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1 **Title:**

2 Body surface temperature responses to food restriction in wild and captive great tits (*Parus major*)

3

4 **Running title:**

5 Body surface temperature in fasting great tits

6

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28

29 Key words (3-6):

30 body temperature, food restriction, heterothermy; thermal imaging, winter

31

32 **Summary statement**

33 We provide evidence that wild and captive great tits reduce temperature of the bill in response to
34 food restriction.

35 **Abstract**

36

37 During winter at temperate and high latitudes, low ambient temperatures, limited food supplies and
38 short foraging periods mean small passerines show behavioural, morphological and physiological
39 adaptations to reduce the risk of facing energy shortages. Peripheral tissues vasoconstrict in low
40 ambient temperatures to reduce heat loss and cold injury. **Peripheral vasoconstriction** has been
41 observed with food restriction in captivity but has yet to be explored in free-ranging animals. We
42 experimentally food restricted both wild and captive great tits during winter months and measured
43 surface temperatures of bill and eye-region using thermal imaging, to investigate if birds show rapid
44 local heterothermic responses, which may reduce **thermoregulatory** costs when facing **a perceived**
45 **imminent** food shortage. Our results **of a continuously-filmed wild population** showed that bill
46 temperature was **immediately reduced in response to** food restriction compared to when food **was** *ad*
47 *libitum*, **an apparent autonomic response. Such immediacy implies a ‘pre-emptive’ response before**
48 **the bird experiences any shortfalls in energy reserves.** We also demonstrate temporal variation in
49 vasoconstriction **of** the bill, with bill temperature gradually rising throughout the food restriction
50 after the initial drop. Eye-region temperature **in the wild birds remained at similar levels throughout**
51 **the food restriction compared to unrestricted birds**, possibly reflecting the need to maintain steady
52 circulation to the central nervous and visual systems. Our findings provide evidence that birds
53 selectively allow the bill to cool when a predictable food supply is suddenly disrupted, likely as a
54 means of **minimising depletion of body reserves for a perceived future shortage in energy.**

55 Introduction

56

57 Winter in seasonal habitats is often challenging for small endotherms as severe weather increases
58 thermoregulatory costs while limited food supply and short foraging periods potentially constrain
59 acquisition of resources to meet these increased costs. It follows that individuals must respond to
60 winter conditions, by morphological, behavioural and physiological adaptations, to avoid facing
61 energetic shortfalls. The thermoneutral zone (TNZ), where heat loss is offset by basal metabolic heat
62 production, for most passerines is 15-35 °C (Gavrilov and Dolnik, 1985). In winter at higher latitudes
63 small birds routinely experience environmental temperatures well below thermoneutrality and
64 therefore to maintain body temperature, metabolic heat production must increase (Scholander et al.,
65 1950; William et al., 1983). A first defence to minimise heat loss are morphological adaptations
66 (e.g., increased insulation from feathers) and behavioural responses (e.g., seeking shelter,
67 ptiloerection) (Nord et al., 2011; Shipley et al., 2019). Physiological adaptations in small endotherms
68 are directed to increasing heat production (Swanson and Vézina, 2015) and insulation *via* local or
69 global heterothermy (e.g. Johnsen *et al.*, 1985; Ruf & Geiser 2015). These responses operate at
70 different temporal scales as seen by long term seasonal acclimatisation (Vezina & Swanson 2015) or
71 through instantaneous responses when there are sudden changes in weather (Marsh and Dawson,
72 1989).

73

74 Reduction in peripheral temperature by shunting blood flow to the core (local heterothermy) can lead
75 to significant energy savings in variable environments (Hagan and Heath, 1980; Steen and Steen,
76 1965; Tattersall et al., 2016). In birds, the legs, bill and eyes are usually unfeathered and are,
77 therefore, key regions of heat transfer. Counter-current vascular arrangements, and sphincteric
78 contractions in major vessels in and around birds' legs, allow the normally uninsulated region to
79 remain at, or close to, ambient temperature (Johansen and Bech, 1983; Midtgård, 1981; Steen and
80 Steen, 1965). This reduces heat loss and prevents cold injury. The bill is highly vascularised but
81 uninsulated, and is known to play a role in thermoregulation particularly in large-billed species in hot
82 climates, though recent work highlights the role of the bill also in cold environments and in small-
83 billed species (Schraft et al., 2019; reviewed by Tattersall et al., 2017). In line with this, bill size
84 declines with decreasing minimum winter temperature (Danner and Greenberg, 2015; Friedman et
85 al., 2017; Symonds and Tattersall, 2010). It is, therefore, a realistic expectation that there will be
86 thermoregulatory responses in the bill (as well as in other peripheral tissues) to manage energetically
87 challenging situations, such as cold snaps and food shortage. Additionally, reduced circulation to the
88 head region might lower evaporative heat loss through uninsulated regions such as the eyes and

