leading to up-regulation of specific genetic pathways for muscle development or fuel
342 mobilisation. For example, McFarlan et al. (2009) showed that white-throated sparrows (*Zonotrichia*
343 *albicollis*) increase gene expression for heart-type FABP and plasma-membrane FABP up to 1,000% relative
344 to pre-migratory resting levels which would facilitate transport of essential fatty acids by up to 110% into the
345 flight muscle to fuel migration. In barnacle geese, FABP in the pectoralis muscle can be twice as high in pre-
346 migratory wild goslings as that of captive goslings and 50% higher in wild than captive adults (Pelters et al.

347 1999). In another study, Price et al. (2011) demonstrated that mRNA expression of Insulin-like growth factor
348 1 (IGF1) was increased in captive white-throated sparrows in migratory condition relative to sparrows in
349 wintering condition. At the same time, it also is clear that training produces muscle hypertrophy and
350 increases in specific gene expression – Price et al. (2011) trained starlings (*Sturnus vulgaris*) in a wind
351 tunnel and demonstrated muscle hypertrophy as well as up-regulation of IGF1 and myostatin immediately
352 following an acute bout of flight. It remains to be demonstrated what proportion of flight muscle hypertrophy
353 may be exercise-induced versus endogenously generated across bird species, but it seems likely that there
354 may be variation in the relative contributions across species and migratory pathways.

355

356 *Expedition style*

357 Waterfowl also generally do not migrate for very long periods at a time, with episodes of flight up to a day
358 long interspersed with stopovers (Arzel et al. 2006). Bar-headed geese migrate up to 5,140 km in as much
359 as 92 days in the spring (Hawkes et al. 2012) which would require them to fly as little as one hour each day
360 (although in reality they make flights of up to 23 hours, interspersed with stopovers of several weeks). Thus,
361 during migration, bar-headed geese have ample time to rest and recover between flights, possibly for days.
362 They may therefore effectively train as they migrate, becoming steadily fitter as they travel and this remains
363 to be investigated. Clearly, the differences in strategies used between human and avian high altitude
364 specialists come about because wild birds do not have access to the facilities, resources and equipment that
365 expedition style human mountaineers do, but also likely come about as a consequence of their differing
366 physiology and evolutionary pressures. Birds, of course, might also make use of assisting wind conditions to
367 reduce flight costs, as has been shown previously (Sherub et al. 2016).

368

369 *The superior avian cardiorespiratory system*

370 The avian respiratory system has evolved to allow birds to take up greater amounts of oxygen per unit of
371 body mass than other vertebrates owing in large part to the huge metabolic demands for oxygen during flight
372 (Cieri et al. 2014; Maina 2000; Ward et al. 2002), although see (McGuire and Guglielmo 2009; Speakman
373 and Thomas 2005) for discussion of flight metabolic rate of bats. It might not be surprising if the *metabolic*
374 costs of flight could be easily met by a migrating bird without much need for training, assuming the flight
375 muscles have sufficient fuel stores. In bar-headed geese, oxygen uptake is thought to be considerably
376 enhanced compared to that of all other birds studied to date, with adaptations for enhanced oxygen uptake
377 throughout the oxygen transport cascade (Butler 2010; Meir and Milsom 2013; Scott 2011; Scott and Milsom
378 2006; 2007; Scott et al. 2011). For example Hawkes et al. (2014) tested the performance of bar-headed

379 geese in severe hypoxia (7% oxygen) and showed that their capacity for endurance exercise is enhanced
380 compared with that of barnacle geese, which are otherwise very similar morphologically, but migrate at low
381 altitudes (Butler et al. 1998; Eichhorn et al. 2009; Prop et al. 2003). It is also worth noting that the majority of
382 work demonstrating the range of adaptations by bar-headed geese to enhance oxygen delivery at multiple
383 steps of the oxygen transport cascade (Butler 2010; Scott 2011; Scott et al. 2015) have been carried out on
384 captive bar-headed geese that had been pinioned and, therefore had never flown, i.e. wild birds might be
385 expected to do better (but see Lague et al. 2016). Bar-headed geese have also been shown to use
386 behavioural adaptations to reduce flight costs, such as staying close to the underlying terrain (Bishop et al.,
387 2015), or flying at night in colder conditions where air density (and therefore oxygen availability) should be
388 higher (Hawkes et al. 2011, 2012).

