Does hyperthermia constrain flight duration in a short–distance migrant?

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Abstract

While some migratory birds perform non–stop flights of over 11,000 km, many species only spend around 15% of the day in flight during migration, posing a question as to why flight times for many species are so short. Here we test the idea that hyperthermia might constrain flight duration in a short–distance migrant using remote biologging technology to measure heart rate, hydrostatic pressure and body temperature in 19 migrating eider ducks (*Somateria mollissima*), a short–distance migrant. Our results reveal a stop–and–go migration strategy where migratory flights

30 were frequent (14 flights per day) and short (15.7 min), together with the fact that

31 body temperature increases by 1°C, on average, during such flights, which equates to

32 a rate of heat storage index (HSI) of 4 °C.h^{-1}. Furthermore, we could not find any

33 evidence that short flights were limited by heart rate, together with the fact that the

34 numerous stops could not be explained by the need to feed, as the frequency of dives

35 and the time spent feeding were comparatively small during the migratory period. We

36 thus conclude that hyperthermia appears to be the predominant determinant of the

37 observed migration strategy, and suggest that such a physiological limitation to flight

38 duration may also occur in other species.

39 **1. Introduction**

40 Long–distance bird migrants seem to be able to achieve the impossible, spending energy at

41 an unmatched rate among vertebrates and dumping large amounts of heat produced while

42 flying, all without dehydrating. Numerous wind–tunnel studies, laboratory and aviary

43 experiments have shown that convection is the main mechanism by which a flying bird

44 maintains heat balance, followed by radiation and evaporation [1-9]. Hyperthermia, a

45 condition that develops when those mechanisms of heat dissipation are not sufficient to

46 maintain heat balance, is barely mentioned as a potential constraint of performance in

47 migrating birds [but see 4 and 10].

48 Forty years ago, it was proposed that cheetahs (*Acinonyx jubatus*) might stop running upon

49 reaching a critical body temperature (T_b) [11, but see 12], which was confirmed further

50 with other mammal models, including rats, antelopes, goats, and dogs [13-16]. Fatigue is

51 a complex multi–faceted phenomenon and one interesting avenue that has been

52 investigated in humans is how body temperature increases during exercise, leading to

53 hyperthermia–induced fatigue [17]. The critical core body temperature hypothesis

stipulates that exercise in the heat ceases at some critical body temperature independently of T_b at the start of exertion [18-20]. Here, the subjects are working at a constant rate until T_b reach some unsustainable level, triggering then a "safety" switch associated with a reduced recruitment of muscles as controlled by the brain [20]. The idea that body temperature may constrain prolonged exercise gained credence upon the experimental work of Danish researchers [21-24] showing that leg blood flow, altered muscle metabolism or skeletal muscle glycogen could not explain the termination of exercise, and that the attainment of critical T_b could severely alter homeostasis. A second hypothesis assumes instead that an exercising subject can modulate exercise intensity in an anticipatory fashion where they would eventually reduce the work load in order to avoid reaching some critical T_b [25,26]. Under this hypothesis, hyperthermia is considered as an evolving condition during exercise and is related to the existence of a continuous thermal signal. In this case, the rate at which heat is stored in the exercising body would be the monitored cue.

The core body temperature of pigeons (*Columbia livia*) during flight under captive conditions has been recorded as high as 43.1 - 44.4ºC, and flight time is reduced during bouts of such high–temperature activity [27]. Such high temperatures in captive experiments may be a result of handling stress [28], and therefore are not necessarily an accurate reflection of body temperatures experienced by birds in flight under field conditions. As such, field experiments are pivotal to truly investigate body temperature changes during natural flight. Captive experiments have also demonstrated that individual birds are reluctant or refuse to fly once a high body temperature has been reached [2,27-34]. Many studies of body temperature during flight, however, have involved very short flights (< 10 minutes) where birds were often tethered to trailing wires.

78 To identify how heat dissipation may constrain prolonged exercise, affect energy

79 expenditure, and play a major determining role in shaping migration strategies, we studied

80 a migratory population of common eiders (*Somateria mollissima*) (herein referred to as

81 eiders), a large marine diving duck [35]. Here, our aim is to investigate if hyperthermia

82 might influence the migration strategy of a large bird and test both the critical body

83 temperature and anticipation hypotheses. Moreover, we investigate two other alternative

84 hypotheses in this paper in order to substantiate further our interpretation, by testing the

85 possibility that the migration strategy observed is limited by the working capacity of the

86 heart or simply by the need to feed. Using remote biologging technology, we recorded

87 heart rate (every 2 s), time spent diving (derived from a pressure sensor measuring dive

88 depth every 2 s) and body temperature (16 s) regularly for every bird for a period of four

89 to 12 months using internally implanted multi–channelled data loggers [35-39]. The

90 sampling rate of these measurements allows the identification of all periods of flight, and

91 the interaction between flight duration and body temperature to be examined at a level of

92 detail not previously explored under natural conditions.

