



HAL
open science

The metabolic cost of subcutaneous and abdominal rewarming in king penguins after long-term immersion in cold water

Agnès Lewden, Batshéva Bonnet, Andreas Nord

► **To cite this version:**

Agnès Lewden, Batshéva Bonnet, Andreas Nord. The metabolic cost of subcutaneous and abdominal rewarming in king penguins after long-term immersion in cold water. *Journal of Thermal Biology*, 2020, 91, pp.102638 -. 10.1016/j.jtherbio.2020.102638 . hal-03491429

HAL Id: hal-03491429

<https://hal.science/hal-03491429>

Submitted on 18 Jul 2022

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 4.0 International License

1 **The metabolic cost of subcutaneous and abdominal rewarming in**
2 **King Penguins after long-term immersion in cold water**

3 Agnès Lewden^{1,2*}

4 Batshéva Bonnet³

5 Andreas Nord^{4,5}

6

7

8

9

10

11

12

13

14 ¹ School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds LS29JT,
15 UK

16 ² Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France

17

18 ³ Centre d'Etudes Biologiques de Chizé, CNRS, UMR 7372, 79360 Villiers en Bois, France.

19

20 ⁴ Department of Biology, Section for Evolutionary Ecology, Lund University, Lund, Sweden, Ecology
21 Building, SE-223 62 Lund, Sweden.

22

23 ⁵ Scottish Centre for Ecology and the Natural Environment, Institute of Biodiversity, Animal Health
24 and Comparative Medicine, University of Glasgow, Rowardennan, G63 0AW, United Kingdom.

25

26 *Author for correspondence: A.M.Lewden@leeds.ac.uk

27 **Abstract**

28 Marine endotherms in the polar regions face a formidable thermal challenge when
29 swimming in cold water. Hence, they use morphological (fat, blubber) adjustment and
30 peripheral vasoconstriction to reduce demands for heat production in water. The animals
31 then regain normothermia when resting ashore. In the king penguin (*Aptenodytes*
32 *patagonicus*) metabolic rate is lower in fed than in fasted individuals during subsequent
33 rewarming on land. This has been suggested to be a consequence of diversion of blood flow
34 to the splanchnic region in fed birds, which reduces peripheral temperatures. However,
35 peripheral temperatures during recovery have never been investigated in birds with
36 different nutritional status. The aim of this study was, therefore, to measure subcutaneous
37 and abdominal temperatures during the rewarming phase on land in fasted and fed king
38 penguins, and investigate to which extent any different rewarming were reflected in
39 recovery metabolic rate (MR_R) after long term immersion in cold water. We hypothesized
40 that fed individuals would have a slower increase of subcutaneous temperatures compared
41 to fasted penguins, and a correspondingly lower MR_R . Subcutaneous tissues reached
42 normothermia after 24.15 (back) and 21.36 min (flank), which was twice as fast as in the
43 abdomen (46.82 min). However, recovery time was not affected by nutritional condition.
44 MR_R during global rewarming ($4.56 \pm 0.42 \text{ W.kg}^{-1}$) was twice as high as resting metabolic rate
45 (RMR ; $2.16 \pm 0.59 \text{ W.kg}^{-1}$). However, MR_R was not dependent on feeding status and was
46 significantly elevated above RMR only until subcutaneous temperature had recovered.
47 Contrary to our prediction, fed individuals did not reduce the subcutaneous circulation
48 compared to fasted penguins and did not show any changes in MR_R during subsequent
49 recovery. It seems likely that lower metabolic rate in fed king penguins on land reported in
50 other studies might not have been caused primarily by increased circulation to the visceral
51 organs.

52

53

54

55 **Keywords:** Subcutaneous temperature, Rewarming, Metabolism, Normothermia

56

57 **1. Introduction**

58 Aquatic endotherms, such as diving birds, experience higher heat loss rate in water than in
59 air of the same temperature, owing to the considerably higher thermal conductivity and
60 specific heat capacity of water (Bullard and Rapp, 1970; Gagge and Nishi, 1977; Dejours,
61 1987). This explains why metabolic rate may be twice as high when the animal is submerged
62 and there is no heat substitution from exercise (Scholander, 1940; Kooyman et al., 1976;
63 Stahel and Nicol, 1982; Ponganis, 2015). Heat loss rate in water is reduced by body insulation
64 provided by subcutaneous fat and a coat that is largely impenetrable water (Kooyman et al.,
65 1976), as well as by counter-current heat exchange in the appendages and vasoconstriction
66 in the trunk that diminishes the thermal gradient between the body and the environment
67 (Bulbard and Rapp, 1970).

68 The king penguin (*Aptenodytes patagonicus*) has a resting metabolic rate (RMR) that is 83%
69 higher in water than in air (Froget et al., 2004). RMR is also higher in fasting compared to fed
70 king penguins (Fahlman et al., 2005). This difference could be explained by lower thermal
71 conductance in fed individuals if they have a thicker layer of subcutaneous fat than fasted
72 birds (Fahlman et al., 2005). However, RMR in fasting king penguins is higher also than in re-
73 fed (*i.e.* fed once after several days of fasting) birds, where there is no difference in thickness
74 of the subcutaneous fat layer between the two groups (Halsey et al., 2008). It is, thus,
75 possible that differences in RMR between birds in different nutritional conditions could be
76 due to adjustments of thermal conductance other than that following changes in body
77 composition. For example, re-fed birds (that need to digest food) may divert most blood
78 flow to the splanchnic region and limit circulation to peripheral tissue, which would result in
79 lower heat loss rate. Moreover, the flexibility of subcutaneous blood perfusion, which is
80 reflected by changes in subcutaneous temperature, is well known in this species. In water,
81 fed adult king penguins maintained lower subcutaneous temperature than fasted individuals
82 (Lewden et al., 2017b), and showed an increase of metabolic rate in parallel to increasing
83 flank temperature (Lewden et al., 2017b). Recently, variation in subcutaneous blood
84 perfusion has also been demonstrated in moulting immature king penguins ashore (Enstipp
85 et al., 2019). However, there has been no measurement of how peripheral blood perfusion
86 changes when king penguins recover normothermia on land after long periods in cold water.

87 This could inform on whether local heterothermy is involved in the lower RMR observed in
88 fed compared to fasted and re-fed king penguins.

89 The aim of this study was to measure subcutaneous and abdominal tissue temperatures
90 during rewarming to normothermia ($\geq 37.5^{\circ}\text{C}$) on land in fasted and fed king penguins, and
91 to determine the energy cost of rewarming in these different nutritional states. To this end,
92 we equipped wild king penguins with four temperatures loggers; one in the abdominal cavity
93 (*i.e.* deep tissue) and three in subcutaneous adipose tissues (flank, back and brood-patch
94 areas). The birds were then maintained in a cold sea water tank for several days without, or
95 with food, where they showed soaking-induced hypothermia (Lewden et al., 2017a). We
96 then removed the birds from the sea water tank and recorded the deep and subcutaneous
97 temperature patterns during recovery to normothermia when they dried on land. We
98 hypothesized that, compared to fasted birds, fed individuals should have a slower recovery
99 of subcutaneous temperatures and, as a result, possibly also a faster recovery of abdominal
100 temperature if warm blood has to be directed to the viscera to aid digestion. Because slower
101 recovery of subcutaneous temperatures would contribute to reduced heat loss rate, a
102 retarded response at the body surface might also convey energy savings during internal
103 recovery. Thus, secondly, we also measured metabolic rate during the recovery phase (MR_R),
104 to test the hypothesis that MR_R is lower before subcutaneous temperatures have recovered,
105 possibly owing to reduced heat loss rate, and that this reduction would be even larger in fed
106 birds.

107

108 **2. Material and methods**

109 King penguins were caught during the courtship phase of their breeding cycle at the colony
110 'La Baie du Marin', on Possession Island, Crozet Archipelago, in the Southern Indian Ocean
111 ($46^{\circ}4' \text{ S}$, $51^{\circ}8' \text{ E}$). The experiments were conducted during three Austral summers
112 (November to March in 2013/2014; 2014/2015 and 2015/2016). Experimental procedures
113 were approved by the French Ethical Committee (APAFIS; permit no. 02015041411414001)
114 and the French Polar Environmental Committee (permit no. 2013-76, 2014-121).

115 On the day of capture, individuals were weighed and equipped with four temperature
116 loggers (iButton MXMDS1922L-F5; AVNET-MEMEC; resolution: $\pm 0.0625^{\circ}\text{C}$; range: $0\text{-}50^{\circ}\text{C}$;

117 accuracy $\pm 0.1^{\circ}\text{C}$) implanted into subcutaneous tissues in the flank, back, and brood patch,
118 and into the abdominal cavity (behind the brood patch), following the surgical procedures
119 described in Lewden et al. (2017a). The abdominal temperature recorded this way is similar
120 to the 'lower abdominal temperature' measured in previous studies on the same species
121 (Fahlman et al. 2005; Halsey et al. 2008). Even if this measurement may not be as close to
122 true core as stomach temperature (cf. Handrich et al. 1997) (see also below), lower
123 abdominal temperature still responds to foraging and digestive activity in a qualitatively
124 similar manner (cf. Lewden et al. 2017a, b). This makes it an appropriate metric when
125 studying thermal and metabolic responses to manipulation of nutritional condition (see 2.1,
126 below). The loggers recorded temperature every 11 min. Following surgery, birds were kept
127 together in pairs in a wooden enclosure on land (3×3 m, without roof) without disturbance,
128 apart from daily feeding, during 6.0 ± 0.1 (s.d.) days. This recovery period was longer than
129 what is required for king penguins to revert to normal behavior after surgery (*i.e.* 2 days;
130 Froget et al., 2004). As a result, our birds had ample time to acclimate to the feeding
131 manipulation. After the recovery period, the penguins were immersed together in a sea
132 water tank (2.5 m length \times 1.3 m width \times 1.2 m height; with a water volume of 2.5 m³) with a
133 constant flow of clean sea water pumped from an adjacent bay. This ensured a stable water
134 temperature of $7.7 \pm 0.6^{\circ}\text{C}$ (s.e.) during the three years of experiment.

