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RESEARCH ARTICLE

# Thermal strategies of king penguins during prolonged fasting in water

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## ABSTRACT

Most animals experience periods of unfavourable conditions, challenging their daily energy balance. During breeding, king penguins fast voluntarily for up to 1.5 months in the colony, after which they replenish their energy stores at sea. However, at sea, birds might encounter periods of low foraging profitability, forcing them to draw from previously stored energy (e.g. subcutaneous fat). Accessing peripheral fat stores requires perfusion, increasing heat loss and thermoregulatory costs. Hence, how these birds balance the conflicting demands of nutritional needs and thermoregulation is unclear. We investigated the physiological responses of king penguins to fasting in cold water by: (1) monitoring tissue temperatures, as a proxy of tissue perfusion, at four distinct sites (deep and peripheral); and (2) recording their oxygen consumption rate while birds floated inside a water tank. Despite frequent oscillations, temperatures of all tissues often reached near-normothermic levels, indicating that birds maintained perfusion to peripheral tissues throughout their fasting period in water. The oxygen consumption rate of birds increased with fasting duration in water, while it was also higher when the flank tissue was warmer, indicating greater perfusion. Hence, fasting king penguins in water maintained peripheral perfusion, despite the associated greater heat loss and, therefore, thermoregulatory costs, probably to access subcutaneous fat stores. Hence, the observed normothermia in peripheral tissues of king penguins at sea, upon completion of a foraging bout, is likely explained by their nutritional needs: depositing free fatty acids (FFA) in subcutaneous tissues after profitable foraging or mobilizing FFA to fuel metabolism when foraging success was insufficient.

**KEY WORDS: Thermoregulation, Seabirds, Subcutaneous fat, Normothermia, Fat mobilization, Heat loss**

## INTRODUCTION

Most animals experience periods of unfavourable conditions, where, e.g. climatic conditions and/or food scarcity might challenge their daily energy balance. If energy expenditure is exceeding energy intake, animals might be able to draw from previously stored energy (e.g. subcutaneous fat) and/or they might change their activity patterns in an attempt to reduce energy expenditure. In the long run, however, the energy budget will have to be balanced to avoid negative consequences for fitness and survival (Drent and Daan, 1980).

In birds, many species regularly encounter voluntary fasting periods (e.g. during reproduction), which range from a few days up to several months. The most extreme example is the 4-month fasting period of male emperor penguins (*Aptenodytes forsteri*) during courtship and incubation in the middle of the Antarctic winter, when air temperatures may reach as low as −40°C and wind speeds as high as 40 m $s^{-1}$ (Stonehouse, 1953; Le Maho et al., 1976). Accumulating fat depots before fasting periods and reducing energy use during these periods by reducing activity or the need for active heat production will all be important strategies to buffer these animals against starvation (Hohtola, 2012). For example, Tattersall et al. (2016) showed that fasting Muscovy ducklings (*Cairina moschata*) at thermoneutrality reduce blood perfusion to thermal windows (e.g. bill surfaces), thereby lowering thermal conductance, allowing birds to maintain their core temperature at lower energetic costs. Similarly, huddling in fasting emperor penguins is a well-known mechanism to reduce heat loss, reducing thermoregulatory costs (Gilbert et al., 2008). Furthermore, allowing a regulated drop in body temperature will reduce the costs of maintaining normothermia below thermal neutrality (Hohtola, 2012). This is due to: (1) a reduction in tissue metabolism due to the temperature dependence of metabolism ($Q_{10}$ effect); and (2) the declining temperature gradient between the animal and its surroundings, reducing heat loss (McCue, 2010). The ability of some bird species to lower their body temperature during periods of food limitation has been known for a long time (Chossat, 1843; Hohtola, 2012). Regulated hypothermia has been observed in many bird species, spanning a body mass ($M_b$) range of between <3 g and ca. 6500 g, and associated reductions in metabolic rate have also been reported (see McKechnie and Lovegrove, 2002 and McCue, 2010 for review).

In large birds, such as geese and penguins, core temperature reductions during fasting are typically small (Le Maho et al., 1981; Groscolas, 1986; Fahlman et al., 2005; but see Butler and Woakes, 2001 and Eichhorn et al., 2011). Nevertheless, these animals usually show a decline in their basal metabolic rate during fasting, most likely due to a decline in peripheral temperature, reducing the body volume that is maintained at normothermia (Dewasmes et al., 1980; Le Maho et al., 1981; Cherel et al., 1988; Fahlman et al., 2005).