93 **2. Material and methods**

94 <u>Model species and methods</u>

95 Common eiders (*Somateria mollissima*) are large (~2 kg) sea ducks that dive for food.

96 They are characterised by short–pointed wings resulting in high wing–loadings and high

97 flight speeds [35-37]. The population studied here breeds in the Baltic and moults in the

98 Wadden Sea [43] and can be characterized as short–distance migrants (200–1100 km)

99 compared to other sub–species where the maximum distance covered by any population

100 between breeding and wintering habitats could be up to 3000 km [43].

101 This study was performed on Christiansø Island (55°19'N, 15°12'E), an old Danish fortress

102 located in the southern Baltic Sea, 18 km from the Danish island of Bornholm. The

103 general approach of our work involved the monitoring and deployment of data loggers on

104 breeding females, using heart rate data to determine the start and the end of each flight,

105 calculating the time spent diving from the depth sensor, and measuring variation of body

106 temperature during flights. All instrumented females of this study were diving during the

107 migratory process, indicating that they were following a coastal route (see Results). As

108 with many other species of waterfowl, eiders undergo a moult migration in late summer.

109 They move from breeding habitats to their moulting areas where they completely lose their

110 wing feathers, leading to a period of flightlessness that lasts 36 days on average [36]. Such

111 simultaneous moulting of the flight feathers has been shown to be energetically

112 demanding in many species of waterfowl [36, 42-45].

113 <u>Deployment of data loggers</u>

114 On Christiansø Island, 45 common eiders were captured in 2003, 2004 and 2005 and

115 implanted with data loggers under license from Dyreforsøgtilsynet (Danish Royal

116 Veterinarian Corporation) and the Canadian Council of Animal Care. All surgical

117 procedures were conducted indoors according to the procedure described by Guillemette et

118 al. [35]. The 45 DLs were 36 mm long (± SD = 0.5) x 28 mm (0.2) wide x 11 mm thick

119 (0.3) and weighed 21 g (0.3), that is 1.2% of body mass at implantation [35]. The DLs

120 were encased in biocompatible material with two asymmetrical leads protruding from

121 them towards the heart. Thirty nine (87%) experimental females returned to the study area

122 one year later, which is similar to previously reported survival rate in that species [46].

123 One year after the implantation, 36 females were re–captured of which 17, 7 and 12

124 (respectively for 2003, 2004 and 2005) had their data logger removed. For all studied

125 years, data loggers recorded pressure and heart rate every 2 s and body temperature every

126 16 s, except for 2003 females as the temperature sensor was not operational for that

127 deployment. We thus analysed for this paper data from 19 females (2004 and 2005

128 deployment only).

129 <u>Time spent flying and feeding</u>

130 Flight schedules (number and duration of flights) were compiled for each bird following

131 the method described by Pelletier *et al.* [37]. This method is based on the dramatic

132 increases during flight where heart rate is typically 3–4 times the resting level. For every

133 female, the daily time spent flying (TSF) was obtained by summing the duration of all

134 flight episodes that occurred during one day.

135 In benthic foragers, the time spent submerged is composed of time traveling back and

136 forth from the surface, and the bottom time where the bird actually searches for and

137 swallows prey items [49-50]. Eiders feed mainly on a very abundant but low quality food,

138 blue mussels (*Mytilus edulis*), and ingest a large number of whole mussels (including

139 shells) during each dive. The pressure sensor encased within the data logger enables the

140 identification of each dive performed by the experimental bird in relation to time. Thus,

141 the daily time spent diving was computed by summing the underwater duration of all dives

142 performed during a migration day for each female.

143 <u>Defining migration</u>

144 Both the daily frequency of flights episodes and the average duration of flights episodes

145 (FD) increases during migrations of female common eiders and is easily recognized on a

146 plot of time spent flying in relation to calendar days [37]. These migration events were

147 associated with at least with one flight > 30 min. We used the occurrence of such flight

148 (30 min duration) as a cut–off point to differentiate migrating from non–migrating birds.

