



**HAL**  
open science

# Changes in surface temperatures reveal the thermal challenge associated with catastrophic moult in captive Gentoo penguins

Agnès Lewden, Tristan Halna Du Fretay, Antoine Stier

► **To cite this version:**

Agnès Lewden, Tristan Halna Du Fretay, Antoine Stier. Changes in surface temperatures reveal the thermal challenge associated with catastrophic moult in captive Gentoo penguins. 2024. hal-04628012v1

**HAL Id: hal-04628012**

**<https://hal.science/hal-04628012v1>**

Preprint submitted on 29 Jan 2024 (v1), last revised 28 Jun 2024 (v2)

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

# Changes in surface temperatures reveal the thermal challenge associated with catastrophic moult in captive Gentoo penguins

Agnès Lewden<sup>1†</sup>, Tristan Halna du Fretay<sup>1</sup>, Antoine Stier<sup>2,3†</sup>

<sup>1</sup> Université de Brest - UMR 6539 CNRS/UBO/IRD/Ifremer, Laboratoire des sciences de

l'environnement marin - IUEM - Rue Dumont D'Urville - 29280 - Plouzané

<sup>2</sup> Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France

<sup>3</sup> Department of Biology, University of Turku, Turku, Finland

<sup>†</sup>: corresponding authors: [agnes.lewden@univ-brest.fr](mailto:agnes.lewden@univ-brest.fr)

<sup>†</sup>: corresponding authors: [anstier@utu.fi](mailto:anstier@utu.fi)

35 **Abstract**

36 Once a year, penguins undergo a catastrophic moult replacing their entire plumage during a  
37 fasting period on land or on sea-ice during which time individuals can lose 45% of their body  
38 mass. In penguins, new feather synthesis precedes the loss of old feathers leading to an  
39 accumulation of two feathers layers (double coat) before the old plumage is shed. We  
40 hypothesize that the combination of the high metabolism required for new feathers synthesis  
41 and the potentially high thermal insulation linked to the double coat could lead to a thermal  
42 challenge requiring additional peripheral circulation to thermal windows to dissipate extra-  
43 heat. To test this hypothesis, we measured the surface temperature of different body regions  
44 of captive Gentoo penguins (*Pygoscelis papua*) throughout the moult under constant  
45 environmental conditions.

46 The surface temperature of the main body trunk decreased during the initial stages of the  
47 moult, therefore suggesting a higher thermal insulation. On the opposite, the periorbital  
48 region, a potential proxy of core temperature in birds, increased during these same early  
49 moulting stages. The surface temperature of bill, flipper and foot (thermal windows) tended  
50 to initially increase during the moult period, highlighting the likely need for extra heat  
51 dissipation in moulting penguins. These results raise questions regarding the thermoregulatory  
52 capacities of wild penguins during the challenging period of moulting on land in the current  
53 context of global warming.

54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67

68 **Keywords:** Thermal challenge - Moult – Thermoregulation – Penguin – Global warming

## 69 Introduction

70

71 In birds, feathers have many functions including flight, thermal insulation,  
72 communication (with plumage coloration; e.g. Bortolotti 2006) as well as tactile sensation  
73 (Cunnighams et al. 2011). Plumage provides thermal insulation for endothermic birds helping  
74 them to maintain a high core body temperature (Prinzinger et al. 1991). Indeed, the feather  
75 layers trap air above the skin (Dawson et al. 1999) and plumage color and microstructure of  
76 plumage elements (Wolf and Walsberg 2000) reduce conductive, convective and radiative heat  
77 loss between bird and the outside environment (e.g. Calder and King 1974; Bakken 1976; Wolf  
78 and Walsberg 2000). This is especially true in aquatic birds such as penguins that show a high  
79 density of downy and contour-feathers increasing the water resistance (Pap et al. 2017; Osváth  
80 et al. 2018). Penguins have a thick and morphologically specialized plumage (Rutschke 1965;  
81 Williams et al. 2015) providing 80-90% of insulation requirements (Le Maho et al. 1976; Le  
82 Maho 1977) that enable them to exist in the harshest climates of Antarctica. It is therefore  
83 important that penguins are able to maintain high quality plumage (Jenni and Winkler 2020),  
84 through the moult: a replacement of the old and damaged feathers by new ones (Humphrey  
85 and Parkes 1959).

86 The moult of penguins is described as “catastrophic” (Davis and Darby 2012) and occurs  
87 once a year during a fasting period on land or on sea-ice where the heat conductance of air is  
88 25 times lower than that of water (de Vries and van Eerden 1995). During this time, individuals  
89 replace their entire plumage in two overlapping stages with the synthesis of new feathers  
90 preceding the loss of old feathers (Groscolas and Cherel 1992; Fig. 1A). New feathers begin to  
91 grow under the skin until they reach 40% of their size, when they emerge through the skin.  
92 Between 40% and 60% of the new feather growth, the old feathers remain attached to the  
93 new feathers and at this stage birds simultaneously have two feather layers (Fig. 1A). The old  
94 feathers then fall off, reducing thermal insulation until the new feathers finish growing  
95 (Groscolas and Cherel 1992; Fig. 1A). Moult is an energetically costly period for penguins  
96 (Croxall 1982; Adams and Brown 1990), despite their low level of activity while fasting on land  
97 (Cherel et al. 1994). Indeed, the metabolic rate increases by a factor of 1.3 and 1.5 in king  
98 penguins (*Aptenodytes patagonicus*; Cherel et al. 1994) and in little penguins (*Eudyptula*  
99 *minor*; Baudinette et al. 1986) respectively. During this fasting period, macaroni (*Eudyptes*  
100 *chrysolophus*) and rockhopper penguins (*E. chrysocome*) lose 44% and 45% of their body mass

101 respectively during a 25-day moult period (Brown 1986). Similarly king and emperor penguins  
102 (*Aptenodytes forsteri*) lose approximately 45% of their body mass in 30 days with a peak of  
103 daily body mass loss during the final stage of feather loss (Groscolas 1978; Cherel et al. 1988).

104 While Groscolas and Cherel (1992) suggested a decrease in thermal insulation during  
105 the loss of old feathers, the preceding overlay of the new and the old feathers could increase  
106 the overall thermal insulation of plumage. Metabolic heat production increases during the  
107 moult due to feather synthesis and increased peripheral blood flow to grow the feather. During  
108 the earlier stages of the moult, when penguins may have a greater thermal insulation due to  
109 the double layer of feathers, penguins may face a thermal challenge (Fig. 1A) by being unable  
110 to efficiently dissipate metabolic heat, potentially leading to a rise in body temperature. To  
111 investigate this hypothesis, we measured surface temperatures of captive Gentoo penguins  
112 (*Pygoscelis papua*) using thermal imaging during the entire moulting period. The captive  
113 conditions allowed us to measure individuals throughout the full moult period at a uniform air  
114 temperature without interferences of solar radiation, wind or precipitations. We measured  
115 surface temperature of old and new plumage to represent well-insulated body regions,  
116 periorbital region as a proxy of core temperature (e.g. Gauchet et al. 2022), while surface  
117 temperature of bill, flipper and the foot correspond to thermal windows (i.e. poorly insulated  
118 body areas governed by a vascular system that controls their surface temperature; Tattersall  
119 et al. 2009; McCafferty et al. 2013; Lewden et al. 2020). Specifically, we predicted that when  
120 penguins possessed two simultaneous feather layers (moulting stages M2 to M5; Fig. 1) there  
121 would be a decrease of plumage surface temperature, an increase in surface temperatures of  
122 thermal windows and a potential rise in the temperature of the periorbital region.