During breeding, king penguins (*Aptenodytes patagonicus* Miller 1778) alternate shifts at the colony with foraging trips at sea. While extended fasting periods at sea are unlikely, birds might have to travel extensively to reach profitable foraging grounds, during which feeding might not be possible. During the Austral winter, the availability of their preferred prey (myctophid fish; Cherel and Ridoux, 1992; Raclot et al., 1998; Bost et al., 2002) declines (Kozlov et al., 1991), so that birds have to roam over greater distances and dive to greater depths to capture prey (Charrassin and Bost, 2001; Charrassin et al., 2002). Furthermore, Bost et al. (2015) showed that during the last decades, frequent large-scale climatic

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**List of abbreviations**

| | |
|---|---|
| FFA | free fatty acids |
| $M_b$ | body mass (kg) |
| RMR | resting metabolic rate |
| $s\dot{V}_{O_2}$ | mass-specific oxygen consumption rate (ml $min^{-1}$ $kg^{-1}$) |
| $T_w$ | water temperature (°C) |
| $\dot{V}_{O_2}$ | oxygen consumption rate (ml $min^{-1}$) |
| $\dot{V}_{CO_2}$ | $CO_2$ production rate (ml $min^{-1}$) |

anomalies occurred in the southern Indian and Atlantic Oceans that shifted the preferred foraging zone of king penguins southward, so that the distance birds had to travel and their foraging depth increased significantly. Hence, foraging might not always be profitable, so that the energy balance of birds on a daily basis might be negative. During these periods, birds have to rely on energy reserves, stored in the abdominal and subcutaneous adipose tissues (Cherel et al., 1994). However, access of these energy stores requires tissue perfusion, which will lead to a warming of these tissues. In the case of the subcutaneous fat store, this will increase the thermal gradient between the periphery and the surrounding water, increasing heat loss, which is considerably greater in water than in air (Kooyman et al., 1976; Dejours, 1987), elevating thermoregulatory costs. Hence, because of the dual function of their subcutaneous adipose tissue, which serves as an energy store but also as an important insulator, king penguins face a conflicting situation during periods of insufficient food intake at sea: (1) maintain peripheral vasoconstriction, as during diving, to reduce heat loss but potentially run out of fuel; or (2) vasodilate the periphery to access the fuel source but increase heat loss and thermoregulatory costs. For juvenile king penguins, during their first months at sea, such an energetic challenge might be crucial (Orgeret et al., 2016; Enstipp et al., 2017).

Previous studies investigated some of these issues in king penguins that floated inside a shallow water channel (at 4°C; Fahlman et al., 2005). During these short trials (~3 h), resting metabolic rate (RMR) was significantly greater in fasted birds when compared with fed birds, most likely because of differences in body insulation. However, when investigating the effect of total fasting duration on penguin RMR in water, Fahlman et al. (2004) found that total fasting duration (in air) had little effect on penguin RMR in water. As birds were maintained in air and only entered the water channel for relatively short periods at a time in these studies, the physiological responses of birds to extended fasting periods in water remain unclear.

Hence, our study investigated the conflicting situation encountered by king penguins when fasting for extended periods in cold water. Implanting temperature loggers into peripheral and deep tissues before birds underwent a specific protocol of fasting in air or water, we studied the changes of tissue temperatures associated with fasting in water as an index of tissue perfusion status. We also investigated the metabolic costs associated with fasting in water, the effect of fasting duration, and the relationship between peripheral tissue temperature and bird metabolic rate. We predicted that birds will initially maintain peripheral perfusion when fasting in water ('short' fast) to mobilize fuel but will reduce peripheral perfusion during a later fasting period ('long' fast). The latter prediction we based on the expectation that birds should maintain a critical fat layer thickness to avoid heat loss and thermoregulatory costs to spiral out of control, as has been suggested for seal pups (Øritsland et al., 1985). Finally, we compared the observed temperature changes of king penguins when fasting in water with changes that occur when birds are feeding in water (Lewden et al., 2017).

## MATERIALS AND METHODS

This study was conducted at the king penguin colony ('La Baie du Marin') on Possession Island, Crozet Archipelago (46°25′34″S, 51°51′36″E); a colony of ~16,000 breeding pairs (Delord et al., 2004). During three consecutive Austral summers, between November 2013 and March 2016, we captured a total of 17 male king penguins (identified by song; Jouventin, 1982) during the courtship phase of their breeding cycle, equipped them with temperature data loggers and maintained them for ~4 weeks each in a captive setting. All birds underwent a specific protocol that consisted of a series of feeding and fasting periods in air and/or in water. In a previous study, we investigated the physiological responses of king penguins to feeding, when maintained inside a shallow seawater tank (see Lewden et al., 2017). Here, we report our investigation into the physiological responses of king penguins to prolonged fasting in water. Both investigations were conducted as a single experiment and the experimental protocol was designed accordingly (Fig. 1). The aim of both investigations was to shed some light into the apparent trade-off between nutritional dynamics and thermoregulation of king penguins at sea. Hence, in the current study, we will contrast and discuss our results from both investigations. All experimental procedures were approved by the French ethics committee (APAFIS, permit no. 02015041411414001) and the French Polar Environmental Committee (permit no. 2013-76, 2014-121).