389

390 *Future directions*

391 Given physiological and behavioural adaptations to reduce the challenge of flight at high altitudes, perhaps
392 the major challenge for migratory bar-headed geese is not in supplying adequate oxygen to the flight
393 muscles or in endogenously gaining flight muscle mass, but in maintaining an adequate supply of fuel to the
394 flight muscles and in preventing dehydration and muscle damage (Butler 2016; Jenni-Eiermann 2017). First,
395 fuel supply and water balance can probably be maintained by migrating bar-headed geese by regular 'stop-
396 overs' to rest and refuel (Hawkes et al. 2012). However, muscle damage might be expected during migratory
397 flights in bar-headed geese, particularly during steep climbing flights from Indian wintering grounds onto the
398 Tibetan Plateau in just seven to eight hours (Hawkes et al. 2011). Both hypoxia and exercise are known to
399 generate high levels of free radical species, which are thought to cause damage in proteins, lipids and
400 muscle myocytes (Zhang et al. 2015, Powers and Jackson 2008). Thus it might be expected that bar-headed
401 geese, or many other high altitude migrants, would have a significant free radical production during
402 migration, yet we lack any understanding of rates of ROS production or their ability for dealing with it, for
403 example through anti-oxidant loading (Powers and Jackson 2008). This is therefore a research priority
404 remaining to be investigated.

405 **FUNDING**

406 The work was supported by a grant from the UK Biotechnology and Biological Sciences Research Council
407 (BBSRC; BB/FO15615/1) to CMB and PJB. Authors were supported by a Natural Sciences and Engineering
408 Research Council of Canada (NSERC) award (WKM), and the FAO through the Animal Health Service
409 EMPRES surveillance program.

410

411 **ACKNOWLEDGEMENTS**

412 Work in Mongolia was conducted with permission from the Wildlife Science and Conservation Centre. We
413 are grateful to the support of all the field team members in Mongolia, to A. Davies for developing the first
414 generation of heart rate–data loggers, to Robin Spivey for developing the logger that we used and the script
415 to interpret the data, and to the work of Beaumaris Instruments Ltd. in the development of housings for the
416 instruments. We thank Lewis Halsey, Gil Bohrer and one anonymous reviewer for comments that improved
417 the manuscript.

418 **REFERENCES**

419

- 420 Arzel C, Elmberg J, Guillemain M. 2006. Ecology of spring-migrating Anatidae: a review. *Journal of*
421 *Ornithology* 147(2):167-184.
- 422 Askew GN, Marsh RL. 2002. Review: Muscle designed for maximum short-term power output: quail flight
423 muscle. *Journal of Experimental Biology* 2015:2153-2160.
- 424 Barton K. 2014. MuMIn: Multi-model inference. R package version 1.10.0. R Foundation for Statistical
425 Computing, Vienna.
- 426 Bärtsch, P, Saltin B. 2008. General introduction to altitude adaptation and mountain sickness. *Scandinavian*
427 *Journal of Medicine & Science in Sports*, 18:1–10.
- 428 Bauchinger U, Biebach H. 2006. Transition between moult and migration in a long-distance migratory
429 passerine: organ flexibility in the African wintering area. *Journal of Ornithology* 147(2):266.
- 430 Bishop CM, Butler PJ, El Haj AJ, Egginton S. 1998. Comparative development in captive and migratory
431 populations of the barnacle goose. *Physiological Zoology* 71:198-207.
- 432 Bishop CM. 1999. The maximum oxygen consumption and aerobic scope of birds and mammals: getting to
433 the heart of the matter. *Proceedings of the Royal Society B* 266:2275-2281
- 434 Bishop CM, Spivey RJ, Hawkes LA, Batbayar N, Chua B, Frappell PB, Milsom WK, Natsagdorj T, Newman
435 SH, Scott GR et al. 2015. The roller coaster flight strategy of bar-headed geese conserves energy
436 during Himalayan migrations. *Science* 347(6219):250-254.
- 437 Bishop DJ, Granata C, Eynon N. 2014. Can we optimise the exercise training prescription to maximise
438 improvements in mitochondria function and content? *Biochimica et Biophysica Acta* 1840(4):1266-
439 1275.
- 440 Butler PJ. 2010. High fliers: The physiology of bar-headed geese. *Comparative Biochemistry and Physiology*
441 *Part A* 156:325-329.
- 442 Butler PJ. 2016. The physiological basis of bird flight. *Philosophical Transactions of the Royal Society B:*
443 *Biological Sciences* 371: 20150384
- 444 Butler PJ, Turner DL. 1988. Effect of training on maximal oxygen-uptake and aerobic capacity of locomotory
445 muscles in tufted ducks, *Aythya fuligula*. *Journal of Physiology* 401:347-359.
- 446 Butler PJ, Woakes AJ, Bishop CM. 1998. Behaviour and physiology of Svalbard barnacle geese *Branta*
447 *leucopsis* during their autumn migration. *Journal of Avian Biology* 29:536-545.
- 448 Caldwell AE. 2016. Physical Activity and Energy Expenditure in Humans. *Human Physical Fitness and*
449 *Activity: An Evolutionary and Life History Perspective*. Springer International Publishing. p. 27-37.