123

## 124 **Material and Methods**

125

### 126 *Study site*

127

128 Twenty-seven Gentoo penguins were studied in captivity at Océanopolis© aquarium, Brest,  
129 France. Individuals were identified by a colored plastic ring on the right flipper, and divided  
130 into two groups, the first of 18 individuals (7 males and 11 females) and the second of 13  
131 individuals (6 males and 7 females). Individuals were maintained within their thermoneutral  
132 zone, that is between 8 and 15°C in Gentoo penguins (Taylor 1985; Wilson et al. 1998), in two  
133 separate enclosures with similar conditions, *i.e.* a permanent access to free water, unfed

134 during moulting period and with the same number of enclosure cleaning and animal keeper  
135 visits. During measurement sessions, air temperature ( $T_a$ ) and relative humidity (RH) were  
136 measured using a weather station Kestrel® 5400 Heat Stress Tracker. The wet-bulb  
137 temperature ( $T_w$ ) was then calculated according to the equation (1) in Stull 2011, to take into  
138 account the cooling effect of higher humidity. Enclosures showed a relatively stable  $T_w$  during  
139 the study period (from July, 30<sup>th</sup> to October, 20<sup>th</sup>) with a range of temperature between 7.20  
140 and 12.56°C in the first group (Group 1) and between 9.19°C and 14.20°C in the second group  
141 (Group 2). However, we measured a small but significant difference between  
142 groups/enclosures with a higher  $T_w$  in Group 2 (mean  $\pm$  standard error of  $10.82 \pm 0.39^\circ\text{C}$ )  
143 compared to Group 1 (mean  $\pm$  standard error of  $9.38 \pm 0.28^\circ\text{C}$ ) ( $P < 0.005$ ). Similarly, the ground  
144 surface temperature ( $T_{\text{ground}}$ ) in contact with penguin's feet was higher in Group 2 (mean  $\pm$   
145 standard error of  $14.65 \pm 0.13^\circ\text{C}$ ) compared to Group 1 (mean  $\pm$  standard error of  $12.93 \pm$   
146  $0.09^\circ\text{C}$ ) ( $P < 0.0001$ ). Moulting lasted  $14.0 \pm 0.66$  days per individual in Group 1 and  $12.8 \pm 0.97$   
147 days per individual in Group 2, without significant difference between group ( $P = 0.85$ ).

148

#### 149 *Moult*

150

151 Penguin surface temperatures were measured once a day in the morning, at least twice to a  
152 maximum of 25 times per individuals with a mean of 11.75 measurements per individual. To  
153 track the progress of the moult, 7 moult stages were characterized (Fig. 1B) ranging from a  
154 uniform old-plumage (Fig. 1B; M1) to a uniform new-plumage (Fig. 1B; M7) and assigned by  
155 the same observer (A.L.) during data collection. The intermediate stages were "pop-corn"  
156 during which individuals carried two feather layers giving them a puffy appearance (Fig. 1B;  
157 M2). The "first fall" stage corresponds to the advanced pop-corn stage with the first fall of old  
158 feathers visible (Fig. 1B; M3). The "25%" stage corresponds to the acceleration of old feather  
159 fall, with at least 25% of the trunk having lost its old plumage and thus presenting a new,  
160 immature plumage (Fig. 1B; M4). The "50%" stage corresponds to the peak of the moult, with  
161 50% of the trunk showing two layers of plumage and 50% showing new immature plumage  
162 (Fig. 1B; M5). At the "75%" stage (Fig. 1B; M6), individuals had lost most of their old plumage  
163 and the new plumage, while not yet fully grown, visibly increased in volume.

164

#### 165 *Thermal image collection and analysis*

166

167 One or two thermal pictures of left or/and right profiles were taken per day of measurement  
168 from the same angle (Playà-Montmany and Tattersall 2021) at a distance of *ca.* 1 meter with  
169 each body area being larger than ten times the spot size (Tabh et al. 2021) of 0.65 mm from  
170 our FLIR E96 thermal camera (640x480 pixel). For each bird, profile pictures were defined as  
171 right (ringed side) or left (non-ringed) side. We hypothesized that the ringed flipper might  
172 show a higher surface temperature induced by a light inflammation linked to ring friction.  
173 Emissivity was set to 0.98 (Whittow 1986; Monteih and Unsworth 1990) whereas  $T_a$  and RH  
174 were set for each picture using Flir ThermaCAM Researcher Professional 2.10 software. Bill,  
175 flipper and foot areas were delineated by tracing a polygon around the edge to extract the  
176 mean surface temperature of each area (hereafter  $T_{bill}$ ,  $T_{flipper}$ ,  $T_{foot}$  respectively). Mean surface  
177 temperature of the ground ( $T_{ground}$ ) was extracted using a standard square size (877 pixel<sup>2</sup>)  
178 situated just below the feet. Head was also delineated and the maximum surface temperature  
179 of this area was extracted corresponding to the periorbital region (hereafter  $T_{eye}$ ; Jerem et al.  
180 2018). As the loss of old plumage on the trunk was not symmetrical on both sides, we could  
181 not determine a representative trunk surface temperature from the profile pictures. This is  
182 why, we calculated an index of (hereafter  $T_{trunk}$ ) using the old and the new plumage surface  
183 temperature ( $T_{old\ plumage}$  and  $T_{new\ plumage}$  respectively) according to the percentage of trunk  
184 recovery as following:

$$185 \quad T_{Trunk} = \text{Percent of feather lost} * T_{new\ plumage} \quad (1)$$
$$186 \quad + (1 - \text{Percent of feather lost}) * T_{old\ plumage}$$

187

188  $T_{old\ plumage}$  and  $T_{new\ plumage}$  corresponded to a mean surface temperature of a standard square  
189 size (438 pixel<sup>2</sup>) positioned on a uniform patch of each kind of plumage. When individuals were  
190 in old plumage (M1) in pop-corn (M2) and in first fall (M3) stages,  $T_{trunk}$  corresponded to  $T_{old}$   
191  $plumage$ . When individuals were in new plumage stage (M7)  $T_{trunk}$  corresponded to  $T_{new\ plumage}$ .  
192 For others moulting stages (M4; M5 and M6),  $T_{trunk}$  was calculated using the equation (1)  
193 determined from the cover of the entire trunk circumference to weight the proportion of each  
194 kind of plumage.

### 195 *Statistical analysis*

196  
197  
198 The effect of plumage color (black or white) on old and new plumage surface temperatures  
199 was initially investigated using an ANOVA, but dropped out of final models since as expected

200 in the absence of solar radiation, we did not find any significant difference in surface  
201 temperature between black and white plumage in both old and new plumage ( $P>0.80$  in both  
202 cases). Similarly, we did not measure any significant effect of the ring on  $T_{\text{flipper}}$  and therefore  
203 excluded this variable from our final statistical models ( $P=0.99$ ).