149 FD was categorized in classes by doubling the upper limit (log 2 scale) of each class (e.g.

150 0–5 and 5–10 min) to ensure a more even number of flights in each category. A similar

151 approach was used for flight segments (FS), defined as the time interval between two

152 specific points (e.g. 0–5 and 5–10 min) in time during flight.

153 <u>Body temperature and heat storage index</u>

154 For each flight episode, the starting (T_{bStart}) and ending (T_{bEnd}) body temperatures (T_b) were

155 obtained together with maximum value (T_{bMax}), whenever it occurred during each episode.

156 We define the overall maximal T_b as the maximum T_b among all flights performed by one

157 individual and averaged over all the 19 females. We used this overall maximal T_b as an

158 approximation of a critical T_b for that species. We define the heat storage index (HSI) as

159 the variation of T_b in relation to time (^{o}C.h^{-1}). More specifically, we calculated a reduced

160 major axis slope of T_b in relation to time for each flight performed by an individual. The

161 T_b sensor time inertia was evaluated to be 3 min, as we observed changes in T_b only after 3

162 min of flight. Thus, all flights < 3 min were excluded from our analysis (n = 412).

163 Three metrics of HSI were used. An average HSI was calculated for all flight episodes

164 performed by an individual and averaged over the 19 females giving a HSI$_{average}$ of 4.1

165 (SD= 1.9) °C.h^{-1}. A categorical HSI was also calculated for each flight and averaged over

166 the 19 females within each FD category in turn (0–5 min, 5–10 min, etc.), which revealed

167 that HSI$_{category}$ varied with FD. In order to reduce the variation due to FD, we also

168 calculated an average individual HSI based on all 5–10 min segments (HSI$_{5-10}$) performed

169 by an individual (all FDs). This was preferable than using a single value per individual as

170 some FD categories were not performed by all individuals. We thus used HSI$_{5-10}$ as an

171 individual index of heat storage.

172

Data analysis

174 Confidence intervals were computed for all delta T_b and HSI values using the bootstrap

175 method and ten thousand re–samplings. We used the Student's *t* version of the bootstrap

176 [51] to compare period of interest. When the 95% confidence intervals of the average

177 deltas excluded 0, the difference was declared significant at the 5% level, otherwise it was

178 concluded that no difference could be detected between the periods being compared. We

179 used a two–way repeated measures ANOVA, with the Satterthwaite's approximation for

180 degrees of freedom, to test the hypothesis that HSIs would vary with both FD and FS.

181 More specifically, we tested the anticipation hypothesis by looking at the interaction term

182 using $P = 0.05$ as the critical value. Finally, the level of significance of correlations were

183 computed using a permutation test.

184 **3. Results**

185 The migration of this population of eiders involved a distance of 714 ± 286 km, taking, on

186 average, 2.6 ± 1.0 days, from the central Baltic region to the Wadden Sea. For each day,

187 the birds spent a total of 205 ± 81 min in flight (14.2 % of total day), comprised of $13.7 \pm$

188 5.7 flights in total per day. Each individual flight averaged 15.7 ± 5.2 min, and of the 655

189 flights recorded from the 19 birds approximately only 10 % lasted more than 40 min, with

190 most (48 %) flights being shorter than 5 min (**Figure 1a**). These longer flights (> 40 min)

191 were most important in terms of contribution to migration effort (flight time of **Fig. 1a**).

192 The critical body temperature hypothesis

193 During flights lasting up to 80 min, the maximum body temperature (T_{bMax}) recorded

194 while the eiders are actively flying increases monotonously with flight duration (**Figure**

195 **1b**). The overall maximal T_b recorded (see Methods) during such flights in the 19 eiders is

196 42.6 ± 0.1 °C, thought to be the critical T_b limit (see Discussion). However, during longer

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197   duration flights (> 80 min, up to 170 min), the patterns of heat gain are quite different

198   from those observed during shorter duration flights. Body temperature, measured at the

199   end of flight, increases linearly up to 30 min but then tends to level off, reaching 41.7 ±

200   0.9 ºC on average (**Figure 1b**). This indicates that after reaching a peak body temperature

201   within the first 30 min of flight, the rate of heat storage decreases as flight duration

202   increases.