89 respiratory heat loss through the nasal passages (Midtgård, 1984). However, while local
90 heterothermic responses carry energetic benefits, the resultant lower tissue temperature in
91 appendages such as the legs and bill, and other peripherally located structures such as the eyes, may
92 reduce ease of locomotion, foraging or sensory perception. Therefore, the use of local heterothermy
93 may be subject to a trade-off between environmental conditions, energetic state and food availability.
94 For example, a study of Muscovy ducklings (*Cairina moschata*) showed cold-acclimated birds had a
95 more stable bill temperature, with evidence of vasoconstriction of the bill, when fasting for relatively
96 long periods, than birds that were kept in thermoneutrality (Tattersall et al., 2016). A recent study on
97 blue tits (*Cyanistes caeruleus*) found that low periorbital temperature was correlated with low body
98 condition (Jerem et al., 2018). Local heterothermy has also been shown to be a response to fasting in
99 several other bird species, and likely explains why in some studies core body temperature remains
100 constant but, nevertheless, energy savings are made (Hohtola, 2012). There is now a need to
101 experimentally test predictions from this work on wild models in their natural environment.

102

103 In this study, we experimentally tested the effects of environmental conditions on peripheral body
104 temperature of wild and captive great tits (*Parus major*) in winter, using thermal imaging. In both
105 settings, we temporarily manipulated access to food and recorded the dynamics of the birds' eye and
106 bill temperatures before, during, and after food restriction. We predicted that peripheral body
107 temperatures would decrease in response to the food restriction, and more so when ambient
108 temperature was lower. We expected to reliably record body surface temperature in uninsulated areas
109 of the body, specifically the bill and eye-region, which are likely key areas of heat-exchange. We did
110 not record responses to food restriction in the uninsulated legs, because previous work in our
111 population has shown that wild parids (including great tits) maintain stable low leg temperatures in
112 winter, even when fed *ad libitum*. By contrast, bill temperature is consistently maintained well above
113 ambient (Nord, A., Huxtable, A., Reilly, H., McCafferty, D. J., in prep.).

114

115

116 **Material and methods**

117

118 The study used great tits in two populations of separate subspecies; one captive (ssp. *newtoni*) and
119 one wild (ssp. *major*). In both populations we compared food-restricted birds to unrestricted control
120 birds. The wild study consisted of continuous filming on days with and without a food restriction
121 experiment (treatment or control days). For the captive study, filming occurred before and after a
122 food restriction event and two consecutive days before the food restriction day. The air temperature

123 range was between -10 and +2°C in the captive study, and +2 to +13°C in the wild study, below the
124 thermoneutral zone of great tits (Broggi et al., 2005).

125

126 *Captive study*

127

128 Fourteen wild great tits were captured near Vomb, Sweden (55°39'N, 13°33'E) and were
129 immediately transferred to four outdoor aviaries (6.0 × 3.0 × 2.5 m; width × length × height) at
130 Stensoffa Ecological Field Station, Sweden (55°42'N, 13°27'E), where they were kept in mixed sex
131 groups from October 2012 to January 2013 and handled as described in Nord *et al.*, (2016). The
132 aviaries contained both a covered and non-covered area, perches and nest boxes for the number of
133 individuals in each aviary. The birds were left for two weeks to acclimate to the aviaries before the
134 start of the experiment. All procedures on the captive birds were approved by the Malmö/Lund
135 Animal Ethics Committee (permit no. M236-10). Catching and ringing of birds was licensed by the
136 Swedish Ringing Centre (license no. 475), and the use of radio transmitters was permitted by the
137 Swedish Post and Telecom Authority (permit no. 12-9096).

138

139

140 Thermal videos were taken at 3 Hz of birds at the feeders at 1.4 m distance using a SC640 FLIR
141 camera (FLIR® Systems, Inc), FOL 76mm lens on three consecutive days (1-3 December). On days
142 1 and 2, food remained *ad libitum* throughout the day (including while filming). On day 3, the food
143 was restricted for three hours (mean: 3hr17min ± 8min) staggered by an hour between aviaries, with
144 the first restriction beginning in the first aviary at 9:00 h (local time) and beginning in the last aviary
145 at 13:00 h. Water was freely available in heated trays (that prevented freezing) throughout the
146 experiment. Thermal imaging took place before the food restriction (data also include the two days
147 prior to the food restriction) and after the food restriction period and lasted for one hour (mean:
148 54mins ± 14mins) at each aviary (for day 2, aviary 4, filming lasted for 4hrs 29mins). A video
149 camera (Panasonic Model: HC-V720, Hamburg, Germany) was used to film the feeder so individual
150 birds could be identified from unique colour ring combinations (birds were also fitted with
151 subcutaneous PIT tags and radio transmitters for other research projects, see Nord et al., 2016).

152

153 Air temperature (accuracy ± 0.5°C, resolution 0.0625°C) was recorded continuously from the centre
154 of the aviary (iButton DS1922-L, Maxim Integrated Products, CA, USA; accuracy ± 0.5°C). Relative
155 humidity was recorded by a weather station at Lund University, 17 km from the study site.

156

157 *Wild study*

158 Data for the wild study was collected in an oak (*Quercus robur*) woodland surrounding the Scottish
159 Centre for Ecology and the Natural Environment on Loch Lomond, Scotland UK (56°3'N, 04°33'W)
160 between January and March 2017. A bird feeder containing peanut granules (Haith's, Grimsby, UK)
161 was provided two months prior to the start of the experiment to attract resident birds.