204 The relationship between four temperature areas (*i.e.* bill, periorbital, flipper and trunk) and  
205 the moult stages were studied using general linear mixed models (GLMM), including penguin  
206 ID as a random intercept, to control for repeated measures and  $T_w$ , Group (1 or 2) and Sex  
207 (male or female) as fixed effects. For the  $T_{\text{foot}}$ ,  $T_{\text{ground}}$  replaced  $T_w$  in the model considering the  
208 large surface of foot in contact with the ground (*i.e.* conductive heat loss). Indeed, the linear  
209 relationship between  $T_w$  and  $T_{\text{foot}}$  ( $R^2= 0.02$ ;  $P=0.004$ ) was markedly weaker than the  
210 relationship between  $T_{\text{ground}}$  and  $T_{\text{foot}}$  ( $R^2=0.38$ ;  $P<0.0001$ ).

211 Differences were then investigated using a Tukey's honestly significant difference (HSD) *post-*  
212 *hoc test* for moult stages effect. Statistical analyses were performed using JMP® v. 13 (SAS  
213 Institute Inc., Cary, North Carolina, USA) and results were reported as mean  $\pm$  standard error  
214 unless specified.

215

## 216 **Results**

217

218 Body surface temperatures were positively related to  $T_w$  or  $T_{\text{ground}}$  (Table 1). The variable  
219 'group' was only significant for  $T_{\text{trunk}}$  ( $P<0.0001$ ) with lower  $T_{\text{trunk}}$  measured in the group  
220 exposed to the slightly colder environment (*i.e.* Group 1). Females had a slightly higher  $T_{\text{eye}}$   
221 than males ( $31.02 \pm 0.11$  and  $30.71 \pm 0.10^\circ\text{C}$  respectively;  $P=0.023$ ). Group and sex did not  
222 explain any significant variation in  $T_{\text{bill}}$ ,  $T_{\text{flipper}}$  and  $T_{\text{foot}}$  (Table 1). However, variation in all body  
223 surface temperatures were significantly influenced by the stage of moult (Table 1).

224  $T_{\text{eye}}$  significantly increased from the old plumage to the pop-corn stage, and stayed elevated  
225 until the 50% moult stage (Fig. 2A).  $T_{\text{eye}}$  then decreased back at 75% of the moult (at a level  
226 similar to the old plumage), and ended up being the lowest at the new plumage stage (Fig.  
227 2A).  $T_{\text{trunk}}$  showed an initial drop from the old plumage stage to the First fall stage (Fig. 3), and  
228 then increased back to its initial level as the moult progressed further towards the new  
229 plumage stage (Fig. 2A).  $T_{\text{trunk}}$  at the new plumage stage ( $15.93 \pm 0.14^\circ\text{C}$ ) did not significantly  
230 differ from the old plumage stage ( $16.48 \pm 0.21^\circ\text{C}$ ;  $P=0.15$ ) (Fig. 2A and 3).



231  $T_{\text{flipper}}$  had the highest temperature at stage 25, 50 and 75% of the moult (Fig. 2B).  $T_{\text{flipper}}$  at  
232 those stages were higher than at first fall and new plumage stages (all  $P < 0.04$ ; Fig. 3), but did  
233 not significantly differ from the old plumage and pop-corn stages (all  $P > 0.30$ ).  $T_{\text{flipper}}$  was  $1.77^{\circ}\text{C}$   
234 colder at new plumage stage compared to old plumage stage ( $P = 0.0002$ ; Fig. 3).

235  $T_{\text{bill}}$  significantly increased from the old plumage to first fall stage, and decreased thereafter  
236 (Fig. 2C) with a maximum difference of  $-5.20^{\circ}\text{C}$  between the first fall and the new plumage  
237 stages ( $P < 0.0001$ ; Fig. 3).  $T_{\text{bill}}$  was also lower at the new plumage stage ( $17.13 \pm 0.31^{\circ}\text{C}$ ) than  
238 at the old plumage stage ( $19.92 \pm 0.52^{\circ}\text{C}$ ;  $P < 0.0001$ ).

239  $T_{\text{foot}}$  initially increased from the old plumage until the First fall stage (Fig. 3), and remained  
240 slightly elevated until the end of the monitoring (i.e. new plumage; Fig. 2D). Yet, there was no  
241 significant difference in  $T_{\text{foot}}$  between new plumage ( $19.29 \pm 0.53^{\circ}\text{C}$ ) compared to old plumage  
242 stages ( $17.99 \pm 0.74^{\circ}\text{C}$ ;  $P = 0.62$ ).

243

## 244 Discussion

245

246 Our study investigated the effect of moulting in captive Gentoo penguins, a fasting  
247 period during which metabolic rate increases (Baudinette et al. 1986; Cherel et al. 1994) while  
248 body insulation is heavily modified in penguin species (Groscolas and Cherel 1992). Our results  
249 showed that at early stages of the moult, when individuals have two feather layers (Stages M2,  
250 M3 and M4; Fig. 1 and 2), plumage insulation was elevated as shown by the lowest  $T_{\text{trunk}}$  (Fig.  
251 2 and 3), while the surface temperatures of thermal windows (bill, flipper and foot) and the  
252 periorbital region were generally elevated at these stages (Fig. 2). This effect was maintained  
253 until approximately 50% percent of the moult, after which surface temperatures of non-  
254 insulated body regions started to decrease (Fig. 2 and 3), such that the mean surface  
255 temperatures of new plumage stage were significantly lower (except for  $T_{\text{foot}}$ ) than at old  
256 plumage stage (Fig. 2).

257

258 Early moulting stages in penguins may therefore provide a thermal challenge for birds  
259 to dissipate extra-heat. Moult is energetically costly (Baudinette et al. 1986; Cherel et al. 1994)  
260 through maintaining a peripheral blood flow for dermal perfusion sustaining feather synthesis.  
261 Simultaneously with this higher heat production, we found that the potential for heat  
262 dissipation is likely to be reduced by the additional insulation resulting from the combination

263 of newly growing and old feathers, as shown by the tendency of  $T_{\text{trunk}}$  to decrease at pop-corn  
264 (M2), first fall (M3) and 25% of old feathers fallen (M4) stages (Fig. 2). Correspondingly,  
265 uninsulated or less insulated body areas from the bill, flipper and foot exhibited the opposite  
266 pattern (Fig. 2 and 3) with an increase of surface temperature allowing greater heat loss  
267 through radiation and convection to the surroundings. Within our captive experimental  
268 conditions, birds were measured within their thermoneutral zone (between 8° and 15°C;  
269 Taylor 1985), which theoretically enable to exclude any changes in metabolic rate associated  
270 with thermoregulation. Thus, our study highlights that blood flow to thermal windows may  
271 help to compensate for greater insulation and heat production associated with feather  
272 synthesis, in order to help maintaining a stable core body temperature during early moulting  
273 stages. Since  $T_{\text{eye}}$  is relatively well correlated to core temperature in chicken (Cândido et al.  
274 2020), budgerigars (*Melopsittacus undulates*; Ikkatai and Watanabe 2015) and wild red-footed  
275 boobies (*Sula sula*; Gauchet et al. 2022), our results suggest that core body temperature  
276 increased during the early stage of moult (+1.61°C of  $T_{\text{eye}}$  between old plumage and 25%  
277 stage). This idea is supported by an increase of *ca.* 0.8°C in core temperature during moult in  
278 Yellow-eyed penguin (*Megadyptes antipodes*; Farner 1958). Yet, blood flow to the eyes,  
279 impacting  $T_{\text{eye}}$ , has been also shown to play a role in reducing brain temperature in pigeons  
280 (*Columba livia*; Pinshow et al. 1982), suggested as heat sink in ostriches (*Struthio camelus*;  
281 Fuller et al. 2003) or to maintain/enhance visual acuity during stress (Winder et al. 2020).  
282 Unfortunately, without core body temperature measurements, it is not possible here to  
283 formally assess if the increased heat dissipation through thermal windows we observed was  
284 sufficient to maintain core body temperature at stable levels, or if the observed increase in  $T_{\text{eye}}$   
285 could reflect the inability to maintain core body temperature at stable levels.