135

136 *2.1. Nutritional state*

137 During the three years, a total of 18 individuals with a mean body mass at capture of $13.6 \pm$
138 0.2 kg (s.e.) were maintained fasting in the sea water tank for $58:44 \pm 04:18$ hours (s.e.;
139 range: 20:32 to 111:13 h) after having completely recover from surgery. During this time,
140 they lost 210 ± 0.02 g (s.e.) body mass daily. After the fasting period, the birds were allowed
141 to recover from soaking-induced hypothermia in the wooden enclosure, ashore at
142 thermoneutrality (following Froget et al., 2002). Mean body mass after fasting was 11.4 ± 0.1
143 kg. During the second and third years of the experiment, we also studied body temperature
144 recovery in 12 individuals that were re-fed once the fasting measurements were completed.
145 Just as when measured in the fasting condition, the fed birds were maintained day and night
146 in the sea water tank, but were briefly (≤ 10 min) removed from the tank to be fed with a
147 fish meal of 0.3 ± 0.1 kg (s.e.) four times daily (*i.e.* 1.2 kg fish per bird and day) distributed

148 evenly throughout the photophase (Lewden et al., 2017a). After each meal, the individuals
149 were immediately put back into the sea water tank. In the second year of the study, the fed
150 birds then fasted for one night (fasting duration of $15:14 \pm 00:03$ h) before being removed
151 from the water and allowed to recover once on land. In the third year, fed penguins were
152 measured more frequently (1 to 6 times per bird) after a shorter mean fasting duration of
153 $06:04 \pm 01:08$. The birds gained 279 ± 0.05 g (s.e.) daily during the feeding treatment. Mean
154 body mass during the whole measurement period in the fed birds (11.9 ± 0.2 kg) did not
155 differ significantly from that during measurements in the fasting condition ($P=0.9$). When
156 averaging the full data set, the fasted birds had not eaten for $58:44 \pm 04:18$ h (range
157 between 20:32 and 111:13), and the fed birds were measured $08:13 \pm 01:07$ h (range: 04:01
158 to 15:25 h) after the last meal.

159

160 *2.2. Body temperatures during recovery*

161 During the first year, we measured recovery temperatures once, after the penguins had
162 spent $39:10 \pm 00:10$ h in the sea water tank. In the second year of the study, the penguins
163 were measured twice; once in a fasted condition after $94:18 \pm 00:07$ h spent in the sea water
164 tank, and once in a fed condition when the birds had been in the sea water tank for $116:38 \pm$
165 $00:19$ h. In the third year, the individuals were removed more frequently (means of 4 times
166 in fasted and 3 times fed condition per bird) to be measured (see above), starting when they
167 had spent $03:17$ h in the sea water tank, and extending until $168:35$ h into the experiment.

168

169 The start of the recovery period was defined as the time when the individual was removed
170 from the sea water tank (time = 0). During the first two years of the study, we analyzed flank
171 (T_{flk}), back (T_{back}), brood patch (T_{brood}) and abdominal cavity (T_{abd}) temperatures during $106 \pm$
172 01 min (s.e.) after the start of recovery (range: 60 to 121 min). Data were collected from $N =$
173 13 birds in $n = 26$ session (*i.e.*, two measurements per bird; Fig. 1). In the third year,
174 measurements ($N = 8$; $n = 40$) were collected during 71 ± 03 min (s.e.) from the start of
175 recovery (range: 33 to 121 min). Normothermia was defined as a tissue temperature \geq
176 37.5°C , which is within $\pm 1^{\circ}\text{C}$ of normothermic tissue temperatures for the four body parts
177 previously measured in dry king penguins resting on land (Lewden et al., 2017a, b).

178

179 2.3. Recovery metabolic rate (MR_R)

180 In the third year of the study, we measured oxygen consumption during the recovery phase
181 (recovery metabolic rate, MR_R) ashore at an air temperature of $8.11 \pm 0.28^\circ\text{C}$ (s.e.).
182 Measurements were performed on 8 individuals in both nutritional conditions (1 to 12
183 measurements per individual; $n = 20$ in each of the fasted and fed groups, respectively)
184 during 60 ± 5 min (s.e.) in the beginning of the recovery period. The first MR_R data were
185 recorded within 2 min of removing the bird from the sea water tank. On average, the fasted
186 birds here had not eaten for $59:50 \pm 05:27$ h (range: 15:21 to 112:09 h) during MR_R
187 recordings, whereas the fed birds were measured $06:59 \pm 00:42$ h after last meal (range:
188 01:32 to 13:34 h). In this sub-sample, fasted individuals (11.5 ± 0.1 kg) had lower body mass
189 than the fed ones (12.0 ± 0.2 kg; paired t -test: $P = 0.0143$). Six of these 8 penguins were also
190 measured once when they were dry, fed and normothermic (*i.e.* all tissues $\geq 37.5^\circ\text{C}$), to
191 collect steady-state data on resting metabolic rate (RMR) for comparison to MR_R .

192

193 We used an open flow respirometry system ('Turbofox'; Sable Systems, Henderson, NV, USA)
194 composed of a cylindrical 60 l metabolic chamber put over the bird like a dome. Ambient air
195 was pulled through the metabolic chamber at rate of 40 l min^{-1} . Flow rate was measured and
196 maintained by the mass flow meter and controller unit in the Turbofox. A sub-sample of 200
197 ml min^{-1} sample air was measured for oxygen and carbon dioxide concentration. These data
198 were recorded every 4 s using the software Expedata (Sable Systems). Baseline air was
199 recorded before and after each measurement session. We calibrated the instruments before
200 each trial as detailed in Lewden et al., 2017b. Briefly, the O_2 analysers was span calibrated to
201 20.95 % O_2 using outside air scrubbed of water vapour, and the CO_2 analyser zero-calibrated
202 using a CO_2 scrubber (Ascarite and soda lime; all chemicals from Sigma–Aldrich Chemie,
203 Lyon, France). Then, the CO_2 analyser was spanned with 0.49% CO_2 (Alphagaz; Air Liquide,
204 Paris, France). We calculated oxygen consumption ($\text{ml.O}_2.\text{min}^{-1}$) using eq. 11.2 and CO_2
205 production using eq. 11.4 in Lighton (2008), and converted these data to metabolic rates (W)
206 using an energy equivalence of 20 J.ml O_2^{-1} (Kleiber, 1961). We used these data to calculated
207 the respiratory quotient (RQ) (*i.e.* $V\text{CO}_2 / V\text{O}_2$). There was no significant difference in RQ in
208 the fasted and fed birds (both 0.74 ± 0.05 ; for 20 fasted and 20 fed measurements) (paired t -
209 test: $P = 0.9$).

210

211 2.4. Data analyses

212 Statistical analyses were conducted in R 3.4.4 (R Core Team 2018) and JMP® v. 13 (SAS
213 Institute Inc., Cary, North Carolina, USA). For each measurement session, we determined
214 individual breakpoint temperatures and times for each tissue using the 'lm.br' function in
215 lm.br package (Adams 2017), to identify the inflection point that marked the end of
216 rewarming. All tissue temperatures were stable after the estimated breakpoint (change 0.00
217 ± 0.00 °C min⁻¹ in all cases). When tissue temperature was stable (± 0.5 °C relative to initial
218 temperature) from the start of the trial onwards, we set breakpoint temperature to equal
219 initial temperature and breakpoint time to 0. To describe body temperature during the
220 rewarming period (*i.e.* before the breakpoint), we separately modelled the initial
221 temperature (T_{initial}), the rewarming rate (°C.min⁻¹ gained before the breakpoint when tissue
222 temperatures increased linearly with time), the time to reach the breakpoint and the
223 temperature at recovery (T_{recovery}) using general linear mixed models (GLMM) including
224 nutritional state (*i.e.* fasted and fed), tissue, the interaction "nutritional state × tissue" and
225 year as fixed factors. Bird ID as a random intercept. Recovery patterns were not affected by
226 respirometry measurement (all $P > 0.1$).