162

163 Nineteen great tits were then caught by mist netting around the feeder from January to February
164 2017, and were fitted with a British Trust for Ornithology (BTO) ring on the right leg and a passive
165 integrated transponder (PIT) tag (EM4102 PIT Tag, Eccel Technology, Leicester, UK), used for
166 identification, on the left leg. A custom-built PIT tag recorder (University of Glasgow Bioelectronics
167 Unit, Glasgow) was attached to the feeder in order to identify birds visiting at a given time. All
168 procedures were approved by BTO ringing permits, and by a UK Home Office Licence.

169

170

171 Thermal video was collected from food-restricted and control birds at 7.5 Hz using a FLIR AX5
172 thermal camera from 0.7 m distance, on nine days between 10 February and 2 March 2017. Food
173 was restricted on five of those days (14, 16, 21, 23 February and 2 March 2017) for three hours
174 (mean: 2hrs 43mins \pm 6mins) between 10:00 and 13:20. On these days, thermal videos were taken
175 for one hour before the food restriction, three hours during the food restriction and an hour and a half
176 after the food restriction (with the exception of 16 February, when due to equipment failure filming
177 occurred only after food restriction). Each food restriction was considered as a stand-alone event as
178 at least one control day separated each day of food restriction. For the remaining four control days
179 (10, 13, 15 and 20 February 2017), where there was no food restriction, filming occurred
180 continuously at the feeder. A dummy camera was deployed five days prior to filming to habituate
181 birds to the presence of the camera and was subsequently returned each day after thermal imaging
182 was completed. Air temperature was measured using a thermocouple attached to the feeder (Tinytag
183 Talk 2, Gemini Data Loggers, Chichester, England). Relative humidity data were available from a
184 MiniMet Automatic Weather Station (Skye Instruments, Powys, UK), within 200 m of the thermal
185 camera.

186

187

188 *Thermal image analysis*

189

190 Individual thermal images (sample sizes shown in Table 1) were extracted and analysed from the
191 thermal videos using FLIR Tools 4.1. Images were selected where a clear lateral view of the head

192 was shown. When a bird visited the feeder, a unique PIT tag code was recorded with the time of
 193 visit. The time could be compared to the thermal imaging video to identify individuals in the wild
 194 study. We only analysed one image per bird within a 10 min period so each image could be
 195 considered as an independent visit to the feeder. As many birds in the wild study could not be
 196 identified when visiting the feeder, we used 41 images from unknown birds. To prevent repeated
 197 measurements of the same bird, we only used images of unknown individuals that were ≥ 10 min in
 198 time apart. For the wild experiment, the entire video was used. For the captive study, we randomly
 199 selected an aviary to be filmed for an hour at the feeder from 8:00-12:00 (before food restriction) and
 200 12:30-15:30 (after food restriction), so that despite a single camera, all aviaries were filmed on each
 201 day.

202

203 **Table 1. Sample sizes in the experiment.** The number of individual birds and images used in the
 204 experiment. Unidentified individuals were used on control days as equipment failure limited our sample size
 205 (see thermal imaging analysis in methods).

		Individuals	Images
Wild	Food restricted days	19 (6 female, 8 male, 5 unknown sex)	126
	Control days	46 (41 unknown IDs, of known: 3 female, 2 male)	55
Captive	Before food restriction	15 (4 female, 11 male)	99
	After food restriction	17 (5 female, 12 male)	52

206

207

208 For each image, the emissivity was set as 0.98 (Best and Fowler, 1981; McCafferty, 2013). Both the
 209 atmospheric and reflected temperatures during image analysis were set as the hourly mean air
 210 temperature obtained from the weather station during recording. Relative humidity equalled the
 211 mean for each recording session.

212

213

214

215 **Fig. 1. Data extraction from thermal image of bird at feeder.** Lateral image of a great tit at the feeder. Bill
 216 temperature was extracted by drawing a line from the base of the nostril to the tip of the bill. Eye region
 217 temperature was extracted by drawing a box around the head to select the hottest pixel inside the box, which
 218 was consistently found on the unfeathered periorbital ring.

219

220 Mean bill temperature (hereafter referred to as “bill temperature”) was measured from the mean
221 surface temperature of a straight line fitted from the base of the nostril to the tip of the bill (Fig. 1).
222 Maximum eye region temperature (hereafter referred to as “eye temperature”) was taken by fitting a
223 rectangle across the head which was large enough to encompass the periorbital ring, where the
224 maximum temperature of the head is typically recorded (see Jerem et al., 2015). Image focus was
225 recorded as a three-level factor. Each image was ranked as “Good” when all edges of the bill were
226 clearly defined in the image, “Medium” when either the tip or base of the bill was not clearly
227 defined, and “Poor” when the edges of the entire bill were undefined. Though images were selected
228 for quality and lateral view of the head, in some images, the head of the bird was slightly turned to
229 one side. As the length of the line along the bill varies depending on the angle of the head, distance
230 from the camera, as well as the individual size of the bird, the pixel length of the bill was recorded as
231 a continuous variable as a proxy of position of the bird (hereafter referred to as “position index”).