286

287 Interestingly, the thermal challenge to dissipate heat described here at  
288 thermoneutrality seems likely to be specific to penguins, due to the accumulation of this  
289 double insulation. Indeed, most studies in birds measured the opposite pattern with an  
290 increase of 30 to 60% in thermal conductance during moult (Lustick 1970; Dietz et al. 1992),  
291 inducing for instance a rise of the lower critical temperature in Long-eared Owl (*Asio otus*;  
292 Wijnandts 1984). In seals, moult represents the renewal not only of the hair but also of the  
293 skin (Ling 1968; 1972). With no accumulation of old and new skin, individuals show an increase  
294 of thermoregulation cost due to higher heat loss (Paterson et al. 2012). In Antarctica Weddell

295 seals (*Leptonychotes weddellii*), Walcott et al. (2020) measured that the energetic costs of  
296 thermoregulation doubled during moulting period, supported by an increase of 25% of heat  
297 loss in early moulting stages. Importantly, no thermal windows were detected in this study  
298 suggesting that individuals were unlikely to overheat (Walcott et al. 2020). Similar results were  
299 obtained in southern elephant seals (*Mirounga leonina*) with an increase of 1.8 x resting  
300 metabolic rate during moult with body surface temperature decreasing throughout the moult  
301 (Paterson et al. 2022), the latter suggesting an energy-saving strategy that seems opposite to  
302 the results obtained in this study in Gentoo penguins maintained within their thermoneutral  
303 zone. Moreover, elephant seals also showed aggregation behavior only during moult on land  
304 suggesting a strategy to reduce heat loss and minimize energy costs (Chaise et al. 2019). In  
305 contrast, penguins have not been shown to exhibit specific aggregation behavior during  
306 moulting to the best of our knowledge.

307 Surface temperatures of new plumage stage did not reach the initial temperature  
308 measured in old plumage and they were either lower ( $T_{\text{bill}}$ ,  $T_{\text{eye}}$  and  $T_{\text{flipper}}$ ) or similar ( $T_{\text{foot}}$ ;  
309  $T_{\text{trunk}}$ ) at the end of the moult. Moult could have started before a visible change in plumage  
310 (i.e. pop-corn stage). In this case, the old plumage stage (M1) could already correspond to an  
311 early stage of moult (i.e. growth of new feathers below the skin, Fig. 1), with a higher  
312 metabolism, rather than a pre-moult stage as initially considered in this study. Secondly, the  
313 new plumage stage (M7) corresponds here to the end of the old feather loss and potentially  
314 not to the end of the new feather growth. The immature new feathers could be less insulated  
315 than the full-length feathers, allowing heat loss without a specific need to maintain blood flow  
316 to thermal windows. Finally, since moulting progression is also associated with the progression  
317 into a more advanced fasting stage, it is possible that individuals reaching the new plumage  
318 stage could use peripheral vasoconstriction as an energy-saving strategy (Kooyman et al. 1976;  
319 Ponganis et al. 2001, 2003; Tattersall et al. 2016) which could also explain the decrease we  
320 observed in  $T_{\text{bill}}$ ,  $T_{\text{eye}}$  and  $T_{\text{flipper}}$ .

321 Our study showed that under nearly constant environmental conditions, moulting  
322 Gentoo penguins increase the surface temperature of poorly insulated regions (thermal  
323 windows) (Fig. 2 and 3) to dissipate extra heat. In the wild, the thermal challenge of carrying  
324 two feather layers could be enhanced by the solar radiation and the increase of air  
325 temperature in the current context of global warming (Ainley et al. 2010; Gorodetskaya et al.  
326 2022). Further studies are needed to better understand the operative temperature (Bakken

327 1976) experienced by wild birds, especially in polar marine species showing adaptations to  
328 reduce heat loss while foraging in cold water.

329

### 330 **Data availability statement**

Attribution CC-BY-NC-ND



331 Anyone can share this material, provided it remains unaltered in any way, this  
332 is not done for commercial purposes, and the original authors are credited and cited.

333 <https://figshare.com/s/c64975557480cc8709f3>

334

### 335 **Acknowledgments**

336

337 We are grateful to Oceanopolis© for providing logistical support and giving us the access the  
338 animals. We would like to thank Christine Dumas, Alexiane Corcuff, Maxence Leroy, Mélanie  
339 Robert and Agathe Lefranc for their help during experiment. We sincerely thank Dominic  
340 McCafferty for his valuable comments improving a previous version of the manuscript. AL was  
341 supported by ISblue project, Interdisciplinary graduate school for the blue planet (ANR-17-  
342 EURE-0015) and co-funded by a grant from the French government under the program  
343 "Investissements d'Avenir" embedded in France 2030. AS was financially supported by the  
344 CNRS and the IdEx Université de Strasbourg.