227
228 We used the T_{flk} and T_{abd} data to estimate the metabolic rates during rewarming (*i.e.*, MR_R)
229 of peripheral and deep tissues, alone and in combination. This was achieved by first defining
230 five categories of body temperature recovery, *viz.* (i) rewarming of both subcutaneous (*i.e.*
231 T_{flk}) and deep (*i.e.* T_{abd}) temperatures (henceforth 'global rewarming'); (ii) rewarming of deep
232 temperature when subcutaneous temperature had recovered (henceforth 'deep
233 rewarming'); or (iii) rewarming of subcutaneous temperature when deep temperature had
234 recovered (henceforth 'subcutaneous rewarming'); (iv) recovered, with both tissues ≥ 37.5
235 °C and (v) stable normothermia throughout the experiment. We then aligned the body
236 temperature and MR data sets by time, and used the breakpoint times defined for tissue
237 temperatures to calculate MR_R as the mean MR during each of the rewarming events.

238

239 We, firstly, tested how MR_R (W) and mass-specific MR_R (*i.e.* W.kg⁻¹) was affected by
240 nutritional state using a GLMM with thermal state, nutritional state ('fasted' or 'fed'), and
241 the interactions "thermal state × nutritional state" as covariates and bird ID as a random

242 factor. We also fitted two separate linear mixed effects model (LMEs) to test how mass-
243 specific MR_R (averaged of rewarming categories) was affected by ΔT_{flk} (*i.e.* recovery $T_{flk} -$
244 initial T_{flk}) and ΔT_{abd} (*i.e.* recovery $T_{abd} -$ initial T_{abd}), using bird ID as random factor. We could
245 not include ΔT_{flk} and ΔT_{abd} in the same model because of collinearity between these
246 variables (VIF > 4).

247

248 The final models were derived using backward elimination of non-significant ($P > 0.05$)
249 terms, starting with the interactions. We used a Tukey's HSD test to assess differences
250 between groups/tissues for significant model terms. All results are reported as predicted
251 means \pm s.e.

252

253 **3. Results**

254

255 *3.1. Body temperatures during recovery*

256

257 We recorded a total of 268 rewarming events in the four tissues (67 events per tissue; Table
258 1). T_{back} showed the highest temperatures (Fig. 1), and was already at normothermia at the
259 first temperature measurement in 49 % of cases. The corresponding values for T_{flk} , T_{abd} and
260 T_{brood} was 19, 10 and 3 %, respectively (Table 1). These events (55 of 268) were not included
261 in the models describing rewarming patterns. Moreover, 12 % of T_{brood} recording and 15% of
262 T_{abd} recordings never reached normothermia, compared to 1 % of events in T_{back} and T_{flk}
263 (Table 1). These data (20 events) were included only when analyzing $T_{initial}$. On average,
264 rewarming of T_{back} and T_{flk} was the fastest, followed by T_{brood} and T_{abd} . There was no effect of
265 year of study on any measurement (Table 2).

266

267 The effect of nutritional state on $T_{initial}$ differed between the tissues ("nutritional state \times
268 tissue" $P < 0.0001$; Table 2). Specifically, neither initial T_{back} nor initial T_{flk} differed between
269 fasted and fed birds (global means: $36.82 \pm 0.97^\circ\text{C}$ and $33.98 \pm 0.86^\circ\text{C}$, respectively; Table 2),
270 but both initial T_{brood} and T_{abd} was lower in fed than in fasted birds (Table 2).

271

272 T_{flk} rewarmed faster ($0.29 \pm 0.02^\circ\text{C min}^{-1}$) than T_{brood} ($0.21 \pm 0.02^\circ\text{C min}^{-1}$), which was still
273 faster than T_{back} and T_{abd} ($0.11 \pm 0.03^\circ\text{C min}^{-1}$ and $0.10 \pm 0.02^\circ\text{C min}^{-1}$ respectively)(Table 2;

274 Fig. 1). However, rewarming rate was not affected by nutritional state (Table 2 and Fig. 2).
275 Nor did nutritional state affect the time needed for recovery (Table 2). However, the
276 different tissues required different times to reach stable temperature ($P < 0.0001$; Table 2).
277 T_{flk} and T_{back} recovered first, with a similar recovery time (21.36 ± 2.76 min and 24.15 ± 3.18
278 min, respectively; Table 2). This was followed by the recovery of T_{brood} some 7 min later
279 (31.22 ± 2.65 min, with no differences between these tissues; Table 2). T_{abd} recovered
280 significantly later than all of the others tissues, after an additional 16 min (46.82 ± 3.00 min;
281 Table 2). It should be noted that while both T_{abdo} and T_{brood} reached stable temperature
282 during the study, neither tissue reached the normothermic values ($\geq 37.5^\circ\text{C}$) that are
283 characteristic of fully recovered birds that have been in the colony for several hours (Lewden
284 et al. 2017a, b).

285
286 When the birds had regained recovered and/or normothermia, temperature differed
287 between tissues depending on nutritional state (“nutritional state \times tissue” Table 2). More
288 precisely, recovery T_{back} and T_{flk} were similar and independent of nutritional state (global
289 means: $38.57 \pm 0.63^\circ\text{C}$ and $38.65 \pm 0.55^\circ\text{C}$, respectively; Table 2). In the fasted birds,
290 recovery T_{brood} and T_{abd} ($37.98 \pm 0.49^\circ\text{C}$ and $38.03 \pm 0.55^\circ\text{C}$, respectively) were similar to
291 recovery T_{back} and T_{flk} (Table 2). In the fed birds, recovery T_{abd} was 1.5°C lower ($36.46 \pm$
292 0.64°C) than in the fasted birds. This was also true for recovery T_{brood} , which was 2.1°C lower
293 in the fed ($35.87 \pm 0.59^\circ\text{C}$) compared to the fasted birds (Table 2).

294

295 3.2. Recovery metabolic rate (MR_R)

296 We measured MR_R of 77 thermal states (Fig. 3A). Of these, 28 (36%) were ‘global
297 rewarming’, and 27 (35%) ‘deep rewarming’, *i.e.* rewarming of deep tissue temperature after
298 the subcutaneous had recovered. There were few observations of ‘subcutaneous
299 rewarming’, *i.e.* rewarming of the subcutaneous once deep temperature had recovered (one
300 each in ‘fasted’ and ‘fed’ conditions; 3% of total). For this reason, these data were not used
301 in the model, but are shown in Fig. 3B for comparison. Finally, we recorded 13 cases (17%)
302 where the birds were categorized as ‘recovered’, and in 6 cases (9%) birds were
303 normothermic (‘stable normothermia’ category) throughout the metabolic measurements.

304 On average, MR_R and mass-specific MR_R during 'global rewarming' (52.46 ± 4.55 W and 4.56
305 ± 0.42 W.kg⁻¹, respectively) were twice as high as RMR during 'stable normothermia' ($25.94 \pm$
306 6.60 W and 2.16 ± 0.59 W.kg⁻¹, respectively).

307 MR_R and mass-specific MR_R were not affected by nutritional state, neither alone nor in
308 combination with thermal state (Table 3). However, MR_R and mass-specific MR_R during
309 'global rewarming' were significantly higher than all other categories (Table 3; Fig. 3B). In
310 fact, MR_R and mass-specific MR_R during 'deep rewarming' was similar to MR_R in the
311 'recovered' thermal state, and also not significantly higher than MR_R at 'normothermia'
312 (Table 3; Fig. 3B). Finally, we measured a significant positive relationship between MR_R and
313 ΔT_{flk} ($F_{1, 70}=12.53$ $P=0.0007$; GLMM $R^2=0.28$ $n=72$; Fig. 3C), but ΔT_{abd} did not affect MR_R
314 ($P=0.3641$; GLMM $R^2=0.09$ $n=72$).

315

316 **4. Discussion**

317 Subcutaneous tissues (back and flank) recovered in half the time (24 ± 3 min and 22 ± 3 min,
318 respectively) taken for internal tissues (abdomen; 47 ± 3 min) when king penguins rewarmed
319 to normothermia after cold water immersion (Fig. 1). As previously measured in water
320 (Lewden et al. 2017a, b), T_{brood} did not follow the same pattern as T_{back} and T_{flk} despite both
321 temperature loggers being implanted subcutaneously. Rather, temperature in this body part
322 was constantly lower than in the three other tissues (Fig. 1), both on land (this study) and in
323 water (Lewden et al. 2017a, b) independent of nutritional state. The subsequent recovery
324 pattern, which was similar to that of T_{abd} , was probably indicative of a response to reduce
325 heat loss from this bare, and richly vascularized, skin area until a more favourable (drier)
326 thermal state had been restored. In contrast, the back region, which was constantly above
327 the surface when the birds rested in the sea water tank (cf. Time -11 in Fig. 1), consistently
328 showed the highest initial temperatures (see also Lewden et al. 2017a, b). MR_R during global
329 rewarming (4.56 ± 0.42 W.kg⁻¹) was about two times higher than RMR at normothermia
330 (2.16 ± 0.59 W.kg⁻¹), which indicates that the birds actively produced heat to rewarm (or to
331 counter increased heat loss when the plumage was still wet) during the initial period on
332 land. Once subcutaneous temperature had recovered, (but the deep tissues were still
333 rewarming), MR_R was not significantly elevated above RMR (Fig. 3). All these changes were

334 independent of nutritional state, despite fed birds having a steeper internal temperature
335 gradient to recover. Hence, a delayed recovery of subcutaneous temperature probably does
336 not explain lower MR observed in fed king penguins in other studies (Fahlman et al., 2005;
337 Halsey et al., 2008).