203   <u>Alternative hypotheses explaining short flights</u>

204   Although reaching a high absolute level of body temperature is a likely signal to stop long

205   flights, such a limit cannot explain why birds stop flying after only a few minutes. For

206   instance, 59 % of the 412 flights, lasting 14 min on average, were stopped while T_b was

207   still increasing. Here we test three alternative hypotheses. The first one is based on the

208   idea that some anticipatory mechanism is used by the migrating birds based on the rate of

209   heat storage [25,26,52,53]. By partitioning each flight into flight duration segments we

210   were able to test the hypothesis that long flights would start with a lower rate of heat

211   storage compared to shorter flights. This hypothesis was supported by our analysis of HSI

212   values as the interaction between the duration of flight episodes (FD) and that of flight

213   segments (FS) was highly significant (Repeated Measures ANOVA, $F_{10,709} = 4.4$, $P <$

214   0.0001). Apart the 0-5 flight segment, the HSI of various flight segments were generally

215   higher for short flights than for longer flights (**Table 1**).

216   From this, we expected migration effort to be modulated at the individual level by the rate

217   of heat storage during flight (**Figure 2**). HSI_{5-10} (see Methods) was negatively correlated

218   with flight duration but not significantly so ($r = –0.317$, $P = 0.092$ from a permutation

219   test). However, the rate of heat storage was significantly and inversely correlated with

220   both the time spent flying (TSF, $r = -0.598$, $P = 0.002$) and maximum flight duration

221   (FD_{max}, $r = -0.734$, $P < 0.001$).

222   The second alternative hypothesis is the cardiac drift hypothesis [54], which stipulates

223   that the stop–and–go strategy of eider ducks may be caused by the limiting capacity of

224   the heart while flying. Indeed, heat accumulation within the body might trigger an

225   increase of blood perfusion to the periphery to enhance heat dumping, thereby causing

226   a reduction of blood pumped at each heart beat. The increase in peripheric perfusion

227   comes from a drop in peripheric resistance. This causes a drop in arterial pressure, and

228   increase in cardiac output, but a decrease in muscle perfusion. More likely, cardiac

229   output is unchanged but increased vasodilation in mucosal and cutaneous surfaces

230   diverts more blood from flight muscles. Hence, an increase in heart rate as a

231   compensation mechanism could have confounded our results. However, average FHR

232   varied very little in relation to flight duration (**Figure 3**) with an average of 351 bpm

233   (sd ± 40) for all FD and individuals. This conclusion was confirmed by an analysis of

234   heart rate acceleration within flight durations categories (heart rate acceleration is the

235   rate at which heart rate changes its velocity (beats per min/min ($beats.min^{-2}$))).

236   Although HR acceleration of 0–5 min flights was highly variable with a positive

237   tendency, it was not significantly different from zero for each flight category in turn

238   (**Figure 3**, CIs include zero).

239   A third and obvious hypothesis to explain the observed migration strategy is that short

240   flights and the numerous stops could have been spent to fuel the high level of exertion.

241   This was not the case as only 22 % of the stops between flights (n = 606) were devoted to

242   feeding. We thus discard the hypothesis that feeding was driving the stop and go strategy

243   of this species during migration.

4. Discussion

Here we have shown that during migratory flights, eiders experience hyperthermia, with body temperature increasing by, on average, 1.0 ºC at the end of a typical flight (16 min). This temperature increase equates to 4.1 ºC.h^{-1}, and corresponds to the storage of a significant fraction (8.2 %) of the body heat produced during flapping flight. Heat stress is a vital component to consider when evaluating migration physiology. Aside from having its own direct effect on flight physiology, hyperthermia will additionally have significant implications for overall body water balance, as it stimulates birds to increase their heat loss through evaporate cooling. Flight hyperthermia is a complex multi–faceted phenomenon, potentially altering homeostasis and causing a multitude of physiological problems. For example, hyperthermia can damage enzymes and other proteins, leading to mitochondrial dysfunction, oxidative stress and cell death in muscle as well as in other organs. This raises the possibility that flight induced hyperthermia is dictating flight duration.

Flight and critical T_b

The observation that T_b increases during flight activity of captive or trained birds has been made several times [27-34]. Indeed, various experiments performed in captivity have shown that individuals birds are reluctant or refuse to fly once a high body temperature have been reached. Birds produced heat while flying at a level one order of magnitude higher than their resting rate, and they do so with a formidable level of insulation as their plumage may act as barrier to heat dissipation [55].