345

## 346 References

- 347 Adams, N. J., & Brown, C. R. (1990). Energetics of molt in penguins. *Penguin biology*, 297-315.
- 348 Ainley, D., Russell, J., Jenouvrier, S., Woehler, E., Lyver, P. O. B., Fraser, W. R., & Kooyman, G. L.  
349 (2010). Antarctic penguin response to habitat change as Earth's troposphere reaches 2 C above  
350 preindustrial levels. *Ecological Monographs*, 80(1), 49-66.
- 351 Bakken, G. S. (1976). A heat transfer analysis of animals: unifying concepts and the application  
352 of metabolism chamber data to field ecology. *Journal of theoretical Biology*, 60(2), 337-384.
- 353 Barbraud, C., Rolland, V., Jenouvrier, S., Nevoux, M., Delord, K., & Weimerskirch, H. (2012).  
354 Effects of climate change and fisheries bycatch on Southern Ocean seabirds: a review. *Marine*  
355 *Ecology Progress Series*, 454, 285-307.
- 356 Bricher, P. K., Lucieer, A., & Woehler, E. J. (2008). Population trends of Adélie penguin  
357 (*Pygoscelis adeliae*) breeding colonies: a spatial analysis of the effects of snow accumulation  
358 and human activities. *Polar Biology*, 31, 1397-1407.
- 359 Brown, C. R. (1986). Feather growth, mass loss and duration of moult in macaroni and rock-  
360 hopper penguins. *Ostrich*, 57(3), 180-184.
- 361 Baudinette, R. V., Gill, P., & O'driscoll, M. (1986). Energetics of the Little Penguin, *Eudyptula*  
362 *minor*: temperature regulation, the calorogenic effect of food, and moulting. *Australian Journal*  
363 *of Zoology*, 34(1), 35-45.
- 364 Bortolotti, G.R. (2006) Natural selection and coloration: protection, concealment,  
365 advertisement, or deception ? Pp 3-35 in G.E. HILL & K. J. MCGRAW (eds): *Birds Colorations*.  
366 Vol 2. Harvard University Press, Cambridge, Massachusetts.
- 367 Calder, W. (1974). Thermal and caloric relations of birds. *Avian biology*, 4, 259-413.
- 368 Cândido, M. G. L., Tinôco, I. F. F., Albino, L. F. T., Freitas, L. C. S. R., Santos, T. C., Cecon, P. R., &  
369 Gates, R. S. (2020). Effects of heat stress on pullet cloacal and body temperature. *Poultry*  
370 *science*, 99(5), 2469-2477.
- 371 Chaise, L. L., McCafferty, D. J., Krellenstein, A., Gallon, S. L., Paterson, W. D., Théry, M., ... &  
372 Gilbert, C. (2019). Environmental and physiological determinants of huddling behavior of  
373 molting female southern elephant seals (*Mirounga leonina*). *Physiology & behavior*, 199, 182-  
374 190.
- 375 Cherel, Y., Leloup, J., & Le Maho, Y. (1988). Fasting in king penguin. II. Hormonal and metabolic  
376 changes during molt. *American Journal of Physiology-Regulatory, Integrative and Comparative*  
377 *Physiology*, 254(2), R178-R184
- 378 Cherel, Y., Charrassin, J., & Challet, E. (1994). Energy and protein requirements for molt in the  
379 king penguin *Aptenodytes patagonicus*. *American Journal of Physiology-Regulatory,*  
380 *Integrative and Comparative Physiology*, 266(4), R1182-R1188.

- 381 Croxall, J. P. (1982). Energy costs of incubation and moult in petrels and penguins. *The Journal*  
382 *of Animal Ecology*, 177-194.
- 383 Cunningham, S. J., Alley, M. R., & Castro, I. (2011). Facial bristle feather histology and  
384 morphology in New Zealand birds: implications for function. *Journal of morphology*, 272(1),  
385 118-128.
- 386 Davis, L. S., & Darby, J. T. (Eds.). (2012). *Penguin biology*. Elsevier.
- 387 Dawson, C., Vincent, J. F., Jeronimidis, G., Rice, G., & Forshaw, P. (1999). Heat transfer through  
388 penguin feathers. *Journal of theoretical biology*, 199(3), 291-295.
- 389 Deppeler, S. L., & Davidson, A. T. (2017). Southern Ocean phytoplankton in a changing  
390 climate. *Frontiers in Marine Science*, 4, 40.
- 391 Descamps, S., Hudson, S., Sulich, J., Wakefield, E., Grémillet, D., Carravieri, A., ... & Steen, H.  
392 (2023). Extreme snowstorms lead to large-scale seabird breeding failures in Antarctica. *Current*  
393 *Biology*, 33(5), R176-R177.
- 394 Emmerson, L., & Southwell, C. (2008). Sea ice cover and its influence on Adelie penguin  
395 reproductive performance. *Ecology*, 89, 2096 – 2102. <https://doi.org/10.1890/08-0011.1>
- 396 Fuller, A., Kamerman, P. R., Maloney, S. K., Mitchell, G., & Mitchell, D. (2003). Variability in brain  
397 and arterial blood temperatures in free-ranging ostriches in their natural habitat. *Journal of*  
398 *experimental biology*, 206(7), 1171-1181.
- 399 Gorman, K. B., Ruck, K. E., Williams, T. D., & Fraser, W. R. (2021). Advancing the Sea Ice  
400 Hypothesis: Trophic interactions among breeding Pygoscelis penguins with divergent  
401 population trends throughout the Western Antarctic Peninsula. *Frontiers in Marine Science*, 8,  
402 526092.
- 403 Gorodetskaya, I. V., Durán-Alarcón, C., González-Herrero, S., Clem, K. R., Zou, X., Rowe, P., ... &  
404 Picard, G. (2023). Record-high Antarctic Peninsula temperatures and surface melt in February  
405 2022: a compound event with an intense atmospheric river. *npj climate and atmospheric*  
406 *science*, 6(1), 202.
- 407 Groscolas, R. (1978). Study of molt pasting followed by an experimental forced fasting in the  
408 emperor penguin *Aptenodytes forsteri*: relationship between feather growth, body weight  
409 loss, body temperature and plasma fuel levels. *Comparative Biochemistry and Physiology Part*  
410 *A: Physiology*, 61(2), 287-295.
- 411 Groscolas, R., & Cherel, Y. (1992). How to molt while fasting in the cold: the metabolic and  
412 hormonal adaptations of emperor and king penguins. *Ornis Scandinavica*, 328-334.
- 413 Humphrey, P. S., & Parkes, K. C. (1959). An approach to the study of molts and plumages. *The*  
414 *Auk*, 76(1), 1-31.
- 415 Ikkatai, Y., & Watanabe, S. (2015). Eye surface temperature detects stress response in  
416 budgerigars (*Melopsittacus undulatus*). *NeuroReport*, 26(11), 642-646.