338
339 The rapid increase of subcutaneous temperatures prior to deep tissue recovery is consistent
340 with the increasing flipper temperature that has been measured in rewarming emperor
341 (*Aptenodytes forsteri*) and king penguins after emergence from water in other studies
342 (Ponganis et al., 2003; Schmidt, 2006; Lewden et al., *submitted*), and suggests that fed king
343 penguins in this study did not use subcutaneous vasoconstriction to reduce heat loss rate
344 during rewarming, as previously hypothesized (Halsey et al., 2008).

345
346 We measured lower initial T_{abd} in fed than in fasted birds (Table 2; Fig. 1). This was probably
347 a consequence of daily ingestion of cold fish meals (Culik et al., 1996; Eichhorn et al., 2011;
348 Lewden et al., 2017a), or a delay of digestive activity (see below). Even so, the rewarming
349 rate and the recovery time was independent of nutritional state (Table 2; Fig. 1). However,
350 both T_{brood} and T_{abd} stabilized at lower temperature in the fed birds (Fig. 2). Thus, these birds
351 would have required a longer time to reach the same high temperature as in the fasted
352 individuals.

353
354 It should be noted that we measured a lower internal temperature than in previous studies
355 (Halsey et al. 2008; Fahlman et al. 2005). This could be an effect of logger placement,
356 because it is known that king penguins may show several temperature differences of several
357 degrees in a small area (*e.g.* Handrich et al., 1997; Fahlman et al. 2005). Our abdominal
358 logger was positioned closer to the 'lower' abdomen measured by Halsey et al. (2008). These
359 authors also measured 'upper' and 'middle abdominal' temperatures, which are likely closer
360 to true core temperature. The differences between the studies could also be explained by
361 variation in the duration in water preceding the recovery measurements. This time was 120
362 and 160 min in the studies by Fahlman et al (2005) and Halsey et al. (2008), but on average
363 5024 min (83 h) in our study. Moreover, we measured that it takes around 5 h of constant
364 immersion before all subcutaneous and deep tissues have reach a low stable temperature
365 (Lewden, A., et al. unpublished data). Thus, the shorter water immersion period of 2-2.5 h in

366 previous studies could explain why a higher 'lower abdominal' than in this study has been
367 reported elsewhere. Even if we assume that T_{abd} as it was measured by us is closer to a
368 'peripheral' tissue than in the previous studies, our conclusion that king penguins do not
369 delay rewarming of the subcutaneous tissues to save energy during recovery remains the
370 same.

371

372 Both physiological and physical factors could explain why we observed a faster recovery of
373 subcutaneous compared to deep temperature (Fig. 2). On the one hand, the rapid increase
374 of subcutaneous temperatures could indicate increased circulation required for fuel
375 mobilisation (Lewden et al., 2017a) to cover energy costs of the rewarming phase. On the
376 other hand, faster subcutaneous rewarming could facilitate evaporation of water within the
377 plumage. Because a dry plumage confers better insulation than a wet one (de Vries and van
378 Eerden, 1995), increased peripheral temperature might actually restore body insulation and,
379 hence, reduce heat loss rate during subsequent rewarming of deep tissues. This is consistent
380 with the drop in MR_R that occurred as soon as the periphery had recovered, from which
381 point onwards it was not different from RMR in a normothermic bird (Fig. 3). Finally, we
382 cannot completely exclude the possibility that there was also a slight effect of heat gain from
383 long-wave and solar radiation in the subcutaneous trunk area when the bird was on land.
384 The contribution of radiative gain to the overall energy budget of rewarming penguins
385 should be quantified in future studies.

386

387 The absence of an effect of nutritional state on MR_R contrasts previous studies (Fahlman et
388 al., 2005; Halsey et al., 2008), despite overall similarly in metabolic rates measured here and
389 elsewhere (Table 4). Thus, both MR_R (global mean across states: 2.52 W.kg^{-1}) and RMR
390 during normothermia (2.09 W.kg^{-1}) was similar to previously measured MR_R (2.76 W.kg^{-1} ;
391 Fahlman et al., 2006) and RMR values (2.16 to 2.72 W.kg^{-1} ; Fahlman et al. 2005, 2014) (Table
392 4). This might be explained by methodological differences between the studies. Our fed
393 group is most similar to 're-fed' birds in Halsey et al. (2008), who force-fed one meal 24 h
394 before the measurements. In contrast, our fed birds ate daily over a period of $47:42 \pm 05:53$
395 h and had ingested their most recent meal 04:01 to 15:25 h before metabolic rate
396 measurements. Even so, MR_R during global rewarming was very similar in the fed birds in our

397 studies and those in the study by Halsey et al. (2008) ($4.15 \text{ W}\cdot\text{kg}^{-1}$ and $4.22 \text{ W}\cdot\text{kg}^{-1}$,
398 respectively) (Table 4).

399
400 Fahlman et al. (2005) hypothesized that differences in MR_R between fasted and fed penguins
401 was caused by differences in body mass. While, on average, body mass differed between
402 nutritional states in the subset where we measured metabolic responses, this difference was
403 only 500 g. This might not have been large enough to detect any effects on the metabolic cost
404 of rewarming. However, one individual lost body mass twice as fast as the others (*i.e.* $628 \pm$
405 $0.19 \text{ g}\cdot\text{day}^{-1}$, compared to $335 \pm 0.08 \text{ g}\cdot\text{day}^{-1}$ for the other penguins) possibly even entering
406 fasting phase III which is characterized by protein catabolism (LeMaho et al., 1988, Cherel et
407 al., 1999; Groscolas et al., 2001). This bird showed a clear pattern of energy conservation,
408 with lower peripheral tissues in the sea water tank (Supp. Fig. 1A), but its rewarming pattern
409 on land did not differ from that in other individuals (Supp. Fig. 1). It would be interesting to
410 repeat this study using a more extensive fasting protocol that more strongly significantly
411 affected nutritional state of the birds to investigate the body mass hypothesis more broadly.

412
413 The similar MR_R in fasted and fed birds could also be due to absence of digestive activity in
414 fed individuals. We do not think that it is likely that the fed birds had already digested the
415 fish meal by the time the measurements started, in which case we would have expected
416 similar T_{abd} between treatments. The capacity to delay digestive activity is well known in this
417 species (Gauthier-Clerc et al., 2000; Thouzeau et al., 2003, 2004). If this is associated with
418 changes to circulation of the abdominal region, delayed digestions could explain the lower
419 T_{abd} observed in our fed birds. This is supported by the similar RQs in the two nutritional
420 conditions (see Materials and Methods). Thus, it is possible that the fed group was not as
421 homogenous with regards to digestive activity as we had intended. To evaluate this
422 possibility, future studies need to measure recovery temperatures and MR_R over a range of
423 time periods from the feeding event onwards.

424
425 Finally, in line with our previous study (Lewden et al., 2017b), we found a positive
426 relationship between MR_R and ΔT_{fjk} during recovery on land (Fig. 3C). However, according to
427 the higher heat loss rate in water than in air, the effect of ΔT_{fjk} on MR_R ashore was more
428 than three times lower ($0.22 \text{ W}\cdot\text{kg}^{-1}\cdot\text{C}^{-1}$) than in water ($0.73 \text{ W}\cdot\text{kg}^{-1}\cdot\text{C}^{-1}$) (Lewden et al.,

429 2017b). In contrast, there was no relationship between MR_R and ΔT_{abd} . These results indicate
430 that while subcutaneous vasodilation increases heat loss rate (and, thus, impacts metabolic
431 rate), temperature change inside the well-insulated trunk does not necessarily increase heat
432 loss from the body. To this end, ΔT_{fik} may be viewed as the driver of the energetic cost
433 needed to rewarm the subcutaneous layer.

434

435 **Acknowledgements**

436 We are grateful to Yves Handrich for his valuable help on this project and comments on a previous
437 version of the manuscript. We also thank Jean-Yves Georges for his material support, Thibaut
438 Hestin, Caroline Bost for their help and all the members of the 53th mission in Crozet for
439 their support in the field.

440 **Funding sources**

441 Logistic support in the field was provided by the Terres Australes et Antarctiques Françaises
442 (TAAF). The research project No. 394 is funded by Institut Polaire Français Paul-Emile Victor
443 and by the Centre National de la Recherche Scientifique (CNRS-INEE). A.L. was the recipient
444 of a scholarship from the French Ministère de l'Éducation Nationale, de la recherche et de la
445 technologie. AN was supported by the Birgit and Hellmuth Hertz Foundation / The Royal
446 Physiographic Society of Lund (grant no. 2017-39034).

447 **Figures:**

448 **Figure 1:** Mean \pm s.e. tissue temperatures recorded every 11 min in subcutaneous tissue on the back
449 (blue), flank (green), and brood patch (pink), and in the abdominal cavity (red) in fasted (A) and fed
450 (B) king penguins. The first measurement (time = -11) is the last temperature recorded before the
451 bird was removed from the cold sea water tank.

452 **Figure 2:** Schematic representation of the rewarming rate in the four tissue from the point in time
453 where the birds were removed from the sea water tank (*i.e.* T_{initial}) to the end of the linear rewarming
454 phase (*i.e.* T_{recovery}). Rewarming rate \pm s.e. ($^{\circ}\text{C}\cdot\text{min}^{-1}$) for each tissue is printed above the curve. There
455 was no effect of nutritional state on T_{flk} and T_{back} , but fed birds (dotted line) had lower T_{brood} and T_{abd}
456 than fasted (dash-dotted line) birds throughout the rewarming phase.