232

233

234 *Statistical analyses*

235

236 All statistical analyses were conducted using R version 3.3.2 (R Development Core Team, 2009).
237 Generalised linear mixed effect models (GLMM) were used to analyse bill and eye region
238 temperatures for both datasets using the *lme4* package (Bates et al., 2015).

239

240 *Captive*

241

242 Bill temperature and eye region temperature were both modelled using air temperature, the position
243 index, treatment (factorial: before/after food restriction). Bird ID with a first order autoregressive
244 (AR1) covariance structure and the aviary ID were tested as random effects in separate models.
245 However, aviary ID did not improve model fit in any case and was removed from all models.

246 Predicted means (\pm standard error) of the bill and eye region temperatures for each treatment in the
247 model described were calculated using the *predictmeans* package (version 1.0.1, Luo et al., 2018).

248

249 *Wild*

250 We tested effects of food restriction in two ways. Firstly, we tested treatment effects in a model with
251 surface temperatures as the dependent variables and “time” (i.e., before, during, or after food-
252 restriction) as a categorical explanatory variable. We calculated predicted means (\pm standard error)

253 of surface temperature from the described model for each of these “times” using the *predictmeans*
254 package (version 1.0.1, Luo et al., 2018). Tukey HSD post hoc tests were used to compare
255 differences between food restriction treatments in both wild and captive birds, using the *stats*
256 package (version 3.5.2, R Development Core Team, 2009). In both tests, we confined the after food-
257 restriction to 1.5 hours from the end of the food restriction to mirror the timings of the captive
258 experiment.

259

260 Secondly, we also used continuous body surface temperature data from before, during and after food
261 restriction. Bill temperature and eye region temperature were both modelled using, as fixed effects,
262 air temperature, the position index, and the interaction between treatment/control day and time of
263 day both as linear and quadratic terms along with their main effects. Bird ID with a covariance
264 structure (AR1 covariance structures) and focus level were random factors. Focus level did not
265 improve fit and was removed from the model.

266

267

268

269 **Results**

270

271 Bill and eye region were linearly related to air temperature in both experiments (Bill: Captive:
272 $p < 0.0001$, Fig. 2A; Wild: $p < 0.0001$, Fig. 2B; Table 2. Eye region: Captive: $p < 0.0001$, Fig. 2C; Wild:
273 $p = 0.03$, Fig. 2D; Table 2).

274

275 The position index also accounted for significant variation in the observed bill temperature for
276 captive ($p < 0.0001$, Table 2) and wild great tits ($p < 0.0001$, Table 2).

277

278

279

280

281 **Fig. 2. The relationship between bill and eye region temperatures and air temperature for captive and**
282 **wild great tits.** Captive ($n = 151$ images of 18 birds [15 before, 17 after food restriction]), and wild ($n = 181$
283 images of 60 (incl. 41 unknown) birds [19 on food restricted days and 46 on control days]). Lines are slopes
284 from linear models of bill and eye region temperatures against air temperature. Shaded regions are 95%
285 confidence intervals.

286

287

288 In the captive study, bill temperature was $1.8 \pm 0.5^{\circ}\text{C}$ greater after food restriction ($p = 0.0008$, Fig.
289 3A, Table 2). In the wild study, bill temperature was significantly lower during the food restriction
290 than both before and after (Before: 14.0 (mean) ± 0.3 (SE), During: 12.7 ± 0.2 , After: 13.9 ± 0.3 ;
291 combined effect: $p < 0.0001$; Fig. 3B, Table 2). Eye region temperature in captive birds was higher
292 after the food restriction compared to before (Before: $20.0 \pm 0.3^{\circ}\text{C}$; After: $20.8 \pm 0.3^{\circ}\text{C}$, $p = 0.04652$,
293 Fig. 3C, Table 2). For the wild study, eye region temperature was significantly lower after the food
294 restriction compared to before (Before: 27.6 ± 0.3 , During: 27.0 ± 0.2 , After: 26.7 ± 0.2 ; combined
295 effect: $p = 0.0023$; Fig. 3D, Table2).

296

297

298

299

300

301 **Fig. 3. Bill and eye region temperature before, during and after food restriction for wild and captive**
302 **great tits.** Only food-restricted days are shown. The wild study is confined to 1.5 hours from the end of the
303 food restriction to maintain a similar timeframe as in the captive study. Boxes are first and third quartiles and
304 whiskers extend to lowest and highest observation within 1.5 times the interquartile range. Observations
305 outside of this range are shown as solid circles. The mean value is indicated by a cross on each box.
306 Significance values are from Tukey HSD. Significance is indicated by brackets with asterisks indicating
307 significance level (* = $p < 0.05$, *** = $p < 0.0001$). Sample size above each plot indicates the number of images
308 used. The number of individual birds in the treatment groups for the wild were, 11 before food-restriction, 17
309 during food-restriction and 9 after food-restriction. In the captive experiment, 15 individuals were measured
310 before food-restriction and 17 after food-restriction.