- 417 Jenni, L., & Winkler, R. (2020). *The biology of moult in birds*. Bloomsbury Publishing.
- 418 Jerem, P., Jenni-Eiermann, S., Herborn, K., McKeegan, D., McCafferty, D. J., & Nager, R. G.  
419 (2018). Eye region surface temperature reflects both energy reserves and circulating  
420 glucocorticoids in a wild bird. *Scientific reports*, 8(1), 1907.
- 421 Kato, A., Ropert - Coudert, Y., & Naito, Y. (2002). Changes in Adelie penguin breeding  
422 populations in Lutzow - Holm Bay, Antarctica, in relation to sea - ice conditions. *Polar Biology*,  
423 25, 934 – 938.
- 424 Kooyman, G. L., Gentry, R. L., Bergman, W. P., & Hammel, H. T. (1976). Heat loss in penguins  
425 during immersion and compression. *Comparative Biochemistry and Physiology A* 54, 75-80.
- 426 Le Maho, Y., Delclitte, P., & Chatonnet, J. (1976). Thermoregulation in fasting emperor penguins  
427 under natural conditions. *American Journal of Physiology-Legacy Content*, 231(3), 913-922.
- 428 Le Maho, Y. (1977). The Emperor Penguin: A Strategy to Live and Breed in the Cold:  
429 Morphology, physiology, ecology, and behavior distinguish the polar emperor penguin from  
430 other penguin species, particularly from its close relative, the king penguin. *American Scientist*,  
431 65, 680–693
- 432 Lee, D. Y., Petersen, M. R., & Lin, W. (2019). The southern annular mode and southern ocean  
433 surface westerly winds in E3SM. *Earth and Space Science*, 6(12), 2624-2643.
- 434 Lewden, A., Nord, A., Bonnet, B., Chauvet, F., Ancel, A., & McCafferty, D. J. (2020). Body surface  
435 rewarming in fully and partially hypothermic king penguins. *Journal of Comparative Physiology*  
436 *B*, 190(5), 597-609.
- 437 Ling, J. K. (1972). Adaptive functions of vertebrate molting cycles. *American Zoologist*, 12(1),  
438 77-93.
- 439 Ling, J. K. (1968). The skin and hair of the Southern Elephant Seal *Mirounga leonina* (L.) III.  
440 morphology of the adult integument. *Australian Journal of Zoology*, 16(4), 629-645.
- 441 Mccafferty, D. J., Gilbert, C., Thierry, A. M., Currie, J., Le Maho, Y., & Ancel, A. (2013). Emperor  
442 penguin body surfaces cool below air temperature. *Biology letters*, 9(3), 20121192.
- 443 Moreau, S., Boyd, P. W., & Strutton, P. G. (2020). Remote assessment of the fate of  
444 phytoplankton in the Southern Ocean sea-ice zone. *Nature communications*, 11(1), 3108.
- 445 Monteith, J., & Unsworth, M. (2013). *Principles of environmental physics: plants, animals, and*  
446 *the atmosphere*. Academic Press.
- 447 Osváth, G., Daubner, T., Dyke, G., Fuisz, T. I., Nord, A., Péntzes, J., ... & Pap, P. L. (2018). How  
448 feathered are birds? Environment predicts both the mass and density of body  
449 feathers. *Functional Ecology*, 32(3), 701-712.
- 450 Pap, P. L., Vincze, O., Wekerle, B., Daubner, T., Vágási, C. I., Nudds, R. L., ... & Osváth, G. (2017).  
451 A phylogenetic comparative analysis reveals correlations between body feather structure and  
452 habitat. *Functional ecology*, 31(6), 1241-1251.

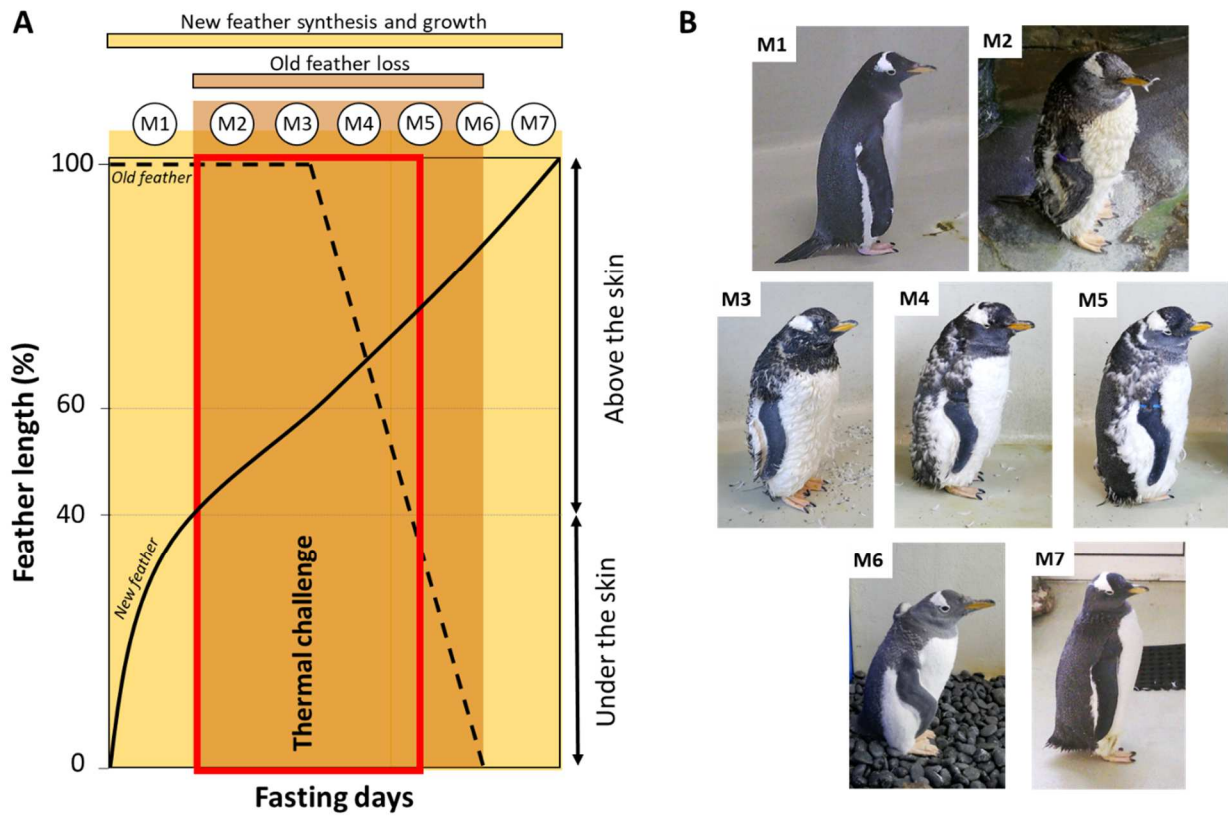
- 453 Paterson, W., Sparling, C. E., Thompson, D., Pomeroy, P. P., Currie, J. I., & McCafferty, D. J.  
454 (2012). Seals like it hot: Changes in surface temperature of harbour seals (*Phoca vitulina*) from  
455 late pregnancy to moult. *Journal of Thermal Biology*, 37(6), 454-461.
- 456 Paterson, W. D., Chaise, L. L., McKnight, C., Currie, J. I., Thompson, D., Ancel, A., ... & Mccafferty,  
457 D. J. (2022). Metabolic heat loss in southern elephant seals (*Mirounga leonina*) differs with  
458 stage of moult and between habitats. *Journal of Thermal Biology*, 104, 103183.
- 459 Pinshow, B., Bernstein, M. H., Lopez, G. E., & Kleinhaus, S. (1982). Regulation of brain  
460 temperature in pigeons: effects of corneal convection. *American Journal of Physiology-  
461 Regulatory, Integrative and Comparative Physiology*, 242(5), R577-R581.
- 462 Playà-Montmany, N., & Tattersall, G. J. (2021). Spot size, distance and emissivity errors in field  
463 applications of infrared thermography. *Methods in Ecology and Evolution*, 12(5), 828-840.
- 464 Prinzinger, R., Preßmar, A., & Schleucher, E. (1991). Body temperature in birds. *Comparative  
465 Biochemistry and Physiology Part A: Physiology*, 99(4), 499-506.
- 466 Ropert-Coudert, Y., Chiaradia, A., Ainley, D., Barbosa, A., Boersma, P. D., Brasso, R., ... &  
467 Trathan, P. N. (2019). Happy feet in a hostile world? The future of penguins depends on  
468 proactive management of current and expected threats. *Frontiers in Marine Science*, 248.
- 469 Ponganis, P. J., van Dam, R. P., Knowler, T., & Levenson, D. H. (2001). Temperature regulation in  
470 emperor penguins foraging under sea ice. *Comparative Biochemistry Physiology A* 129, 811-  
471 820.
- 472 Ponganis, P. J., van Dam, R. P., Levenson, D. H., Knowler, T., Ponganis, K. V., & Marshall, G. (2003).  
473 Regional heterothermy and conservation of core temperature in emperor penguins diving  
474 under sea ice. *Comparative Biochemistry Physiology A* 135, 477-487.
- 475 Rutschke, E. (1965). Beiträge zur morphologie der pinguinfeder. *Zeitschrift für Morphologie  
476 und Ökologie der Tiere*, 55(7), 835-858.
- 477 Tattersall, G. J., Andrade, D. V., & Abe, A. S. (2009). Heat exchange from the toucan bill reveals  
478 a controllable vascular thermal radiator. *science*, 325(5939), 468-470.
- 479 Tattersall, G. J., Roussel, D., Voituron, Y., & Teulier, L. (2016). Novel energy-saving strategies to  
480 multiple stressors in birds: the ultradian regulation of body temperature. *Proceedings of the  
481 Royal Society B: Biological Sciences*, 283(1839), 20161551.
- 482 Trivelpiece, W. Z., Hinke, J. T., Miller, A. K., Reiss, C. S., Trivelpiece, S. G., & Watters, G. M. (2011).  
483 Variability in krill biomass links harvesting and climate warming to penguin population changes  
484 in Antarctica. *Proceedings of the National Academy of Sciences*, 108(18), 7625-7628.
- 485 Turner, J., Phillips, T., Thamban, M., Rahaman, W., Marshall, G. J., Wille, J. D., ... & Lachlan-  
486 Cope, T. (2019). The dominant role of extreme precipitation events in Antarctic snowfall  
487 variability. *Geophysical Research Letters*, 46(6), 3502-3511.