450 Cerretelli P. 1976. Limiting factors to oxygen transport on Mount Everest. *J Appl Physiol* 40:658-67.

451 Chaplin SB, Munson MM, Knuth ST. 1997. The effect of exercise and restraint on pectoral muscle
452 metabolism in pigeons. *Journal of Comparative Physiology B* 167(3):197-203.

453 Chapman RF, Karlsen T, Ge RL, Stray-Gunderson J, Levine B. 2015. Living altitude influences endurance
454 exercise performance change over time at altitude. *Journal of Applied Physiology* 120:1151-1158.

455 Chapman BB, Hulthén K, Wellenreuther M, Hansson LA, Nilsson JA, Brönmark C. 2014. Patterns of animal
456 migration. In: Hansson LA, Åkesson S, editors. *Animal Movement Across Scales*. Oxford University
457 Press. p. 11-35.

458 Cieri RL, Craven BA, Schachner ER, Farmer CG. 2014. New insight into the evolution of the vertebrate
459 respiratory system and the discovery of unidirectional airflow in iguana lungs. *Proceedings of the*
460 *National Academy of Sciences* 111(48):17218-17223.

461 Dietz MW, Piersma T, Dekinga A. 1999. Body-building without power training: endogenously regulated
462 pectoral muscle hypertrophy in confined shorebirds. *Journal of Experimental Biology* 202(20):2831-
463 2837.

464 Dubé JJ, Broskey NT, Despines AA, Stefanovic-Racic M, Toledo FGS, Goodpaster BH, Amati F. 2016.
465 Muscle characteristics and substrate energetics in lifelong endurance athletes. *Medicine and science*
466 *in sports and exercise* 48(3):472-480.

467 Duriez O, Kato A, Tromp C, Dell'Omo G, Vyssotski AL, Sarrazin F, Ropert-Coudert Y. 2014. How Cheap Is
468 Soaring Flight in Raptors? A Preliminary Investigation in Freely-Flying Vultures. *PLOS ONE*
469 9(1):e84887.

470 Egevang C, Stenhouse IJ, Phillips RA, Petersen A, Fox JW, Silk JRD. 2010. Tracking of Arctic terns *Sterna*
471 *paradisaea* reveals longest animal migration. *Proceedings of the National Academy of Sciences*
472 107(5):2078-2081.

473 Eichhorn G, Drent RH, Stahl J, Leito A, Alerstam T. 2009. Skipping the Baltic: the emergence of a dichotomy
474 of alternative spring migration strategies in Russian barnacle geese. *Journal of Animal Ecology*
475 78:63-72.

476 Gaunt AS, Hikida RS, Jehl JR, Fenbert L. 1990. Rapid Atrophy and Hypertrophy of an Avian Flight Muscle.
477 *The Auk* 107(4):649-659.

478 Gill RE, Tibbitts TL, Douglas DC, Handel CM, Mulcahy DM, Gottschalck JC, Warnock N, McCaffery BJ,
479 Battley PF, Piersma T. 2009. Extreme endurance flights by landbirds crossing the Pacific Ocean:
480 ecological corridor rather than barrier? *Proceedings of the Royal Society B: Biological Sciences*
481 276(1656):447-457.