### Temperature loggers and surgical procedure

Individual penguins were equipped with four temperature loggers (iButton, MXMDS1922L-F5; Maxim Integrated, San Jose, CA, USA; resolution ±0.0625°C; range 0–50°C; diameter and height 17.4×5.9 mm; mass 2.9 g; recalibrated in a water bath with an absolute accuracy of ±0.1°C) at four distinct locations, which recorded instant temperature every 11 min. Three of the four temperature loggers were implanted into the subcutaneous fat layer within (1) the flank, (2) the back and (3) the brood patch, while the last logger (4) was placed into the abdominal cavity, lateral to the brood patch. The three peripheral loggers were positioned about halfway between the muscle and the skin, and care was taken to achieve comparable logger positioning in all birds. During experimentation, we also recorded ambient air temperature in the enclosure and water temperatures ($T_w$) within the tank (monitored with iButtons; in water submerged to 0.8 m). Temperature loggers were implanted on the day of capture under general anaesthesia, as detailed in Lewden et al. (2017). After surgery, birds were maintained inside a wooden enclosure (3×3 m, no roof) for an average of 6.0 ±0.1 days for recovery, before experimentation started.

### Experimental design

To investigate the physiological responses of king penguins to fasting in cold water, all birds underwent a specific protocol that consisted of two 2-day fasting periods inside a shallow seawater tank, which were separated by fasting periods of 4–7 days in air (see Figs 1 and 2 for an outline of the temporal organization of our experiment). Two birds at a time were maintained inside this shallow seawater tank (2.5×1.3×1.2 m; length, width and height, filled to a depth of 0.8 m) that was supplied continuously with fresh seawater from the adjacent bay (mean $T_w$ in tank: 7.7±0.9°C; see Lewden et al., 2017 for details). When initially introduced to the tank, birds conducted a few brief and shallow dives, after which they

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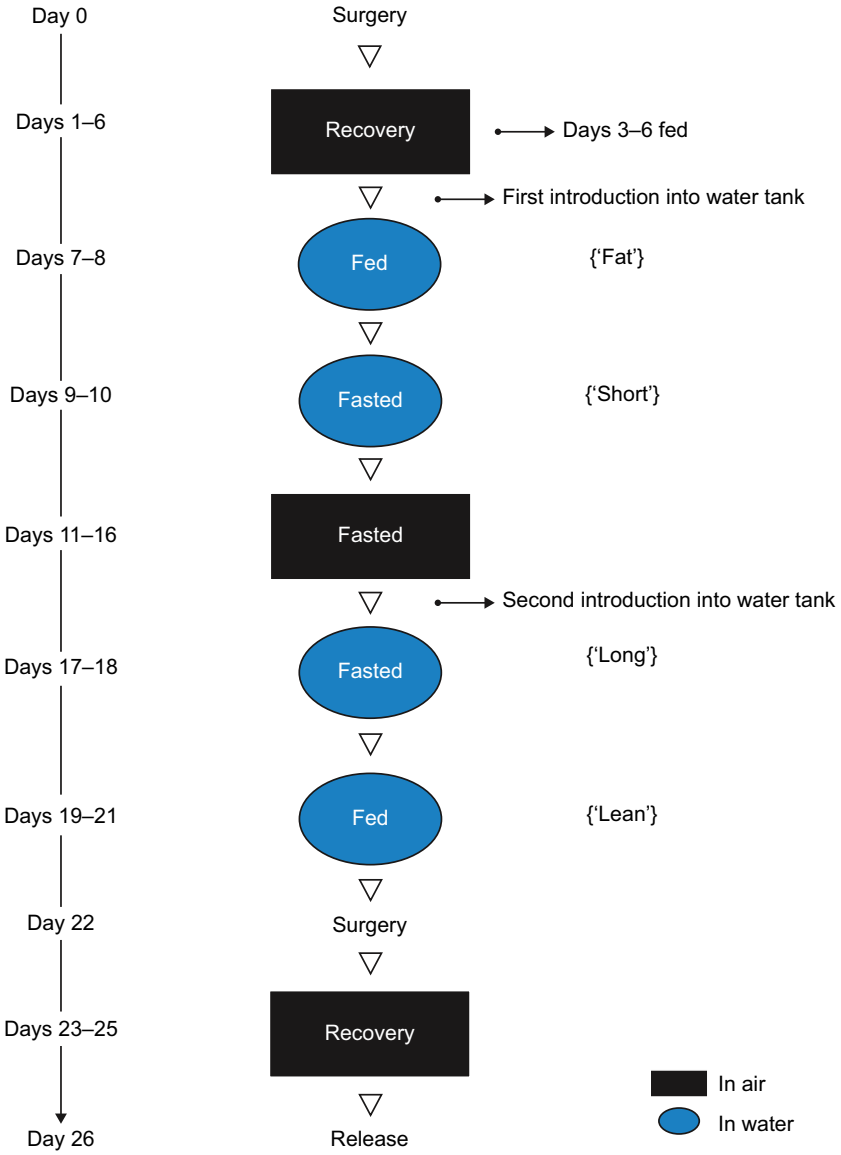