311

312

313 In the wild study, bill temperature was measured continuously from the start of recording and was
314 found to vary temporally between food restricted and food available days (Fig. 4, Table 2). During
315 food restriction, bill temperature was $1.3 \pm 0.3^{\circ}\text{C}$ below bill temperature on food available days at
316 the corresponding time period when ambient temperature was accounted for (Fig. 4). After the initial
317 decrease, however, the bill temperature of food restricted birds increased throughout the food
318 restriction period and was similar to that in birds on food available days at the end of the observation
319 period, unlike in the captive birds. Before and after food restriction temperatures were, thus, similar
320 for both food restricted and food available days.

321

322

323

324 **Fig. 4. Effects of food restriction on bill temperature for wild great tits.** Food restricted days are shown in
325 blue (n = 126 images, 19 birds) and days where food was available are shown in orange (n = 55 images, 46
326 birds). The smooth curve line and 95% confidence intervals are fitted from locally estimated scatterplot
327 smoothing. The grey shaded region indicates the food restriction period (variation in start and end time
328 between days was < 15 min).

329

330

331 Eye region temperature in the wild study was not significantly influenced by food restriction (Fig. 5,
332 Table 2), and the 95% confidence intervals overlapped between food restricted and food available
333 days throughout the experiment. There was a general decrease in eye temperature throughout the
334 experiment, however, as this was true for both food restricted and food available days, this trend was
335 not driven by the food restriction event.

336

337

338

339

340 **Fig. 5. Effects of food restriction on eye temperature for wild great tits.** Food restricted days are shown
341 in blue (n = 126 images, 19 birds) and days where food was available are shown in orange (n = 55 images, 46
342 birds). The smooth curve line and the 95% confidence intervals are fitted from locally estimated scatterplot
343 smoothing. The grey shaded region indicates the food restriction period (variation in start and end time
344 between days was < 15 min).

345

346

347

348 **Table 2. Model outputs of bill temperature for wild and captive great tits.** For the captive study, filming
349 occurred before and after a food restriction event and two consecutive days before the food restriction day
350 (included in the control group) (see methods section). The models used are described in the table with the
351 response variable and fixed effects (all models were mixed effects and details of random effect can be found
352 in the methods). Interactions are represented by “x” between variables. Estimates are the change in the
353 response variable (i.e., surface temperature) per unit increase in the parameter, or for categorical variables,
354 per unit increase when the baseline equals zero. Baseline levels for categorical variables are indicated by ^a.
355 For interactions, the estimates give the change in slope from the regression of the response for each
356 treatment level compared to the baseline treatment level.

357

358

	Model	Parameter	Estimate	SE	F-value	d.f.	P	
Bill temperature	Captive $T_{bill} \sim T_{air} +$ treatment category + position index	Intercept	-0.12	1.42	220.51	1, 130	<0.0001	
		Air temperature	0.83	0.08	142.83	1, 130	<0.0001	
		Treatment: Before ^a / after food restriction	Before: 4.32 ± 0.39 After: 6.11 ± 0.45	1.79	0.50	14.69	1, 130	0.0008
		Position index	0.32	0.06	30.39	1, 130	<0.0001	
	Wild	Intercept	7.26	0.88	5055.80	1, 61	<0.0001	
		Air temperature	0.62	0.09	106.38	1, 61	<0.0001	
		Treatment: Before ^a / during/ after food restriction	Before: 14.01 ± 0.28 During: 12.71 ± 0.22 After: 13.92 ± 0.27	(During) -1.20 (After) -0.09	(During) 0.31 (After) 0.35	20.64	1, 61	<0.0001
		Position index	0.17	0.05	9.69	1, 61	0.0028	
		Wild	Intercept	24.67	7.43	6708.43	68	<0.0001
	$T_{bill} \sim T_{air} +$ treatment category + time + position index + treatment category x time + treatment category x time ²	Air temperature	0.42	0.05	107.25	1, 68	<0.0001	
		Treatment: food restricted ^a / food available day	-6.88	15.43	3.31	1, 68	0.0731	

Time of day	-3.31	1.29	0.01	68	0.9177
Position index	0.23	0.05	24.31	1,68	<0.0001
Treatment x Time of day	1.63	2.72	3.11	1,68	0.0823
Treatment x Time of day ²	(Food restricted) 0.15 (Food available) 0.07	0.06 0.1	3.78	2,68	0.0279

Eye region temperature

Captive $T_{eye} \sim T_{air} + \text{treatment category} + \text{position index}$	Intercept	19.42	1.07	6117.29	1, 107	<0.0001	
	Air temperature	0.49	0.06	78.66	1, 107	<0.0001	
	Treatment: Before ^a / after food restriction	Before: 20.03 ± 0.29 After: 20.81 ± 0.34	0.78	0.37	5.52	1, 107	0.04652
	Position index	0.10	0.04	5.08	1, 107	0.02868	
Wild $T_{eye} \sim T_{air} + \text{treatment category} + \text{position index}$	Intercept	22.25	0.90	40586.53	1, 61	<0.0001	
	Air temperature	0.44	0.08	42.31	1, 61	<0.0001	
	Treatment: Before ^a / during/ after food restriction	Before: 27.61 ± 0.26 During: 26.97 ± 0.18 After: 26.69 ± 0.24	(During) -0.64 (After) -0.92	(During) 0.32 (After) 0.36	6.74	1, 61	0.0023