482 Goutianos G, Tzioura A, Kyparos A, Paschalis V, Margaritelis NV, Veskokouk AS, Zafeiridis A, Dipla K,
483 Nikolaidis MG, Vrabas IS. 2015. The rat adequately reflects human responses to exercise in blood
484 biochemical profile: a comparative study. *Physiological Reports* 3(2):e12293.

485 Guglielmo CG, Haunerland NH, Hochachka PW, Williams TD. 2002. Seasonal dynamics of flight muscle
486 fatty acid binding protein and catabolic enzymes in a migratory shorebird. *American Journal of*
487 *Physiology - Regulatory, Integrative and Comparative Physiology* 282(5):R1405-R1413.

488 Gwinner, E. 1968. Circannuale Periodik als Grundlage des jahreszeitlichen Funktionswandels bei
489 Zugvögeln. Untersuchungen am Fitis (*Phylloscopus trochilus*) und am Waldlaubsänger (*P. sibilatrix*).
490 *J. Ornithol.* 109:70-95.

491 Halsey LG. 2016. Do animals exercise to keep fit? *Journal of Animal Ecology* 85:614-620.

492 Hawkes LA, Balachandran S, Batbayar N, Butler PJ, Chua B, Douglas DC, Frappell PB, Hou Y, Milsom WK,
493 Newman SH et al. 2012. The paradox of extreme high-altitude migration in bar-headed geese *Anser*
494 *indicus*. *Proceedings of the Royal Society B: Biological Sciences* 280:20122114

495 Hawkes LA, Balachandran S, Batbayar N, Butler PJ, Frappell PB, Milsom WK, Tseveenmyadag N, Newman
496 SH, Scott GR, Sathiyaselvam P et al. 2011. The trans-Himalayan flights of bar-headed geese (*Anser*
497 *indicus*). *Proceedings of the National Academy of Sciences* 108(23):9516-9519.

498 Hawkes LA, Butler PJ, Frappell PB, Meir JU, Milsom WK, Scott GR, Bishop CM. 2014. Maximum Running
499 Speed of Captive Bar-Headed Geese Is Unaffected by Severe Hypoxia. *PLOS ONE* 9(4):e94015.

500 Hellsten Y, Nyberg M. 2011. Cardiovascular Adaptations to Exercise Training. *Comprehensive Physiology*
501 6(1):1-32.

502 Hoffman J. 2014. *Physiological Aspects of Sport Training and Performance*. Illinois, USA: Human Kinetics. p.
503 520 pp.

504 Ivy C, Scott GR. 2015. Control of breathing and adaptation to high-altitude hypoxia in deer mice
505 (*Peromyscus maniculatus*). *FASEB Journal* 29:686.4

506 Jacobs RA, Lundby AM, Fenk S, Gehrig S, Siebenmann C, Flück D, Kirk N, Hilty MP, Lundby C. 2016.
507 Twenty-eight days of exposure to 3454 m increases mitochondrial volume density in human skeletal
508 muscle. *Journal of Physiology* 594:1151-1166.

509 Jehl J 1997. Cyclical changes in body composition in the annual cycle and migration of the eared grebe
510 *Podiceps nigricollis*. *Journal of Avian Biology* 28:132-142.

511 Jenni-Eiermann S. 2017. Energy metabolism during endurance flight and the post-flight recovery phase.
512 *Journal of Comparative Physiology A(1-8)*: doi:10.1007/s00359-017-1150-3.

513 Jones AM, Carter H. 2000a. The Effect of Endurance Training on Parameters of Aerobic Fitness. Sports
514 Medicine 29(6):373-386.

515 Jones, A. M. and H. Carter 2000b. "The Effect of Endurance Training on Parameters of Aerobic Fitness."
516 Sports Medicine 29(6): 373-386.

517 Joyner M. and Coyle EF. 2008. Endurance exercise performance: the physiology of champions. Journal of
518 Physiology 585:35-44.

519 Kays R, Crofoot MC, Jetz W, Wikelski M. 2015. Terrestrial animal tracking as an eye on life and planet.
520 Science 348(6240):aaa2478.