**Fig. 1. Flow chart illustrating the experimental protocol to investigate the physiological responses of king penguins to feeding and fasting in water.** The fasting duration in air differed between trials (range 7–10 days), while birds fasted on average for 10 days between feeding sessions in the tank (range 8–11 days). 'Fat' and 'Lean' refer to different body condition states during feeding trials in water (see Lewden et al., 2017), whereas 'Short' and 'Long' refer to different fasting durations in the current study.

typically floated calmly. No antagonistic behaviour between birds was observed. The experimental protocol was designed to facilitate both investigations (the physiological effect of feeding and fasting in water) and an idealized outline is presented in Fig. 1. However, the experimental protocol varied between years, allowing the effect of total fasting duration (in air and water) on peripheral perfusion to be investigated, as visible in Fig. 2: during year 1 ($N$=6), stage 2, the first fast in the water tank started with birds that had fasted in air for 6 days (Fig. 2A), whereas during year 2 ($N$=7), stage 2, started with birds that had been fed inside the water tank (Fig. 2B). Accordingly, fasting duration in air (7–10 days) and total fasting duration (11–14 days) varied between years, whereas the fasting time in water was identical in all cases, i.e. 4 days (Figs 1 and 2). The accumulative time spent fasting during each year (in air/water) is indicated in Fig. 2 ('Total fasting duration'). Concerning the current investigation, our protocol included four distinct periods, during which we recorded tissue temperatures in birds: (1) stage 1 (not shown in Fig. 2 and not included in our analysis), during which birds fasted for 6 days inside the wooden enclosure (but see above); (2) stage 2, during which birds spent two consecutive days fasting inside the water tank (experimental days I–II; 'short' fast); (3) stage 3, during which birds fasted for 4–7 days inside the wooden enclosure in air; and (4) stage 4, during which birds spent another two consecutive days fasting inside the water tank (experimental days III–IV; 'long' fast; Figs 1 and 2).

When inside the water tank, disturbance of birds was kept to a minimum. However, each bird was captured and removed briefly (<5 min) from the tank once every other day for weighing ($M_b$). During the third year ($N$=4 birds), the focus was on respirometry trials, which were conducted with single birds in the morning and afternoon. Accordingly, one of the birds was removed from the tank during these periods. During this last year, two birds also underwent a fasting period of 6 days in air before introduction to the water tank, while the other two birds were fed up to the day before introduction. Upon introduction during that year, birds were fasted within the water tank for a continuous period of up to 4 days.

### Respirometry trials

During the third year, we measured oxygen consumption rates ($\dot{V}_{O_2}$) in four penguins, when they floated calmly inside the seawater tank, using an open-circuit respirometry system ('Turbofox'; Sable Systems, Henderson, NV, USA). Respirometry trials were