- 488 Walcott, S. M., Kirkham, A. L., & Burns, J. M. (2020). Thermoregulatory costs in molting  
489 Antarctic Weddell seals: impacts of physiological and environmental conditions. *Conservation*  
490 *physiology*, 8(1), coaa022.
- 491 Williams, C. L., Hagelin, J. C., & Kooyman, G. L. (2015). Hidden keys to survival: the type,  
492 density, pattern and functional role of emperor penguin body feathers. *Proceedings of the*  
493 *Royal Society B: Biological Sciences*, 282(1817), 20152033.
- 494 Wilson, R. P., Adelung, D., & Latorre, L. (1998). Radiative heat loss in gentoo penguin  
495 (*Pygoscelis papua*) adults and chicks and the importance of warm feet. *Physiological*  
496 *zoology*, 71(5), 524-533.
- 497 Winder, L. A., White, S. A., Nord, A., Helm, B., & McCafferty, D. J. (2020). Body surface  
498 temperature responses to food restriction in wild and captive great tits. *Journal of*  
499 *Experimental Biology*, 223(8), jeb220046.
- 500 Whittow, G. C. (1986). Regulation of body temperature. In *Avian physiology* (pp. 221-252).  
501 New York, NY: Springer New York.
- 502 Wolf, B. O., & Walsberg, G. E. (2000). The role of the plumage in heat transfer processes of  
503 birds. *American Zoologist*, 40(4), 575-584.
- 504