521 Kemi OJ, Haram PM, Wisløff U, Ellingsen Ø. 2004. Aerobic Fitness Is Associated With Cardiomyocyte
522 Contractile Capacity and Endothelial Function in Exercise Training and Detraining. Circulation
523 109(23):2897-2904.

524 Klaassen RHG, Alerstam T, Carlsson P, Fox JW, Lindström Å. 2011. Great flights by great snipes: long and
525 fast non-stop migration over benign habitats. Biology Letters 7(6):833-835.

526 Kuzmiak-Glancy S, Willis WT. 2014. Skeletal muscle fuel selection occurs at the mitochondrial level. The
527 Journal of Experimental Biology 217(11):1993-2003.

528 Lague S, Chua B, Farrell AP, Wang Y, Milsom WK. 2016. Altitude matters: differences in cardiovascular and
529 respiratory responses to hypoxia in bar-headed geese reared at high and low altitudes. Journal of
530 Experimental Biology 219:1974-1984.

531 Lindstrom A, Kvist A, Piersma T, Dekinga A, Dietz MW. 2000. Avian pectoral muscle size rapidly tracks body
532 mass changes during flight, fasting and fuelling. Journal of Experimental Biology 203(5):913-919.

533 Mahalingam, S., McClelland, G. B. and Scott, G. R. (2017). Evolved changes in the intracellular distribution
534 and physiology of muscle mitochondria in high-altitude native deer mice. Journal of Physiology
535 doi:10.1113/JP274130.

536 Maina JN. 2000. What it takes to fly: the structural and functional respiratory refinements in birds and bats.
537 Journal of Experimental Biology 203(20):3045-3064.

538 Mairböurl H. 2013. Red blood cells in sports: effects of exercise and training on oxygen supply by red blood
539 cells. Frontiers in Physiology 4:332.

540 Marsh RL. 1984. Adaptations of the Gray Catbird *Dumetella carolinensis* to Long-Distance Migration: Flight
541 Muscle Hypertrophy Associated with Elevated Body Mass. Physiological Zoology 57(1):105-117.

542 Marsh RL, Storer RW. 1981. Short Communications: Correlation of Flight-Muscle Size and Body Mass in
543 Cooper's Hawks: A Natural Analogue of Power Training. Journal of Experimental Biology 91(1):363-
544 368.

545 McFarlan JT, Bonen A, Guglielmo CG. 2009. Seasonal upregulation of fatty acid transporters in flight
546 muscles of migratory white-throated sparrows (*Zonotrichia albicollis*). Journal of Experimental
547 Biology 212(18):2934-2940.

548 McGuire LP, Guglielmo CG. 2009. What Can Birds Tell Us about the Migration Physiology of Bats? Journal
549 of Mammalogy 90(6):1290-1297.

550 Meijer JH, Robbers Y. 2014. Wheel running in the wild. Proceedings of the Royal Society B: Biological
551 Sciences 281(1786).

552 Meir JU, Milsom WK. 2013. High thermal sensitivity of blood enhances oxygen delivery in the high-flying bar-
553 headed goose. Journal of Experimental Biology 216:2172-2175.

554 Mitchell CJ, Churchward-Venne TA, Bellamy L, Parise G, Baker SK, Phillips SM. 2013. Muscular and
555 Systemic Correlates of Resistance Training-Induced Muscle Hypertrophy. PLOS ONE 8(10):e78636.

556 Muheim R, Henshaw I, Sjöberg S, Deutschlander ME. 2014. BirdOriTrack: a new video-tracking program for
557 orientation research with migratory birds. Journal of Field Ornithology 85(1):91-105.

558 Norberg UM. 1996. Energetics of flight. In: Carey C, editor. Avian Energetics and Nutritional Ecology.
559 London: Chapman & Hall. p. 199–249.

560 Nourani E, Yamaguchi NM. 2017. The Effects of Atmospheric Currents on the Migratory Behavior of Soaring
561 Birds: A Review. Ornithological Science 16(1):5-15.

562 Pelters MMAL, Butler PJ, Bishop CM, Glatz JFC. 1999. Fatty acid binding protein in heart and skeletal
563 muscles of the migratory barnacle goose throughout development. Am. J. Physiol. (Regulatory
564 Integrative Comp. Physiol.) 276:R637-R643.