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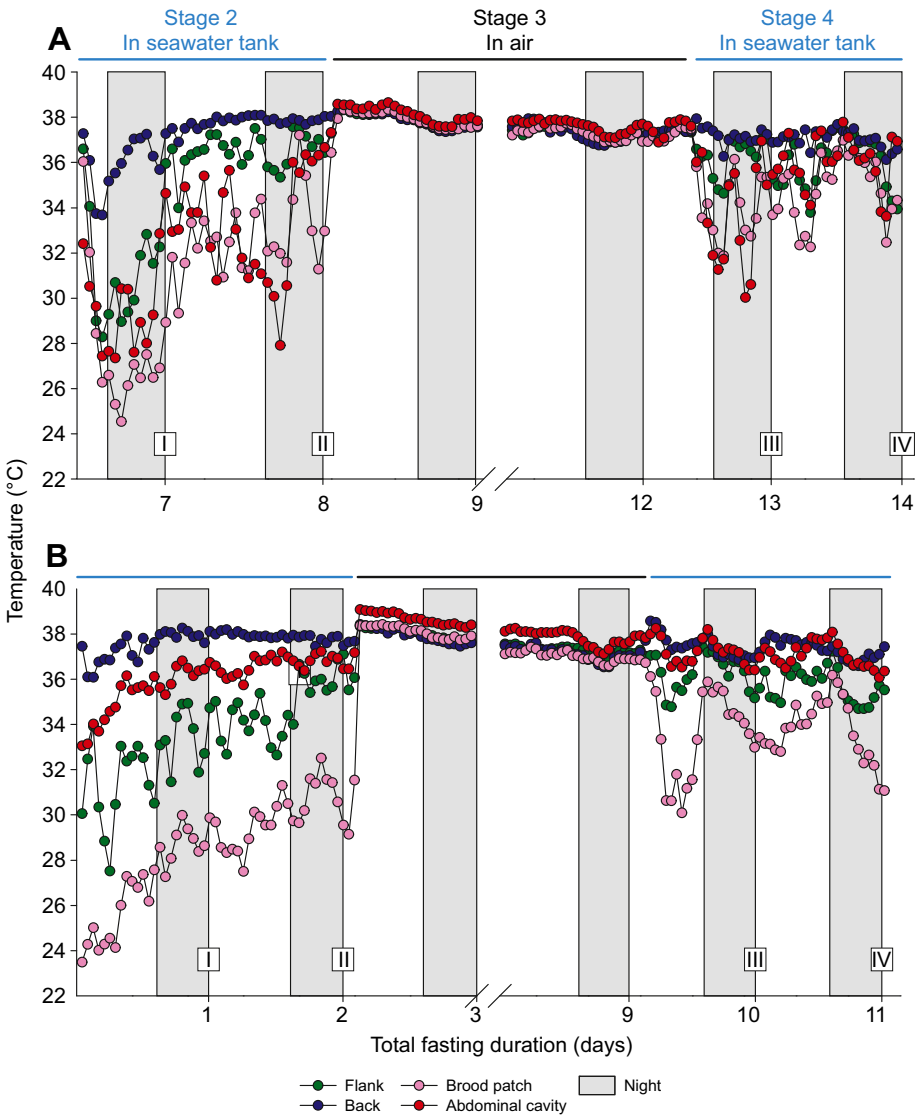


**Fig. 2. Tissue temperatures recorded in four tissues of king penguins throughout experimentation.** Birds fasted in a seawater tank during stages 2 and 4, while they fasted on land during stage 3. Values are hourly means shown separately for year 1 (A) and year 2 (B). *N*=6 and 7 birds for year 1 and year 2, respectively. The total fasting duration of birds (in water and air) is indicated in Arabic numbers (position of the numbers corresponds with the end of the indicated fasting day), while the fasting days in water (experimental days) are indicated by Roman numbers (I–IV). Night periods are indicated by the grey shading.

conducted throughout the fasting period, after birds had fasted between 0.58 and 3.91 days within the seawater tank (between 0.58 and 8.22 days of total fasting duration). We conducted a total of 13 trials (between two and six trials per bird) that lasted on average 1.5 h. Penguins were trained to remain and breathe inside a transparent Plexiglas dome in the shape of a diamond with a sloped ceiling (44×44×15–20 cm, long×wide×high; volume ∼30 l), which served as the respirometry chamber. The remaining water surface was covered with mesh, to prevent surfacing outside the chamber, in case birds submerged. The open bottom end of the chamber was slightly submerged within the seawater tank to provide a seal. Three small holes near the bottom of the chamber allowed ambient air to enter the dome. During a trial, the primary flow control unit of the Turbofox pulled air through the chamber at a rate of 50 l $min^{-1}$ [automatically corrected to standard temperature and pressure (STP), 273 K and 101.3 kPa]. A subsample (200 ml $min^{-1}$) was passed through a humidity meter (RH-300), a $CO_2$ analyser and a fuel cell oxygen analyser. Oxygen and $CO_2$ concentrations within the chamber, main flow rate through the chamber, humidity of the gas sample and barometric pressure were recorded every 4 s onto a laptop computer using ExpeData (Sable Systems). All connections between the various components of the respirometry system were made using gas-impermeable tubing. The gas analysers were calibrated before each trial using ambient air scrubbed of water vapour (magnesium perchlorate) and $CO_2$ (Ascarite and Soda lime; all chemicals from Sigma–Aldrich Chemie, Lyon, France), while the $CO_2$ analyser was spanned with 0.49% $CO_2$ (Alphagaz; Air Liquide, Paris, France). The humidity meter was calibrated weekly, according to recommendations of the manufacturer. We used wet and dried (using magnesium perchlorate) ambient air to set the span and zero water vapour pressure reading (kPa), respectively.