505

## Figures



506

507

508

509

510

511

512

513

514

515

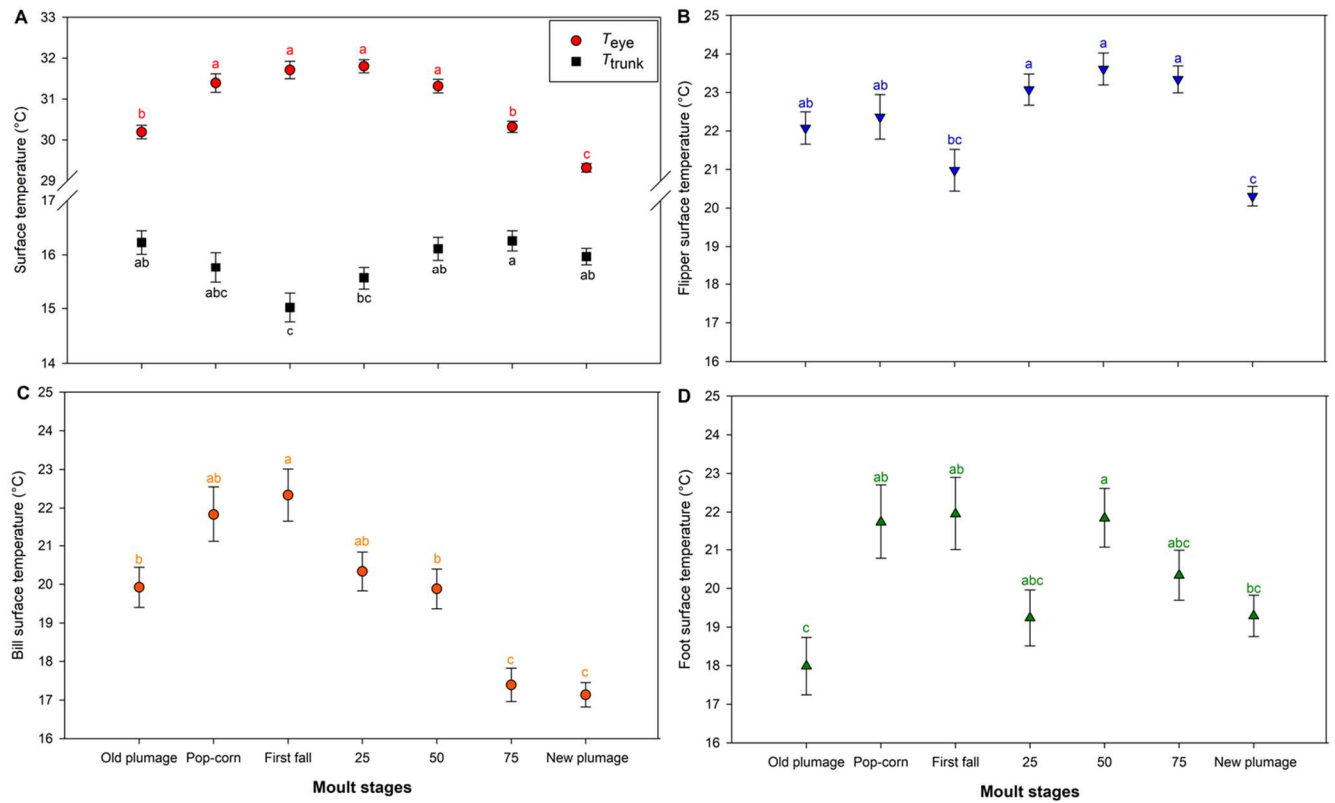
516

517

518

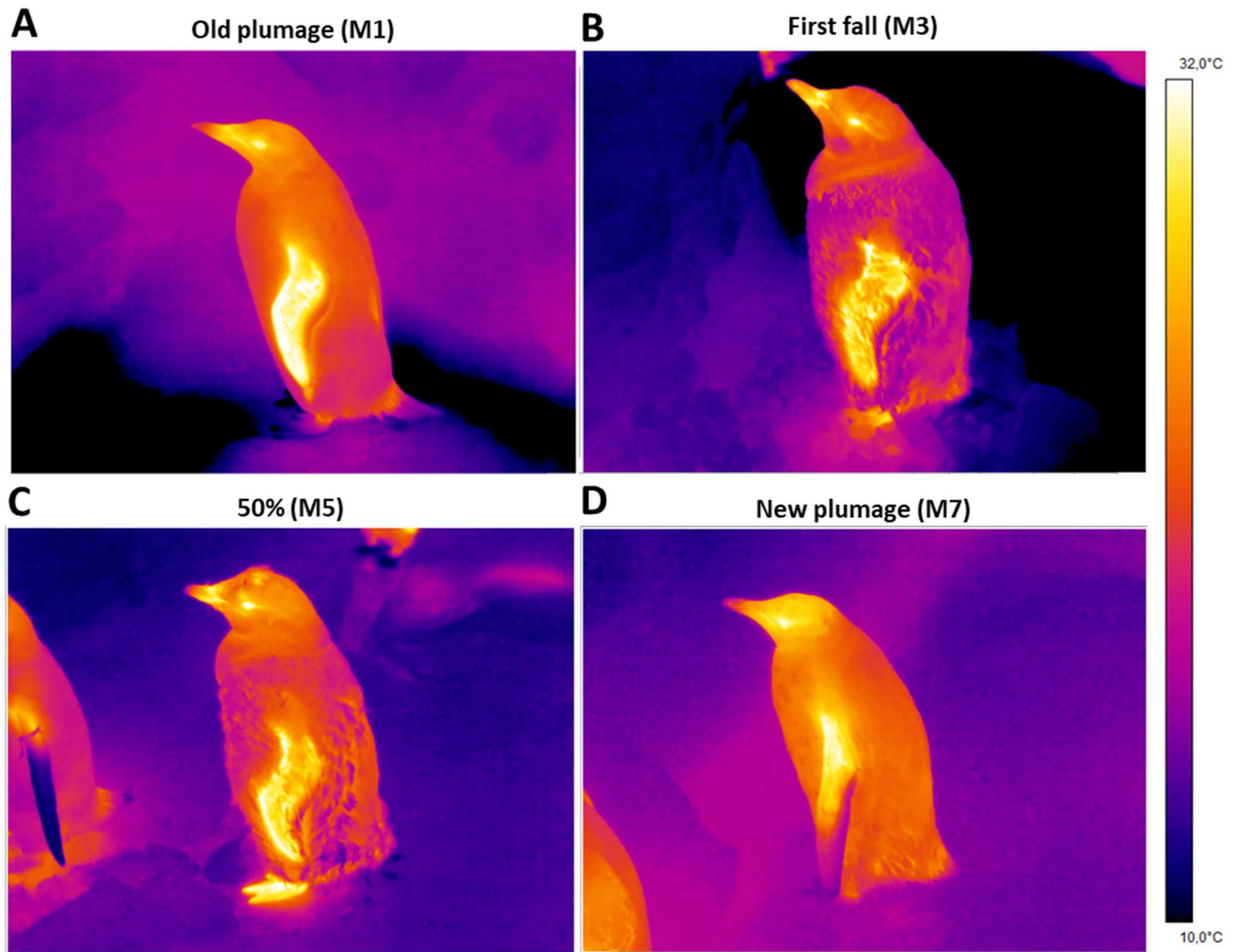
519

**Fig. 1: Identification of the seven main moulting stages (stages M1 to M7) characterized during the Gentoo moult in captivity.** The schematic representation of the penguin moult (**A**) shows the new feathers (straight-line) grow beneath the outer skin layer until 40% of their total length. Between 40 and 60% of new feather growth, penguins have a double feather layer, old (dashed line) and new. These two feather layers could lead to a thermal challenge for heat dissipation (red box). After 60% of new feather growth, the old feathers are starting to fall-off. Adapted from Groscolas and Cherel (1992). During moult, visual plumages changes can be noticed (**B**) and characterized into seven different stages: M1 = uniform old plumage, M2 = 'pop-corn' (superposition of old and new immature feathers), M3 = first fall of old plumage, M4 = 25% of old feathers fallen, M5 = 50% of old feathers fallen, M6 = 75% of old feathers fallen and M7 = uniform new plumage corresponding at the end of the monitoring. See the main text for more details ©A. Lewden



520

521 **Fig. 2: Least-squared mean surface temperatures during the seven moulting stages.** Values  
522 for the periorbital region (red) and the trunk (black) (A), the flipper (B), the bill (C), and the  
523 foot (D) are shown. Values that do not share the same letter are significantly different from  
524 each other (post-hoc Tukey's HSD test;  $P < 0.05$ ).



525

526

527

528

529

530

531

532

533

**Fig. 3: Visual comparison of four Gentoo penguins measured during moult by thermal imaging.** Individuals were measured through the moult including before moult in old plumage (A), at moulting stage first fall (B) and 50% (C) and in new plumage (D). Images illustrate lower  $T_{\text{trunk}}$  at first fall (M3), lower  $T_{\text{flipper}}$  in new plumage (M7) compared to others stages and higher, higher  $T_{\text{bill}}$  at first fall (M3) than in new plumage (M7) and higher  $T_{\text{foot}}$  during moult (M3 and M5) than before and at the last stage of the moult (M1 and M7).

534 **Table:**

535 Table 1: Summary of the general linear mixed models used to investigate variation in body surface temperatures during moult in captive Gentoo  
 536 penguins  
 537

	$T_{\text{eye}}$			$T_{\text{trunk}}$			$T_{\text{bill}}$			$T_{\text{flipper}}$			$T_{\text{foot}}$		
	R <sup>2</sup> =0.55 N= 339			R <sup>2</sup> =0.68 N= 340			R <sup>2</sup> = 0.36 N= 340			R <sup>2</sup> =0.31 N= 340			R <sup>2</sup> =0.60 N= 327		
<b>Random effect :</b>	<i>Variance</i>			<i>Variance</i>			<i>Variance</i>			<i>Variance</i>			<i>Variance</i>		
Bird ID	0.05			0.29			0.25			0.16			3.49		
Residual	0.91			1.18			9.38			6.05			14.03		
<b>Fixed effect :</b>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Ta or Tground	1,328	6.05	<b>0.014</b>	1,320	42.39	<b>&lt;.0001</b>	1,330	20.81	<b>&lt;.0001</b>	1,330	18.66	<b>&lt;.0001</b>	1,316	172.19	<b>&lt;.0001</b>
Group	1,64	0.75	0.38	1,103	104.33	<b>&lt;.0001</b>	1,84	0.10	0.75	1,55	1.61	0.21	1,107	2.59	0.11
Sex	1,37	5.59	<b>0.023</b>	1,71	1.26	0.27	1,48	3.48	0.068	1,30	1.07	0.31	1,70	1.76	0.19
Molt stages	6,323	49.67	<b>&lt;.0001</b>	6,321	4.94	<b>&lt;.0001</b>	6,324	16.47	<b>&lt;.0001</b>	6,319	15.23	<b>&lt;.0001</b>	6,309	5.29	<b>&lt;.0001</b>

538

539