We determined the highest value of body temperature (Tb_{max}) for each female. The overall average of Tb_{max} among females, which we presumed to be the highest T_b

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268   tolerated (Tb$_{crit}$), was 42.6 °C, 2.4 °C higher than the average. An average T$_{bCrit}$ of

269   43.7 °C can be estimated (n = 5, range 43,1–44,5 °C) for pigeons (*Columba livia*)

270   reluctant to fly in captive conditions [7,29,31,32], which is 1.1 °C higher than our

271   estimate of T$_{bCrit}$ in eiders. However, it is well known that T$_b$ of birds is inversely

272   related to body mass [56], and pigeons, with a much lower body mass (340-369 g)

273   should have a higher T$_b$ compared to eider ducks. Moreover, T$_{bCrit}$ during flight may

274   be influenced by habitats features and the thermic regime of their environment [57].

275   Therefore, it is highly unlikely that a single value of T$_{bCrit}$ might apply to all species

276   and conditions. In a very different situation, captive eiders suffering handling stress

277   experienced increase in body temperature of 2 °C from 41.5 °C, plateauing at 43.5 °C

278   [28]. More importantly, allowing body temperature to reach a point at which

279   homeostasis is not maintained is expected to have negative survival consequences.

280   We thus conclude that although it must be a critical T$_b$ at which a bird stops flying,

281   the notion of a critical T$_b$ alone cannot fully explain the migration strategy of eider

282   ducks.

283

284   <u>Flight and the rate of heat storage</u>

285   The anticipation hypothesis, derived from experiments conducted on humans and other

286   mammals, stipulates that an animal can modulate exercise intensity in an anticipatory

287   fashion, where an individual would eventually reduce the work load in order to avoid

288   reaching some critical T$_b$ [25,26,58]. In this case, the rate at which heat is stored in the

289   exercising body would be the monitored cue. As predicted by this hypothesis, the HSI of

290   various flight stages/durations were higher for short flights than for longer flights in eider

291   ducks (**Table 1**). The main underlying assumption of that hypothesis is that an individual

292   should be able to regulate its working level, which in turn would stabilize or even decrease

293   T_b while flying, and then avoid reaching some critical level of T_b. However, the ability of

294   eiders ducks to modulate their work level (see below) seems to be limited by their wing

295   morphology and a high wing–load [41], which in turn produce one of the highest flight

296   speed amongst birds using flapping flight [40,59]. We thus suggest that the main avenue to

297   vary the work load while flying for an eider duck is to stop flying. A second avenue would

298   be to select favorable wind conditions that would minimize the work load and the rate of

299   heat storage, for instance flights assisted by tail winds [40]. Thirdly, individual HSIs were

300   negatively correlated to the daily time spent flying and maximum flight duration,

301   suggesting that heat storage has the potential to shape migration strategy in this species.

302   <u>Alternative hypotheses to hyperthermia constrained flight duration</u>

303   We could not find any evidence for the cardiac drift hypothesis (see Results) or any

304   relationship between heart rate and T_b (**Figure 1b** and **3**). The latter is puzzling especially

305   when compared to a recent study on Bar–headed geese (*Anser indicus*) where FHR and T_b

306   were apparently tightly related in four migrating individuals (Figure 1, [60]). One possible

307   reason to explain the apparent discrepancy is the high wing–loading of eider ducks

308   compared to geese. In theory, the relationship between flight speed and power output is a

309   U–shape curve where low and high flight speeds would be associated with a high level of

310   exertion. Following Bishop (2005) [61] and Pelletier (2006) [62], calculations of available

311   power (P_{max}) over required power (P_{min}) suggest that the power margin, and the possibility

312   to modulate (air) flight speed, is weak or almost null in eider duck. In other words, eiders

313   would have little aerobic capacity above their minimum requirements, which would

314   reduce their capacity to modulate their working level while flying. In contrast to these

315   theoretical considerations, FHR of eiders can vary noticeably within a FD category

316   (**Figure 3**) and it was observed that T_b can be down regulated during long flights (**Figure**

317   **1b**). Bowlin et al. [63] have shown the heart rate of wild thrushes (*Catharus spp*.), a

13

passerine bird, to be tied to wing–beat frequency. Unfortunately, we did not record wing–beat frequency in this study and we suggest that without such information, it is difficult to test the main assumption of the anticipation model when applied to eider ducks. Nevertheless, we conclude again that the main option available to eider ducks to vary the work level during migration is to stop flying.

Another and final consideration is the observation that only 22 % of the stops involved diving, thereby refuting the possibility that feeding was driving the stop–and–go strategy of migrating eiders. Indeed, the birds spent only 2.3 % of their time (per 24 h) diving during the migration period compared to 6.3 % of their time, on average, during a full annual cycle [43]. With the increase in energetic requirements due to the migratory flights [35], this time spent diving is likely to be insufficient to be replenishing lost fat stores, and the short dives likely perform an alternate function.