### Data analysis and statistics

In our analysis of body temperature variation, we first calculated mean hourly values for each bird and all tissues throughout stages 2–4 (experimental days I–II, fasting in air, and experimental days III–IV), from which we calculated grand means from all birds during year 1 and year 2 (Fig. 2). For our statistical analysis, values from all birds during the first two years were then pooled (*N*=13 birds), while the temperature values from the four birds during year 3 were excluded from this part of the analysis, as the experimental protocol differed (focus on respirometry). In a second step, we calculated mean tissue temperatures for each experimental day, split into daytime (08:00 h to 22:00 h) and nighttime periods (22:00 h to 08:00 h). As tissue temperatures declined strongly when birds were

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first introduced to the water tank, after which they recovered (Fig. 2), we excluded the daytime part of experimental days I and III from our statistical analysis. Thirdly, to investigate temperature variations throughout the penguin body during fasting, we selected the highest (maximum; in air) and highest/lowest (maximum/minimum; in water) mean hourly temperatures recorded across all tissues (grand means from all birds) throughout the fasting period. Temperature variations were investigated separately for the fasting periods in air (stage 3) and in water (stages 2 and 4). In a further step, we focused on the flank tissue and investigated episodes when flank tissue temperatures of birds were ≥36°C while fasting in water (stages 2 and 4), which we defined as normothermic events. For these events, we recorded the maximum temperature per event, event duration (minutes) and the total number of events per day/night period. From this, we calculated the mean maximum flank temperature during these episodes for day and night periods (experimental days I–IV) and their cumulative duration (expressed as a percentage of day/night periods spent at normothermia). Lastly, to compare tissue temperature changes in king penguins associated with fasting conditions with those of feeding (see Lewden et al., 2017), we plotted the mean hourly temperatures for all tissues from all birds (grand means from 13 birds) when fasting throughout stages 2–4 in comparison with those of seven birds that were fed throughout these stages (data from Lewden et al., 2017).

Respirometry data were analysed using ExpeData (Sable Systems). Gas analyser drift and lag time of the respirometry system were corrected for. Main flow rate was corrected to STP dry (STPD) using eqn 8.6 in Lighton (2008). Similarly, we did not scrub water vapour before gas analysis but corrected for this dilution effect during data analysis using eqn 15.3 in Lighton (2008). Oxygen consumption rate ($\dot{V}_{O_2}$) and $CO_2$ production rate ($\dot{V}_{CO_2}$) were calculated using eqns 11.7 and 11.8 in Lighton (2008), respectively. To investigate the link between fasting time inside the water tank and $\dot{V}_{O_2}$, we selected a stable 10 min segment of $\dot{V}_{O_2}$ data from each respirometry trial, when birds floated calmly inside the respirometry chamber, to represent the metabolic rate of the bird for that trial. To investigate the link between flank temperature and metabolic rate, when birds fasted inside the water tank, we selected multiple periods throughout each trial when $\dot{V}_{O_2}$ was stable for at least 5 min ($n$=12 observations in six trials, $N$=1 bird) and plotted $\dot{V}_{O_2}$ against the corresponding flank temperatures for each period. As instantaneous temperatures were recorded every 11 min, we linearly interpolated between two recorded values to acquire temperature values for each minute. Unfortunately, the temperature logger inside the flank tissue failed in three out of the four birds that participated in respirometry trials. Hence, we were only able to investigate this relationship in one bird. $\dot{V}_{O_2}$ values are presented mass specifically as $s\dot{V}_{O_2}$ (ml $min^{-1}$ $kg^{-1}$).

All statistical analyses were conducted in JMP® (Version Pro 11.2.0, SAS Institute Inc., Cary, NC, USA). All investigations concerning: (1) $M_b$ changes; (2) the effects of various parameters on tissue temperatures; (3) the effect of fasting time in water/total fasting duration on $\dot{V}_{O_2}$; and (4) the relationship between flank temperature and $\dot{V}_{O_2}$ were conducted using linear mixed models (LMMs). Where appropriate, interaction terms were first included in the respective model and removed if not significant. If interactions were significant, we conducted Tukey's HSD test for *post hoc* analysis.

In our investigation of $M_b$ changes, we included year and stage (capture, after stage 2, after stage 4) and the interaction year×stage as fixed effects, while bird ID was included as a random effect. To test for the effects of various parameters on the temperatures of tissues throughout fasting periods in water, we ran separate LMMs for each tissue, including experimental day (I–IV) as a non-linear factor, year, period (day/night), $M_b$ loss and $T_w$ as fixed effects, while bird ID was included as a random effect. Tukey's HSD test was used for *post hoc* analysis. To distinguish between the effects of total fasting duration (in air and water) and fasting duration in water only (experimental day) on tissue temperatures, we ran separate LMMs for each tissue, testing their effects on tissue temperatures during experimental days III–IV. Similarly, LMM analysis was used to test for the effect of fasting time in water/total fasting duration (fixed effect) on bird oxygen consumption and for the relationship between flank temperature and oxygen consumption (fixed effect). Significance for all statistical tests was accepted at $P$<0.05. All mean values are presented with standard error (s.e.m.).