	Position index	0.16	0.06	7.67	1, 68	0.0074
Wild	Intercept	20.97	7.5	38927.14	1, 68	<0.0001
	Air temperature	0.1	0.05	5	1, 68	0.0286
	Treatment: food restricted ^a / food available day	31.66	14.78	1.53	1, 68	0.22
	Time of day	0.35	1.3	2.19	1, 68	0.1434
	Position index	0.25	0.05	24.15	1, 68	<0.0001
	Treatment x Time of day	-5.5	2.61	0.27	1, 68	0.6062
	Treatment x Time of day ²	(Food restricted) -0.02 (Food available) 0.22	0.06 0.1	2.52	2, 68	0.088

$$T_{eye} \sim T_{air} + \text{treatment category} + \text{time} + \text{position index} + \text{treatment category} \times \text{time} + \text{treatment category} \times \text{time}^2$$

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363 Discussion

364 We found that the bill temperature of **free-ranging** great tits decreased significantly during periods of
 365 food restriction compared to periods when supplemented food was available to birds. As bill
 366 temperature returned to before-food-restriction temperature (or higher, in the case of the captive
 367 birds) on food available days, we are confident that the reduction in bill temperature was a direct
 368 response to the removal of a reliable food source. The relative immediacy (the lowest temperatures

369 occurs in less than an hour from the beginning of the restriction) of the reduction in bill temperature
370 indicates control of vasoconstriction by the bird, rather than reductions in temperature due to lower
371 metabolic heat production as a result of the lack of food. This is suggestive of a cautionary measure,
372 as an autonomic response, to minimize subsequent energetic shortfalls, should the lack of food
373 persist. The putative mechanism, constriction of the blood vessels that supply the bill (cf., Midtgård,
374 1984), reduces the tissue-skin gradient and hence heat loss rate. Tattersall et al., (2017) suggest that
375 small birds are disproportionately more affected by heat loss from uninsulated regions compared to
376 larger birds. Therefore, vasoconstriction of the bill is likely an important energy-saving response for
377 small passerines in cold environments.

378

379 Conversely, we found no difference in eye region temperature when wild birds were food restricted
380 compared to periods when food was available. This suggests that the bill temperature response was
381 caused by local vasoconstriction, and not by reduced circulation to the entire head region. A possible
382 cause for maintaining eye region temperature could be the close proximity of the eye to the brain,
383 which must receive a continuous supply of warm blood to maintain function. Likewise, steady, high,
384 temperature in the eye region is likely of value for visual acuity, and hence beneficial for maintained
385 foraging efficiency in a visually guided bird such as the great tit. The relatively long duration the bill
386 was at a lower temperature on food restricted days compared to food available days indicates that
387 vasoconstriction of the bill was not driven by an acute stress response triggered by the experiment. If
388 so, we would have expected to see a considerably faster return to before-food restriction values than
389 in this study, based on the timeline of the thermal response to an acute stressor in periorbital skin in
390 the closely related blue tit (*Cyanistes caeruleus*) (Jerem et al., 2019). This provides evidence for
391 selective vasoconstriction of the bill as opposed to a global drop in peripheral temperature as is
392 expected in response to an acute stressor (e.g., Herborn et al., 2015; Nord and Folkow, 2019;
393 Robertson et al., 2020).

394

395 The blood supply to the bill must also serve some purpose in functionality, or else it would remain
396 permanently low when the bird is below the thermoneutral zone, even when food is plentiful. It
397 follows that even though vasoconstriction of the bill is likely reflecting a first major defence against
398 energetic shortfalls, it is conceivable that the bird will act to minimise periods of reduced bill
399 function. This could explain why, in the wild, bill temperature gradually increased throughout the
400 food restriction period following the initial drop. This gradual increase in temperature throughout the
401 food restriction may, in part, be through increased activity as birds tried to locate, and potentially
402 ingested, alternative food sources. This is supported by surface temperature increases seen in non-

403 manipulated wild birds throughout the morning, likely from activity-generated heat. **Though no**
404 **filming occurred during the food restriction in the captive study, the significantly higher bill and eye**
405 **temperatures in these birds after the food restriction, compared to before, is likely due to increased**
406 **activity and/or metabolic heat production when re-fed (Zhou and Yamamoto, 1997).**

407

408 Bill and eye temperature of wild and captive great tits decreased with air temperature, which we
409 believe was largely due to greater heat loss to the environment. Similar trends have been observed in
410 other studies of birds at varying environmental temperatures (McCafferty et al., 2011; Robinson et
411 al., 1976; Tattersall et al., 2016). It is important to note the effect of air temperature on body surface
412 temperature occurred regardless of whether food was being restricted at the time or not. Our data,
413 and those of other studies, highlight the role of the bill in thermoregulation. Under low ambient
414 temperatures, heat loss through the bill is reduced by vasoconstriction; conversely, at high ambient
415 temperatures there is increased circulation to the bill to facilitate heat loss (Tattersall et al., 2009;
416 Wolf and Walsberg, 1996). This thermoregulatory role of the bill, consolidated by our data, should
417 be taken into account when interpreting recently described adaptive changes in bill size, notably in
418 great tits (Bosse et al., 2017; Danner and Greenberg, 2015; Friedman et al., 2017; Symonds and
419 Tattersall, 2010; **Tattersall et al., 2017**).