565 Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RDC. 2011. nlme: linear and nonlinear mixed effects
566 models. R package version 3.1-98. R Foundation for Statistical Computing, Vienna.

567 Portugal SJ, Green JA, White CR, Guillemette M, Butler PJ. 2011. Wild geese do not increase flight
568 behaviour prior to migration. Biology Letters doi:10.1098/rsbl.2011.0975.

569 Portugal SJ, Thorpe SKS, Green JA, Myatt JP, Butler PJ. 2009. Testing the use/disuse hypothesis: pectoral
570 and leg muscle changes in captive barnacle geese *Branta leucopsis* during wing moult. The Journal
571 of Experimental Biology 212(15):2403-2410.

572 Powers SK, Jackson MJ. 2008. Exercise-induced Oxidative Stress: Cellular Mechanisms and Impact on
573 Muscle Force Production. Physiological Reviews 88:1243-1276.

574 Price ER, Bauchinger U, Zajac DM, Cerasale DJ, McFarlan JT, Gerson AR, McWilliams SR, Guglielmo CG.
575 2011. Migration- and exercise-induced changes to flight muscle size in migratory birds and

576 association with *IGF1* and *myostatin* mRNA expression. The Journal of Experimental Biology
577 214(17):2823-2831.

578 Prins HHT, Namgail T. 2017. Bird Migration Across the Himalayas. Cambridge University Press, Cambridge,
579 United Kingdom.

580 Prop J, Black JM, Shimmings P. 2003. Travel schedules to the high arctic: barnacle geese trade-off the
581 timing of migration with accumulation of fat deposits. Oikos 103:403-414.

582 Qasem L, Cardew A, Wilson A, Griffiths I, Halsey LG, Shepard ELC, Gleiss AC, Wilson RP. 2012. Tri-axial
583 dynamic acceleration as a proxy for animal energy expenditure; should we be summing values or
584 calculating the vector? PLOS ONE 7:e31187.

585 Saunders DK, Klemm RD. 1994. Seasonal changes in the metabolic properties of muscle in blue-winged
586 teal, *Anas discors*. Comparative Biochemistry and Physiology Part A: Physiology 107(1):63-68.

587 Scott GR. 2011. Elevated performance: the unique physiology of birds that fly at high altitudes. The Journal
588 of Experimental Biology 214(15):2455-2462.

589 Scott GR, Hawkes LA, Frappell PB, Butler PJ, Bishop CM, Milsom WK. 2015. How Bar-Headed Geese Fly
590 Over the Himalayas. Physiology 30(2):107-115.

591 Scott GR, Milsom WK. 2006. Flying high: A theoretical analysis of the factors limiting exercise performance
592 in birds at altitude. Respiratory Physiology & Neurobiology 154:284-301.

593 Scott GR, Milsom WK. 2007. Control of breathing and adaptation to high altitude in the bar-headed goose.
594 American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 293:R379-
595 R391.

596 Scott GR, Schulte PM, Egginton S, Scott ALM, Richards JG, Milsom WK. 2011. Molecular Evolution of
597 Cytochrome c Oxidase Underlies High-Altitude Adaptation in the Bar-Headed Goose. Molecular
598 Biology and Evolution 28:351-363.

599 Sherub S, Bohrer G, Wikelski M, Weinzierl R. 2016. Behavioural adaptations to flight into thin air. Biology
600 Letters 12(10): doi: 10.1098/rsbl.2016.0432

601 Smith KJ, MacLeod D, Willie CK, Lewis NCS, Hoiland RL, Ikeda K, Tymko MM, Donnelly J, Day TA,
602 MacLeod N, Lucas SJE, Ainslie PN. 2015. Influence of high altitude on cerebral blood flow and fuel
603 utilisation during exercise and recovery. Journal of Physiology 592:5507-5527.

604 Speakman JR, Thomas DW. 2005. Physiological ecology and energetics of bats. In: Kunz TH, Fenton MB,
605 editors. Bat ecology. University of Chicago Press. p. 430-490.

606 Spivey RJ, Bishop CM. 2014. An implantable instrument for studying the long-term flight biology of migratory
607 birds. Review of Scientific Instruments 85(1):014301.

608 Tucker VA. 1968. Respiratory physiology of house sparrows in relation to high-altitude flight. Journal of
609 Experimental Biology 48, 55-66.