## RESULTS

$M_b$ of king penguins at first capture was higher during the first year (13.8±0.3 kg), when compared with the second year (12.4±0.3 kg, $P$<0.0001). However, $M_b$ development throughout experimentation did not differ between years ($P$=0.9, *post hoc* Tukey's HSD). $M_b$ declined significantly between first capture (13.1±0.2 kg) and stages 2 (12.1±02 kg) and 4 (11.0±0.2 kg) ($P$<0.0001; $N$=13 birds, $n$=39 observations).

### Tissue temperature variations

When fasting in cold water, the thermal response was complex. During stage 2 (experimental days I–II), the mean hourly temperatures of all tissues increased (Fig. 2), after the initial decline associated with introduction to the water tank (Fig. 2A). During the second period inside the water tank (stage 4; experimental days III–IV), temperatures of all tissues declined again upon introduction to the water tank but to a lesser extent than during stage 2 (Fig. 2). Hereafter, tissue temperatures recovered quickly and generally stabilized at an elevated level, while frequent temperature oscillations continued to occur, especially in the brood patch and the flank tissue (Fig. 2). This general pattern of temperature development becomes even clearer when looking at the mean temperature values computed by LMM analysis for each experimental day (Fig. 3, data from both years merged). This shows that temperatures for all tissues except the back tissue increased significantly throughout stage 2, while during stage 4 they were maintained at an elevated level ($P$<0.0001 for the flank and brood patch, $P$=0.02 for abdominal cavity; Table 1). In the back tissue, temperature was elevated throughout experimental days I–III and declined marginally but significantly during experimental day IV (Fig. 3B; $P$=0.003; Table 1). Our LMM analysis further showed that experimental day (i.e. fasting duration in water) had the strongest effect on tissue temperatures, while other factors considered were less consistent (year, period, $M_b$ loss, $T_w$; Table 1). A further LMM analysis showed that tissue temperatures during stage 4 (experimental days III–IV) were not affected by total fasting duration and did not differ between birds that had fasted for 11 or 14 days ($P$=0.39 for the flank, $P$=0.28 for the brood patch, $P$=0.11 for abdominal cavity and $P$=0.08 for the back). Of all the tissues investigated, the back tissue clearly contrasted with the rest, as its temperature remained fairly stable throughout fasting in water, with slightly lower mean temperatures during stage 4 (when compared with stage 2; Fig. 3B) and during the night, when compared with the day (37.2±0.1°C versus 37.6±0.2°C). Plotting the maximum and minimum mean hourly temperatures observed across tissues against total fasting duration, when birds fasted in water (stages 2 and 4), shows that the maximum temperatures declined slightly with fasting

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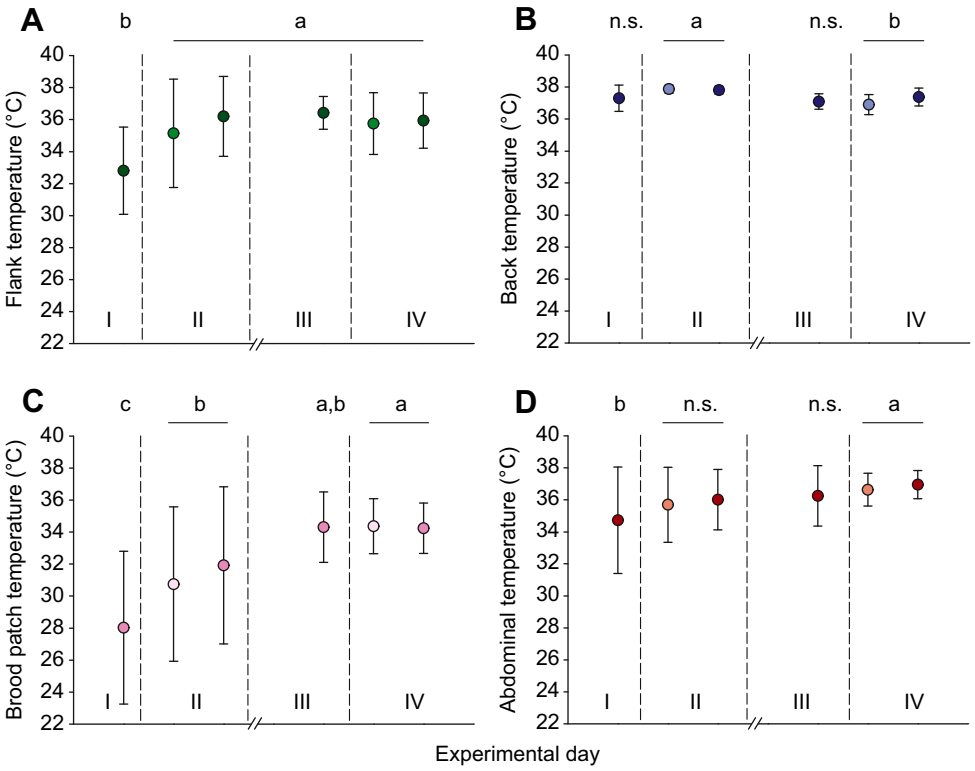