457 **Figure 3:** (A) Metabolic rate (MR_R , W) \pm s.e. and mass-specific MR_R ($\text{W}\cdot\text{kg}^{-1}$) \pm s.e in each thermal state
458 during recovery to normothermia on land in king penguins after long-term immersion in cold.
459 'Subcutaneous rewarming' was not included in the statistical model on account of very few
460 occurrences, as described in the main text. The number of segments representing each category is
461 indicated on the bars, and different letters denote significant differences between categories. MR_R is
462 delimited by the horizontal line in each bar. Mass-specific MR_R is represented by the upper range of
463 the bars. (B) Temporal changes in mean \pm s.e. T_{flk} (green), T_{abd} (red), and metabolic rate (black) in the
464 sea water tank immediately before the recovery measurements started (Time = -11), and during
465 subsequent recovery to normothermia on land (starting at Time =0). Metabolic rate in the sea water
466 tank before recovery started (hexagon) was measured in different individuals but during the same
467 experiment (N = 4; n = 13; Lewden et al. 2017b). MR_R recovery (downward facing triangle) was
468 calculated as every 10 min means during all thermal states (cf. panel A) (N = 8; n = 40). RMR (upward
469 facing triangle) was measured in dry birds at normothermia (N = 6; n = 6). (C) MR_R in relation to the
470 change in flank temperature (ΔT_{flk}) during rewarming. Thermal states are indicated with different
471 symbols for reference only.

472

473

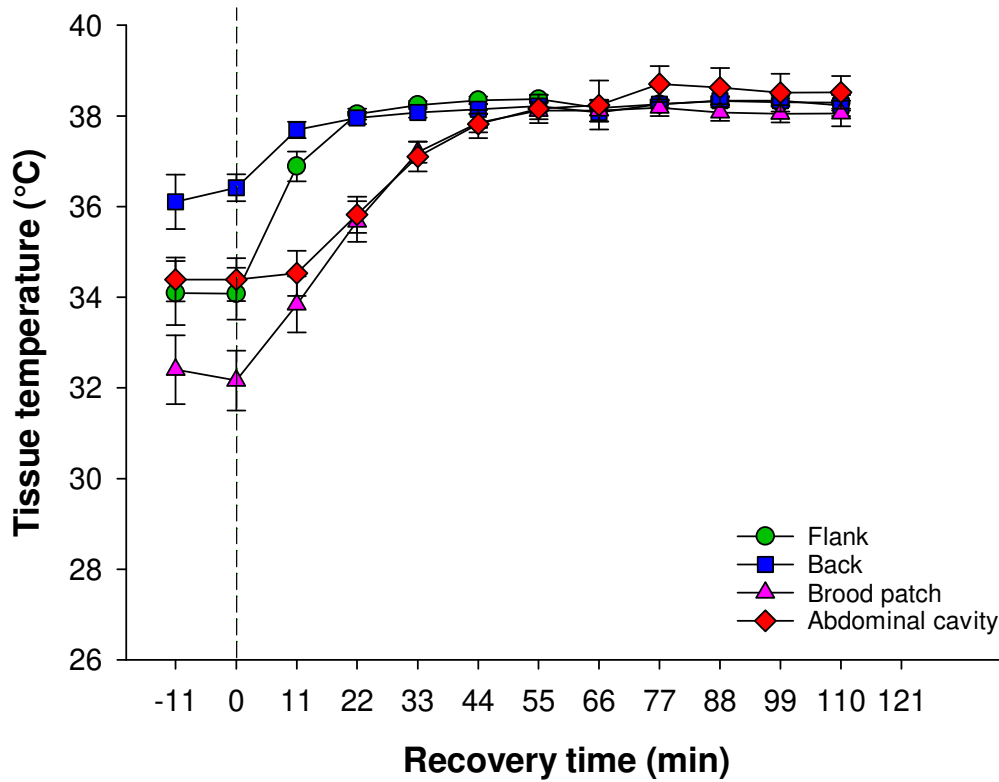
474

475

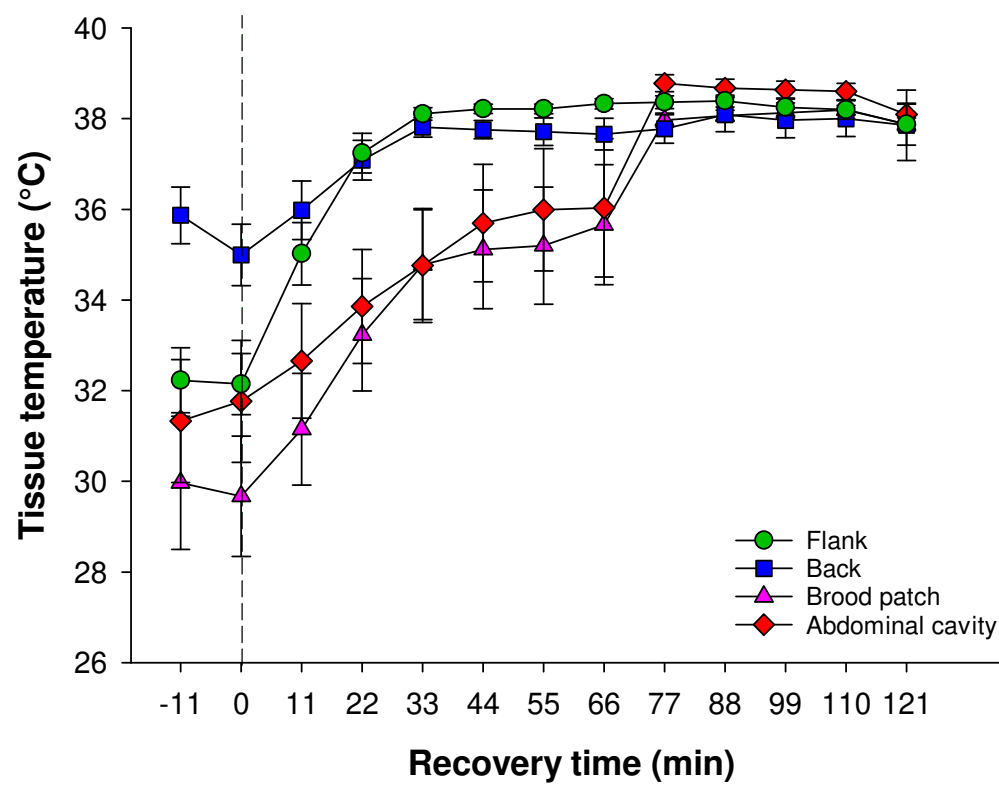
476

477 **Figure 1:**

A - Fasted condition (N=18 individuals, n=36 rewarming events)



B - Fed condition (N=13 individuals, n=28 rewarming events)