610 Vézina F, Jalvingh KM, Dekinga A, Piersma T. 2007. Thermogenic side effects to migratory predisposition in
611 shorebirds. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292:R1287-R1297.

612 Ward S, Bishop CM, Woakes AJ, Butler PJ. 2002. Heart rate and the rate of oxygen consumption of flying
613 and walking barnacle geese (*Branta leucopsis*) and bar-headed geese (*Anser indicus*). Journal of
614 Experimental Biology 205(21):3347-3356.

615 Waters RE, Rotevatn S, Li P, Annex BH, Yan Z. 2004. Voluntary running induces fibre-type-specific
616 angiogenesis in mouse skeletal muscle. American Journal of Physiology - Cell Physiology
617 287:C1342-1348.

618 Wehrlin JP, Marti B, Hallén J. 2016. Hemoglobin mass and aerobic performance at moderate altitude in elite
619 athletes. Chapter 24 in: Roach RC, Wagner PD, Hackett PH (Eds): Hypoxia. Springer Verlag, New
620 York, pp.357-374.

621 Weiner RB, DeLuca JR, Wang F, Lin J, Wasfy MM, Berkstresser B, Stöhr E, Shave R, Lewis GD, Hutter AM
622 et al. 2015. Exercise-Induced Left Ventricular Remodeling Among Competitive Athletes. Circulation:
623 Cardiovascular Imaging 8(12): doi: [10.1161/CIRCIMAGING.115.003651](https://doi.org/10.1161/CIRCIMAGING.115.003651)

624 Zhang Y, Liang S, He J, Bai Y, Niu Y, Tang X, Li D, Chen Q 2015. Oxidative stress and antioxidant status in
625 a lizard *Phrynocephalus vlangalii* at different altitudes or acclimated to hypoxia. Comparative
626 Biochemistry and Physiology Part A 190:9-14.

627 Zúñiga D, Falconer J, Fudickar AM, Jensen W, Schmidt A, Wikelski M, Partecke J. 2016. Abrupt switch to
628 migratory night flight in a wild migratory songbird. Scientific Reports 6:34207.

629 Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. Mixed effects models and extensions in
630 ecology with R. New York, NY: Springer.

631

632

633 **SUPPLEMENTAL FIGURE LEGENDS**

634 Figure S1. Plot showing the onset of migration for four geese (A, B, C, D), where periods of migratory flight
635 can be easily distinguished from the pre-migratory period with marked changes in altitude accompanied by
636 peaks in activity, marked with a vertical red line for each goose.

637

638 Figure S2. Plots showing (A, C, E, G, left panels) daily minimum body temperature and (B, D, F, H, right
639 panels) daily range of body temperatures for four bar-headed geese (each in a separate row) over the period
640 between device deployment and migratory departure, shown as days prior to departure, where 0 is the day
641 of departure.

642

643 Figure S3. Plots showing (a) altitude, (b) heart rate, (c) VeDBA and (d) body temperature for three weeks
644 leading up to migration (which occurs immediately after the last data point in the plot) for one bar-headed
645 goose (P35). Data are averaged into two-minute bins. Diurnal patterns in heart rate, VeDBA and body
646 temperature can be seen as regular increases during the day and decreases overnight. Grey vertical boxes
647 show night-time for all figure parts.

648

649 Figure S4. Plots showing (a) altitude, (b) heart rate, (c) VeDBA and (d) body temperature for three weeks
650 leading up to migration (which occurs immediately after the last data point in the plot) for one bar-headed
651 goose (P41). Data are averaged into two-minute bins. Diurnal patterns in heart rate, VeDBA and body
652 temperature can be seen as regular increases during the day and decreases overnight. Grey vertical boxes
653 show night-time for all figure parts.

654

655 Figure S5. Plots showing (a) altitude, (b) heart rate, (c) VeDBA and (d) body temperature for three weeks
656 leading up to migration (which occurs immediately after the last data point in the plot) for one bar-headed
657 goose (P43). Data are averaged into two-minute bins. Diurnal patterns in heart rate, VeDBA and body
658 temperature can be seen as regular increases during the day and decreases overnight. Grey vertical boxes
659 show night-time for all figure parts.