<u>What about other migratory birds?</u>

This paper is the first report of direct and detailed measurements of the daily time spent flying and the time spent feeding of a migrating bird together with measurements of body temperature. We would like here to consider how our findings might apply to other bird species. We thus postulate that birds prone to hyperthermia during migration are associated with high wing–loadings, large body masses and frequenting aquatic habitats. First, hyperthermia during migration should apply to diving birds with high wing-loading and continuous, fast-flapping flight such as diving ducks and alcids because of their high wing–loadings dictating high flight costs [40,41,59], which would generate large amount of heat while flying. The only other study we aware of that measured T_b during flight of diving birds has been done with shags (*Phalacrocorax georgianus*, [64]), where the rate of T_b increase (16 $°C.h^{-1}$) was much higher to what we have quantified for eider ducks; although this was only for short flights (< 5 min). Second, birds exchange heat with their

343 surroundings at rates proportional to their surface areas while heat storage is proportional

344 to body mass [65], rendering heat storage more probable in large species, while the

345 insulation level (low conductance) also increases with body mass [66]. We thus predict

346 that the likelihood of hyperthermia will vary with body size, where large migratory birds

347 are the most likely candidates to store heat while flying. Thirdly, aquatic birds migrating

348 over the water may be more inclined to let hyperthermia happen as the cooling power of

349 water is much larger than that of air (in terrestrial habitats).

350 Finally, migration strategies are diverse and we expect birds to use various behavioral

351 strategies to limit the extent of hyperthermia during migration. For example, three

352 common strategies used by migratory birds are nocturnal travelling, flying at high

353 altitudes, and V–formation flocking [10,67-70]. This could lessen considerably the

354 possible outcome of hyperthermia and facilitate the thermoregulatory balance during

355 flight, particularly as nocturnal flight and altitude are associated with a cooler air and an

356 increase of convection. On the other hand, many other species of birds migrate during the

357 day at low altitude [67]. We thus consider that only recent and future advances of

358 biologging capabilities will offer the opportunity to determine the role that hyperthermia

359 may play during flight and migration of these various species.

360 **Data accessibility**

361 Supplementary   Information   are   available   with   the   paper
362 http://dx.doi.org/10.1098/rstb.2015.0386.

363 **Ethics**

364 This study was done under the approval of the Canadian Council of Animal Care # CPA
365 16–03–07–01.

366 **Competing interests**

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367 No competing interest to declare.

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Acronyms

DL   Data Loggers
FD   Flight Duration
FS   Flight Segments
HSI   Heat Storage Index

Figure Legends

Figure 1. A. Flight frequency and total flight time in relation to flight duration (FD). Most flights performed during moult migration were short duration but those contributing the most to migration effort were long flights. **B**) Mean body temperature (T_b) at the beginning (T_{bStart}) and at the end (T_{bEnd}) of a flight together with (T_{bMax}) while aloft in relation to flight duration. Errors bars depict 95 % confidence intervals. Critical T_b was assessed as the overall maximal T_b among all flights performed by one individual and averaged over all the 19 females (42.6 ± (SD) 0.1 °C).

Figure 2. Scatterplot relating the individual (see Methods) heat storage index averaged over all flight segments (HSI_{5-10}) performed by an individual in relation to (**A**) average flight duration, (**B**) average daily time spent flying and (**C**) maximum flight duration (one flight per individual). Pearson correlation coefficients are shown together with probability levels determined from permutation tests.

Figure 3. Mean flight heart rate (FHR ± 95 % CI) and mean heart rate acceleration (HRA ± 95 % CI) in relation to flight duration. HRA was calculated for each category as an average reduced major axis slope relating time and HR together with 95 % CIs using the bootstrap approach. When a CI excludes the zero line, the average slope is considered to be significantly different from zero ($P < 0.05$).

559 **Table 1**. Average (bold) values of heat storage index (HSI in $°C.h^{-1}$), with 95%

560 confidence intervals (CI) in relation to flight durations (FD) and flight segments (FS),

561 to test the hypothesis that long flights are characterized by a lower level of heat

562 storage than short flights (see text). The table reads from left to right going through

563 flight duration classes from a single flight segment classe. Only late segments of flight

564 for longer flights have HSI non-significantly different from zero (shaded values).