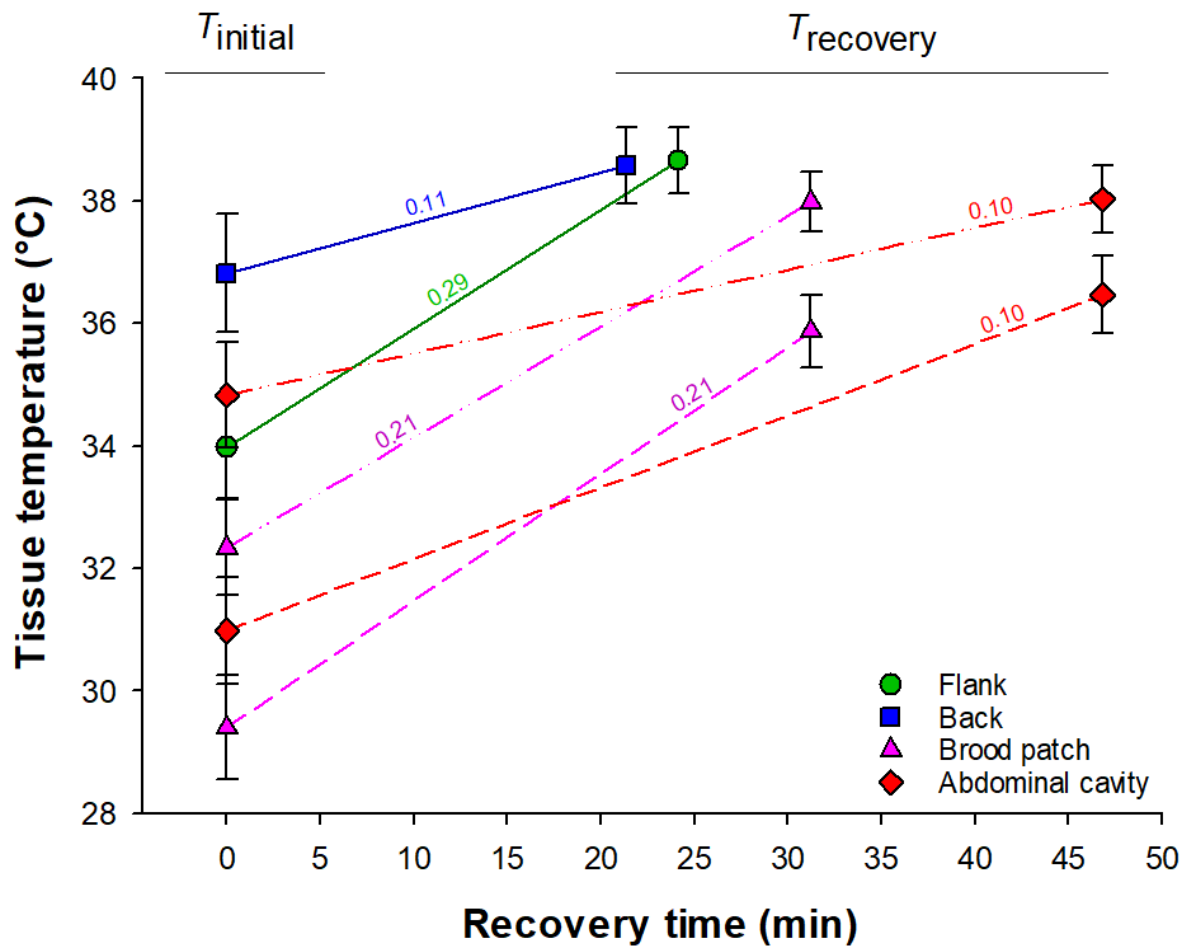
478

479

480

481 **Figure 2:**

482



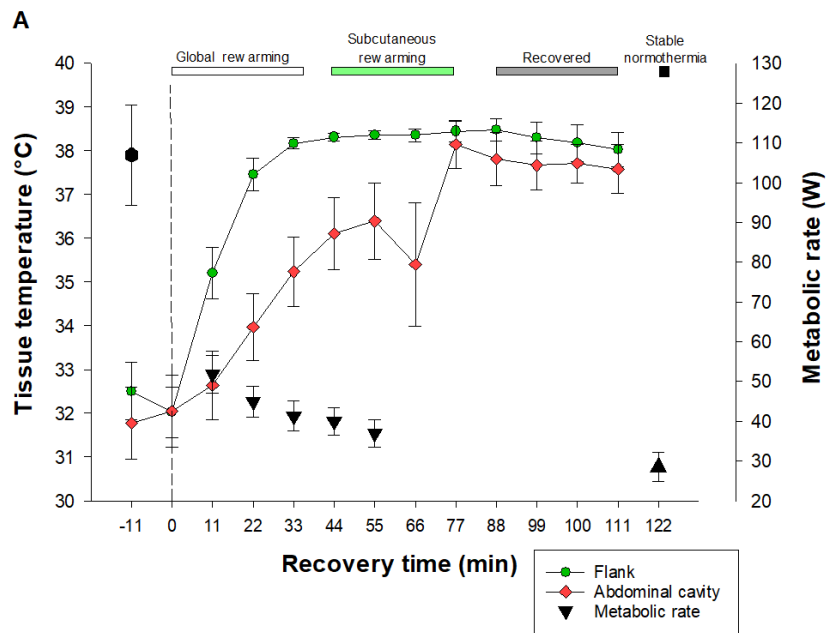
483

484

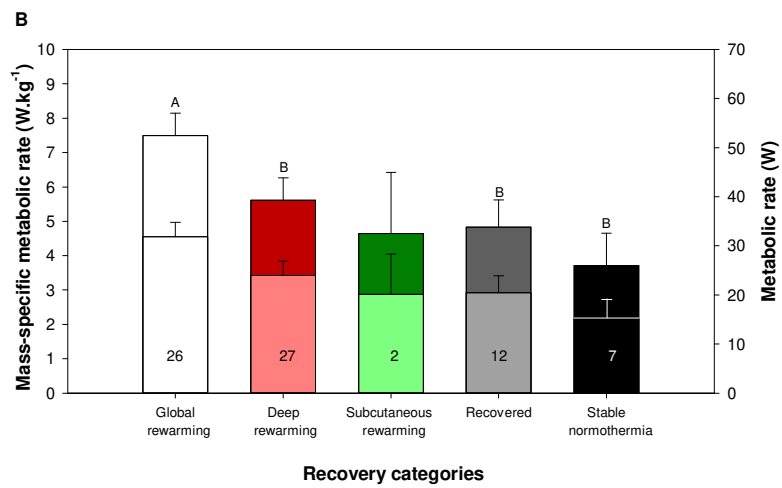
485

486

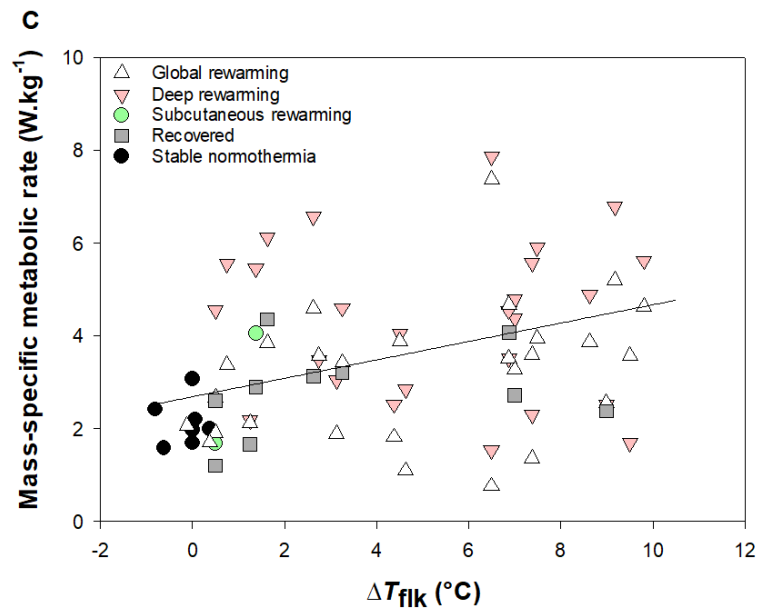
487 **Figure 3:**



488



489



490

491 **Table 1:**

492 Distribution of rewarming events recorded in four tissues of king penguins during the three years. In total, 67 rewarming
 493 events were recorded. However, due to device failure ('without data' category) there are missing data for some tissues.
 494 Rewarming events where a given tissue was already at normothermia before recovery (*i.e.* ' $T_{\text{initial}} \geq 37.5^{\circ}\text{C}$ ' category) were
 495 not included in the statistical analyses. Nor were data where the tissue never reached stable temperature during
 496 rewarming the event ('Never reached stable state' category). Thus, we analysed 73% of the 67 rewarming events recorded
 497 in the flank, 48% of those in the back tissue, and 79% and 60% of those recorded in the brood patch and abdominal cavity,
 498 respectively.

499

	T_{flk}	T_{back}	T_{brood}	T_{abd}
Rewarming events - n (%)	49 (73)	32 (48)	53 (79)	40 (60)
Without data* - n (%)	4 (6)	1 (1)	4 (6)	10 (15)
T_{initial} ≥ 37.5°C - n (%)	13 (19)	33 (49)	2 (3)	7 (10)
Never reached stable state - n (%)	1 (1)	1 (1)	8 (12)	10 (15)
Total events - n	67	67	67	67

* due to device failure

500

501

502

503 **Table 2:**

504 Effects of nutritional states, tissues, the interaction tissues x nutritional states and year on $T_{initial}$, $T_{recovery}$, rewarming rate and recovery time in
 505 king penguins recovering normothermia on land after long immersion in cold water. Data were analyzed using general linear mixed effects
 506 models with bird ID as a random intercept, with separate models for each parameter Model estimates are not provided for main effects in the
 507 presence of significant interactions.

508

	$T_{initial}$ (°C)					$T_{recovery}$ (°C)				
	$R^2 = 0.63$ N = 20 n = 194					$R^2 = 0.48$ N = 20 n = 174				
	df	F	Prob. > F	Prediction ± s.e.	Post-hoc test	df	F	Prob. > F	Prediction ± s.e.	Post-hoc test
Nutritional state	1.184	8.10	0.0049			1.166	2.01	0.1575		
Tissue	3.172	28.92	<0.0001			3.155	7.84	<0.0001		
Nutritional state x Tissue	3.172	5.89	0.0008			3.154	5.49	0.0013		
Back Fasted				36.30 ± 0.95	ab				38.21 ± 0.61	ab
Back Fed				37.34 ± 0.99	a				38.94 ± 0.65	a
Flank Fasted				34.13 ± 0.85	bc				38.28 ± 0.54	ab
Flank Fed				33.83 ± 0.86	bc				39.03 ± 0.55	a
Brood patch Fasted				32.33 ± 0.78	cb				37.98 ± 0.49	ab
Brood patch Fed				29.40 ± 0.85	e				35.87 ± 0.59	c
Abdominal cavity Fasted				34.82 ± 0.86	abc				38.03 ± 0.55	ab
Abdominal cavity Fed				30.98 ± 0.87	de				36.47 ± 0.64	c
Year	-	-	0.1892			-	-	0.223		

Rewarming rate (°C.min⁻¹)

R² = 0.32 N = 20 n = 174

	df	F	Prob. > F	Prediction ± s.e.	Post-hoc test
Nutritional state	-	-	0.6224		
Tissue	3.16	19.33	<0.0001		
Back				0.11 ± 0.03	c
Flank				0.29 ± 0.02	a
Brood patch				0.21 ± 0.02	b
Abdominal cavity				0.10 ± 0.02	c
Nutritional state x Tissue	-	-	0.7119		
Year	-	-	0.3536		

Recovery time (min)

R² = 0.40 N = 20 n = 174

	df	F	Prob. > F	Prediction ± s.e.	Post-hoc test
Nutritional state	-	-	0.7555		
Tissue	3.155	21.76	<0.0001		
Back				24.15 ± 3.18	bc
Flank				21.36 ± 2.76	c
Brood patch				31.22 ± 2.65	b
Abdominal cavity				46.82 ± 3.00	a
Nutritional state x Tissue	-	-	0.864		
Year	-	-	0.5406		

509

510

511

512

513

514 **Table 3:**

515 Effects of thermal state and nutritional condition on recovery metabolic rate (MR_R) and mass-specific MR_R during the four thermal states in fed
 516 and fasted king penguins that recovered from soaking-induced hypothermia on land. Thermal states were assigned using inflection points in T_{flk}
 517 and T_{abd} recordings, as detailed in the main text.