420

421 *Conclusion*

422 We have shown the bill plays a key role in the thermoregulatory response to a sudden drop in food
423 availability in wild passerines. **This is probably a pre-emptive response by the bird to prevent future**
424 **energetic shortfalls by immediately reducing thermoregulatory costs.** In addition, our results also
425 suggest that the level of vasoconstriction is flexible, as bill temperature increased throughout the
426 food restriction, possibly through active control to allow resumed functionality of the bill, or through
427 increased activity to locate alternate food sources. This study gives novel insight into the
428 thermoregulatory responses of birds to meet immediate changes to prospects of energy acquisition.

429

430

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432

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438

439 **Competing interests**

440

441 The authors declare no competing or financial interests.

442

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Bx1

33.0°C

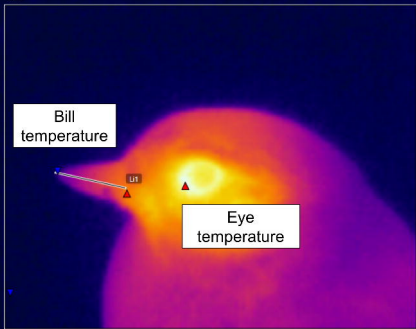
Bill
temperature

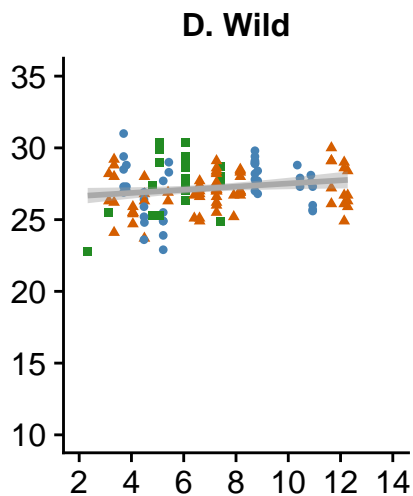
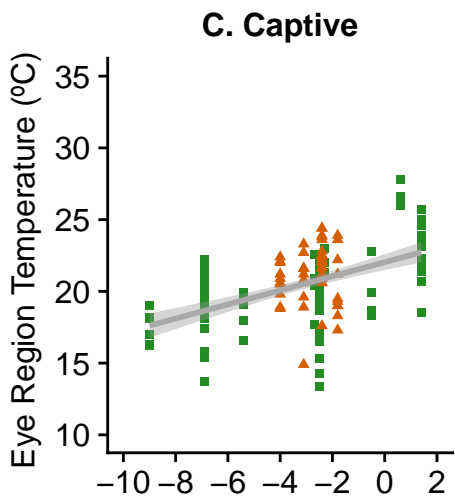
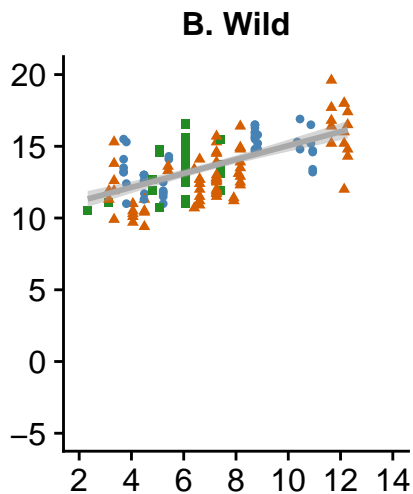
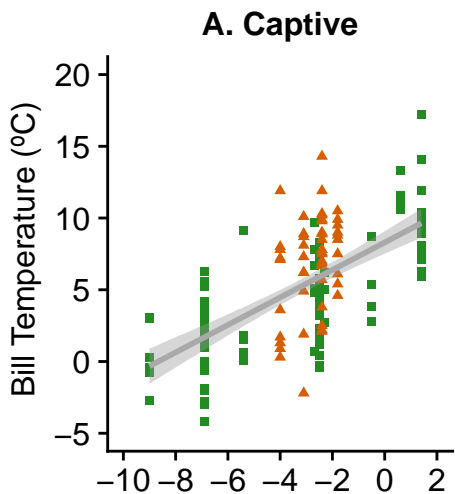