518

	MR_R (W)					Mass-specific MR_R (W.kg⁻¹)				
	R ² = 0.35 N = 8 n = 72					R ² = 0.35 N = 8 n = 72				
	df	F	Prob. > F	Prediction ± s.e.	Post-hoc test	df	F	Prob. > F	Prediction ± s.e.	Post-hoc test
Nutritional state	-	-	0.2164			-	-	0.3449		
Thermal state	3.65	7.57	0.0002			3.64	7.64	0.0002		
Global rewarming				4.56 ± 0.42	a				52.46 ± 4.55	a
Deep rewarming				3.42 ± 0.42	b				39.31 ± 4.54	b
Recovered				2.92 ± 0.50	b				33.84 ± 5.49	b
Stable normothermia				2.16 ± 0.59	b				25.94 ± 6.60	b

519

520

521

522 **Table 4:**

523 Summary table of field metabolic rate (FMR), field metabolic rate estimate (FMR_{est}), resting metabolic rate (RMR) and
 524 recovery metabolic rate (MR_R) in free-ranging or temporarily captive, wild king penguins in different nutritional conditions.
 525 Bold values correspond to results obtained in the current study.

<u>Condition</u>	<u>Methods</u>	<u>Metabolism</u>	<u>Nutritional condition</u>	<u>RQ</u>	<u>Body mass</u>	<u>VO₂ (ml.min⁻¹)</u>	<u>W.kg⁻¹</u>	
Captivity	Respirometry	FMR		-	-	-	2.80	LeM
	Respirometry	FMR		-	-	-	3.00	Barro
Free-ranging	Doubly labelled water	FMR		-	-	-	3.30	Kooy
	Heart rate	FMR _{est}		-	-	122.1	3.15	Frog
<hr/>								
Captivity	Respirometry	RMR	Fasted	0.76	13.8	127.6	3.05	
				0.76	12.6	107.2	2.81	
				0.74	11.5	95.3	2.72	Fahl
				0.75	10.7	79.1	2.44	
				0.69	9.8	65.8	2.16	
				-	11.1	86.9	2.55	Fahl
				0.72	11.5	69.0	2.09	This
<hr/>								
Captivity	Respirometry	RMR	'Fed' (after foraging trip)	-	13.9	117.1	2.76	Fahl
			Fed (manually fed)	0.74	12.0	91.03	2.52	This
<hr/>								

Captivity	Respirometry	MR _R	Fasted	0.74	10.6	83.6	3.58	Hals
				0.74	11.5	122.7	3.56	This
Captivity	Respirometry	MR _R	Fed (after foraging trip)	0.8	13.1	121.5	2.51	Hals
			Fed (manually fed)	0.73	12.0	149.5	4.15	This
			Re-fed (manually fed)	0.78	10.4	94.3	4.22	Hals

526

527 **References**

- 528 Adams, M., 2017. lm.br: Linear Model with Breakpoint. R package version 2.9.3. [https://CRA](https://CRAN.R-project.org/package=lm.br)
529 [N.R-project.org/package=lm.br](https://CRAN.R-project.org/package=lm.br)
- 530 Baudinette, R.V., Gill, P., O'Driscoll, M., 1986. Energetics of the little penguin, *Eudyptula*
531 *minor*: temperature regulation, the calorogenic effect of food, and moulting. *Aust. J. Zool.* 34,
532 35-45.
- 533 Bech, C., Præsteng, K.E., 2004. Thermoregulatory use of heat increment of feeding in the
534 tawny owl (*Strix aluco*). *J. Therm. Biol.* 29, 649-654.
535 <https://doi.org/10.1016/j.jtherbio.2004.08.034>
- 536 Bullard, R.W., Rapp, G.M., 1970. Problems of body heat loss in water immersion. *Aerosp.*
537 *Med.* 41, 1269-1277.
- 538 Chappell, M. A., Bachman, G. C., Hammond, K. A., 1997. The heat increment of feeding in
539 house wren chicks: magnitude, duration, and substitution for thermostatic costs. *J. Comp.*
540 *Physiol. B* 167, 313-318.
- 541 Cherel, Y., Tremblay, Y., Guinard, E., Georges, J.Y., 1999. Diving behaviour of female northern
542 rockhopper penguins, *Eudyptes chrysocome moseleyi*, during the brooding period at
543 Amsterdam Island (Southern Indian Ocean). *Mar. Biol.* 134, 375-385.
- 544 Dejours, P., 1987. Water and air physical characteristics and their physiological
545 consequences. In *Comparative Physiology: Life in Water and on Land* (ed. P. Dejours, L. Bolis,
546 C. R. Taylor and E. R. Weibel), pp. 3-11. Berlin: Springer Verlag.
- 547 de Vries, J., van Eerden, M.R., 1995. Thermal Conductance in Aquatic Birds in Relation to the
548 Degree of Water Contact, Body Mass, and Body Fat: Energetic Implications of Living in a
549 Strong Cooling Environment. *Physiol. Zool.* 68, 1143-1163.
- 550 Enstipp, M.R., Grémillet, D., Jones, D.R., 2008. Heat increment of feeding in double-crested
551 cormorants (*Phalacrocorax auritus*) and its potential for thermal substitution. *J. Exp. Biol.*
552 211, 49-57. <https://doi.org/10.1242/jeb.012229>.
- 553 Enstipp, M.R., Bost, C.A., Le Bohec, C., Bost, C., Laesser, R., Le Maho, Y., ... Handrich, Y. 2019.
554 The dive performance of immature king penguins following their annual molt suggests
555 physiological constraints. *J. Exp. Biol.* 222. <https://doi.org/10.1242/jeb.208900>.
- 556 Fahlman, A., Schmidt, A., Handrich, Y., Woakes, A.J., Butler, P.J., 2005. Metabolism and
557 thermoregulation during fasting in king penguins, *Aptenodytes patagonicus*, in air and water.
558 *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, 670-679.
559 <https://doi.org/10.1152/ajpregu.00130.2005>
- 560 Froget, G., Handrich, Y., Le Maho, Y., Rouanet, J.L., Woakes, A.J., Butler, P.J., 2002. The heart
561 rate/oxygen consumption relationship during cold exposure of the king penguin: a
562 comparison with that during exercise. *J. Exp. Biol.* 205, 2511-2517.

563 Froget, G., Butler, P.J., Woakes, A.J., Fahlman, A., Kuntz, G., Le Maho, Y., Handrich, Y., 2004.
564 Heart rate and energetics of free-ranging king penguins (*Aptenodytes patagonicus*). J. Exp.
565 Biol. 207, 3917-3926. <https://doi.org/10.1242/jeb.01232>

566 Gagge, A.P., Nishi, Y., 1977. Heat exchange between human skin surface and thermal
567 environment. In: Handbook of Physiology: Reactions to Environmental Agents, edited by
568 Lee DHK, Falk HL, and Murphy SD. Bethesda: American Physiological Society. pp. 69-92.

569 Gauthier-clerc, M., Le Maho, Y., Clerquin, Y., Drault, S., Griffin, T. M., Kram, R. 2000. Penguin
570 fathers preserve food for their chicks. Nature 408, 928-929.

571 Groscolas, R., Robin, J.P., 2001. Long-term fasting and refeeding in penguins. Comp.
572 Biochem. Physiol. A. Mol. Integr. Physiol. 128, 645-655. [https://doi.org/10.1016/S1095-](https://doi.org/10.1016/S1095-6433(00)00341-X)
573 [6433\(00\)00341-X](https://doi.org/10.1016/S1095-6433(00)00341-X)

574 Halsey, L.G., Handrich, Y., Rey, B., Fahlman, A., Woakes, A.J., Butler, P.J., 2008. Recovery
575 from swimming-induced hypothermia in king penguins: effects of nutritional condition.
576 Physiol. Biochem. Zool. 81, 434-41.

577 Handrich, Y., Bevan, R. M., Charrassin, J-B., Butler, P. J., Ptz, K., Woakes, A. J., Lage, J., Le
578 Maho Y. 1997. Hypothermia in foraging king penguins. Nature 388, 6637-6400.

579 Hawkins, P., Butler, P., Woakes, A., Gabrielsen, G., 1997. Heat increment of feeding in
580 Brunnich's guillemot. J. Exp. Biol. 200, 1757-1763.

581 Kleiber, M., 1961. The Fire of Life. An Introduction to Animal Energetics. John Willey &
582 Sons. Inc., New York-London, 44-59.

583 Kooyman, G.L., Gentry, R.L., Bergman, W.P., Hammel, H.T., 1976. Heat loss in penguins
584 during immersion and compression. Comp. Biochem. Physiol. A Physiol. 54, 75-80.

585 Le Maho, Y., Robin, J.P., Cherel, Y., 1988. Starvation as a treatment for obesity: the need to
586 conserve body protein. News. Physiol. Sci. 3, 21-24.

587 Lewden, A., Enstipp, M.R., Picard, B., van Walsum, T., Handrich, Y., 2017a. High peripheral
588 temperatures in king penguins while resting at sea: thermoregulation versus fat deposition.
589 J. Exp. Biol. 220, 3084-3094. <https://doi.org/10.1242/jeb.158980>

590 Lewden, A., Enstipp, M. R., Bonnet, B., Bost, C., Georges, J.-Y., Handrich, Y., 2017b. Thermal
591 strategies of king penguins during prolonged fasting in water. J. Exp. Biol. 220, 4600-4611.
592 <https://doi.org/10.1242/jeb.168807>

593 Lighton, J. R. B., 2008. Measuring metabolic rates - a manual for scientists. Oxford: Oxford
594 Univ. Press.

595 Lovvorn, J.R., 2007. Thermal substitution and aerobic efficiency: measuring and predicting
596 effects of heat balance on endotherm diving energetics. Philos. Trans. R. Soc. Lond. B. Biol.
597 Sci. 362, 2079-2093. <https://doi.org/10.1098/rstb.2007.2110>

598 Masman, D., Daan, S., Dietz, M., 1989. Heat increment of feeding in the kestrel, *Falco*
599 *tinnunculus*, and its natural seasonal variation. In: Physiology of cold adaptation in birds, ed.
600 C. Bech and R. E. Reinertsen. NewYork, NY, Plenum Press. pp 123-135.

601 Ponganis, P. J., Van Dam, R.P., Levenson, D.H., Knowler, T., Ponganis, K.V., Marshall, G., 2003.
602 Regional heterothermy and conservation of core temperature in emperor penguins diving
603 under sea ice. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 135, 477-487.
604 [https://doi.org/10.1016/S1095-6433\(03\)00133-8](https://doi.org/10.1016/S1095-6433(03)00133-8)

605 Ponganis, P.J., 2015. Diving physiology in marine mammals and seabirds. Cambridge
606 university press.

607 Roussel, D., Boël, M., Romestaing, C., 2018. Fasting enhances mitochondrial efficiency in
608 duckling skeletal muscle by acting on the substrate oxidation system. *J. Exp. Biol.* 221.
609 <https://doi.org/10.1242/jeb.172213>.

610 Scholander, P.F., 1940. Experimental investigations on the respiratory function in diving
611 mammals and birds. *Hvalrad- ets Skrifter* 22, 1-131.

612 Schmidt, A. 2006 Etude de la thermorégulation en mer chez le manchot royal : Mécanismes
613 et conséquences énergétiques. Thesis, University of Strasbourg.

614 Schmidt, A., Alard, F., Handrich, Y. 2006. Changes in body temperature in king penguins at
615 sea: the result of fine adjustments in peripheral heat loss? *Am. J. Physiol. Regul. Integr.*
616 *Comp. Physiol.* 291, 608-618. <https://doi.org/10.1152/ajpregu.00826.2005>

617 Seagram, R., Adams, N., Slotow, R., 2001. Time of feeding and possible associated
618 thermoregulatory benefits in bronze mannikins *Lonchura cucullata*. *Comp. Biochem. Physiol.*
619 *A. Mol. Integr. Physiol.* 130, 809-818. [https://doi.org/10.1016/S1095-6433\(01\)00407-X](https://doi.org/10.1016/S1095-6433(01)00407-X)

620 Stahel, C. D. and Nicol, S. C. (1982). Temperature regulation in the little penguin, *Eudyptula*
621 *minor*, in air and water. *J. Comp. Physiol. B* 148, 93-100.

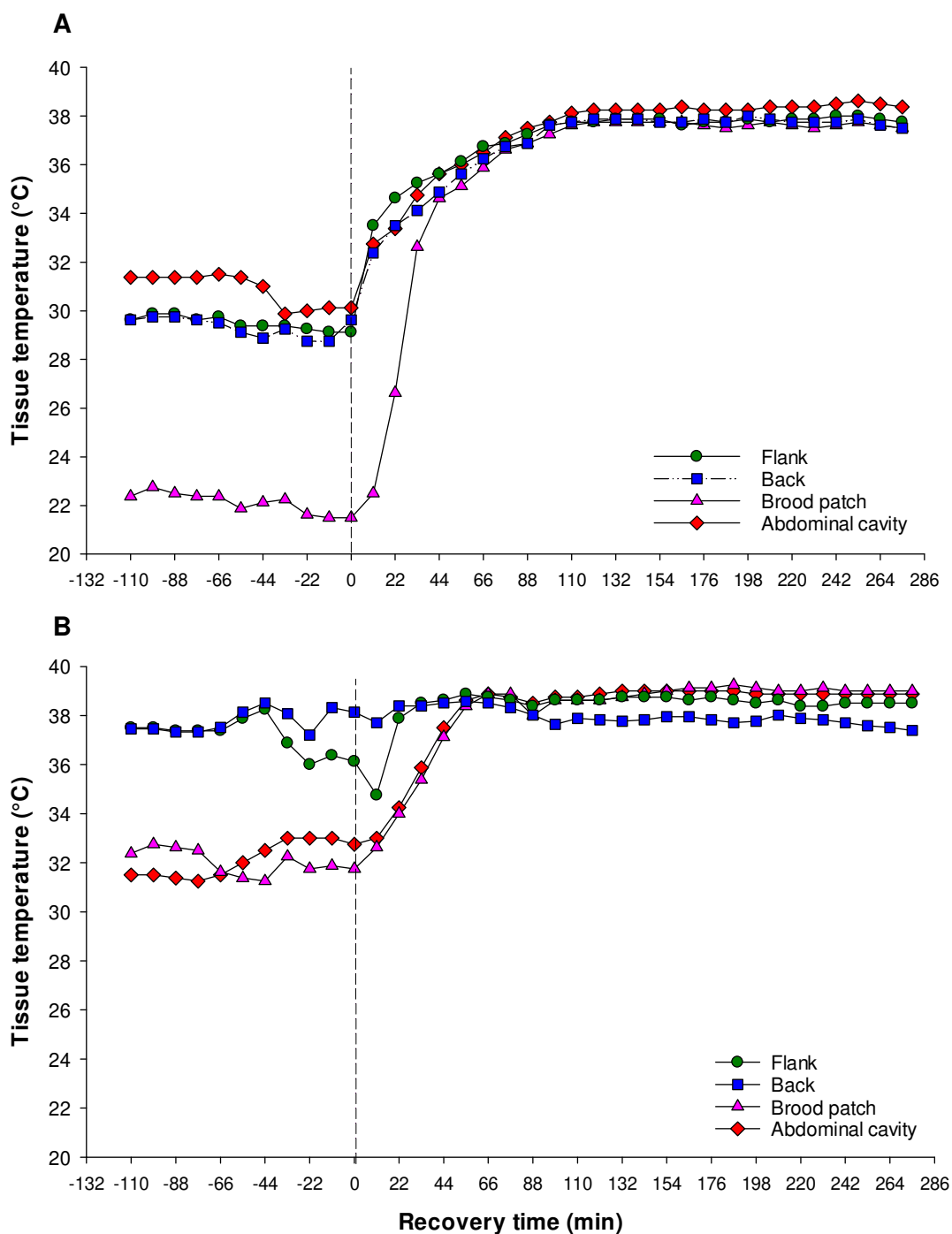
622 Thouzeau, C., Froget, G., Monteil, H., Le Maho, Y., Harf-Monteil, C., 2003. Evidence of stress
623 in bacteria associated with long-term preservation of food in the stomach of incubating king
624 penguins (*Aptenodytes patagonicus*). *Polar. Biol.* 26, 115-123.

625 Thouzeau, C., Peters, G., Le Bohec, C. and Le Maho, Y., 2004. Adjustments of gastric pH,
626 motility and temperature during long-term preservation of stomach contents in free-ranging
627 incubating king penguins. *J. Exp. Biol.* 207, 2715-2725.

628

629 **Supplementary material**

630 Fig. S1: Temperatures recorded subcutaneously in the flank, back, brood patch, and
631 abdominal cavity, in king penguin A (A) that weighed 9.33 kg after having lost 628 ± 0.19
632 $\text{g}\cdot\text{day}^{-1}$ during fasting (and might have been metabolizing protein), compared to king penguin
633 B (B) weighing 11.40 kg after having lost $335 \pm 0.08 \text{ g}\cdot\text{day}^{-1}$ over the same fasting period.
634 Both individuals were maintained fasting together in a sea water tank for four days, after
635 which they were allowed to recover to normothermia on land (starting at time = 0 min).



636