Eye
temperature



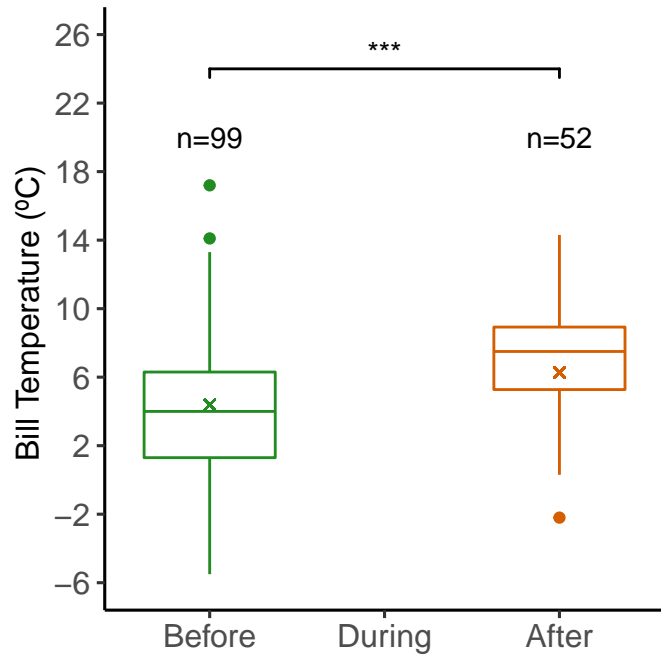
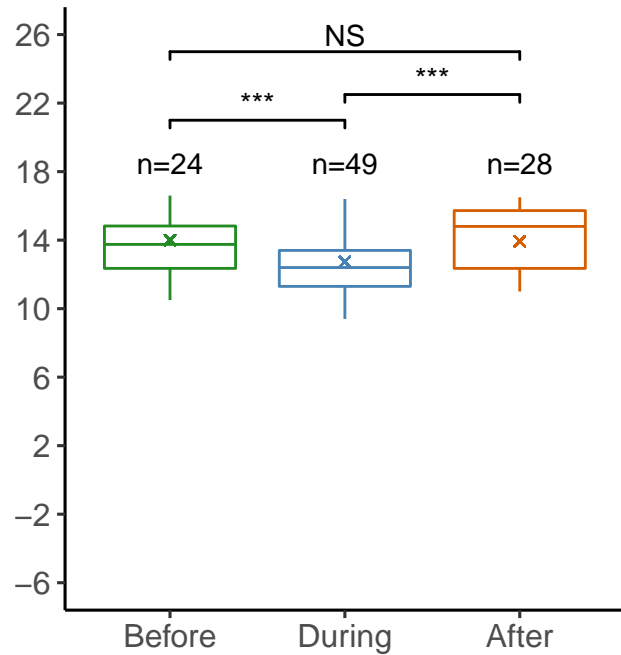
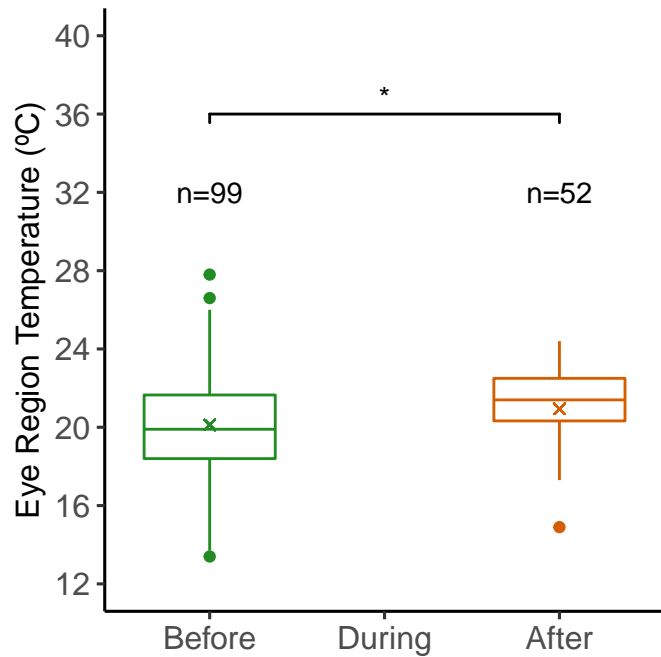
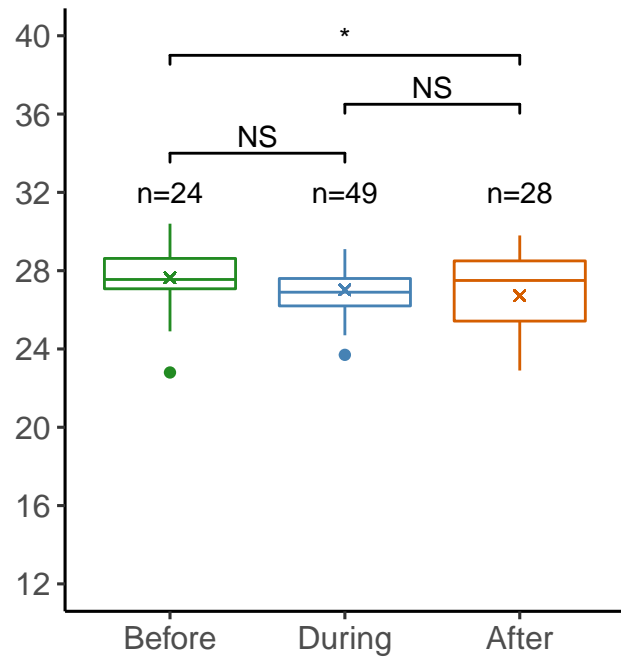
12.0°C





- Before
- During
- ▲ After

Air Temperature (°C)

A. Captive**B. Wild****C. Captive****D. Wild**

Food Restriction Treatment