**Fig. 3. Marginal means (±s.e.m.) of temperatures for four tissues in 13 king penguins while fasting in water.** Values for the flank (A), the back (B), the brood patch (C) and the abdominal cavity (D) are shown, which were computed from separate LMMs for daytime (light colour) and nighttime periods (dark colour; Table 1, see text). Values that do not share the same letter are significantly different from each other (*post hoc* Tukey's HSD test; $P<0.05$). n.s., not significant.

duration ($P<0.0001$ for year 2), while at the same time, the minimum temperatures increased ($P<0.0001$ for both years; Fig. 4A). In the latter case, temperature increased on average by 0.56°C per day fasting (total fasting duration).

In the flank tissue, the number of normothermic events (periods with temperatures ≥36°C) increased with the number of fasting days spent in water (experimental days I–IV; Table 2). This is true for day and night periods, while, in general, a greater number of events was observed during the day, when compared with the night. The relative time spent at normothermia during the day and night also increased during stage 2 (experimental days I–II), while it levelled off or even decreased slightly during stage 4 (experimental days III–IV; Table 2). In some individuals, flank temperature was at normothermia for entire day and/or night periods. Mean maximum temperature during these normothermic events was similar throughout all fasting days in water, ranging between 37.6°C and 38.0°C (Table 2).

By contrast, when fasting in air (stage 3), temperatures of all tissues investigated declined over time (Fig. 2). Plotting the highest mean hourly temperatures observed across tissues against total fasting duration shows that these temperatures declined significantly over time during both years (stage 3, fasting in air; Fig. 4A; $P<0.0001$ for both years). The average temperature decline was −0.19°C per day fasting (total fasting duration).

**Table 1. Effects of various parameters on tissue temperature variations in king penguins fasting in water (LMM analysis)**

| | Flank $R^2$=0.52 | Back $R^2$=0.54 | Brood patch $R^2$=0.70 | Abdominal cavity $R^2$=0.59 |
|---|---|---|---|---|
| Experimental day | <0.0001*** | 0.0029** | <0.0001*** | 0.0235** |
| Year | n.s. | n.s. | n.s. | 0.0139* |
| Period (day versus night) | n.s. | 0.0230** | n.s. | n.s. |
| $M_b$ loss | n.s. | n.s. | 0.0125 | n.s. |
| $T_w$ | n.s. | 0.0433 * | n.s. | n.s. |

The model was run separately for each tissue with bird ID as a random factor. *$P<0.05$; **$P<0.005$; ***$P<0.001$. $M_b$, body mass; $T_w$, water temperature; n.s., not significant.

### Metabolism

We found a strong positive relationship between the $s\dot{V}_{O_2}$ of birds when floating in the tank and both total fasting duration (in air and water; $P=0.0012$) and their fasting time in water only ($P=0.0008$; Fig. 5A). During experimental day I, $s\dot{V}_{O_2}$ averaged 20.1±7.2 ml min$^{-1}$ kg$^{-1}$, while during experimental day IV it averaged 45.4±0.3 ml min$^{-1}$ kg$^{-1}$. We also observed a significant positive relationship between $s\dot{V}_{O_2}$ and flank temperature, when birds fasted inside the water tank, so that $s\dot{V}_{O_2}$ was higher when flank temperature was higher ($N=1$ bird, $P=0.01$; Fig. 5B). Lastly, flank temperature during these selected periods increased with fasting duration in water ($N=1$ bird, $P<0.0001$).

### Fasting versus feeding

Comparing the tissue temperature development of birds during stages 2 and 4 (in water) when fasting (current study) and when feeding (Lewden et al., 2017) shows that temperatures of all tissues were higher in the fasting condition, when compared with the fed condition (Fig. 6). When maintained in air (stage 3), the temperature of all tissues declined slowly over time (Fig. 6). During this latter stage, birds were fasted in both investigations and, hence, no comparison with a fed condition is possible.

## DISCUSSION

Our study found that deep and peripheral tissue temperatures of king penguins increased during the initial 2-day fasting period in water, and were maintained at a near-normothermic level during the second, later fasting period in water (Figs 2 and 3). Similarly, when fasting in water, minimum tissue temperature across all tissues

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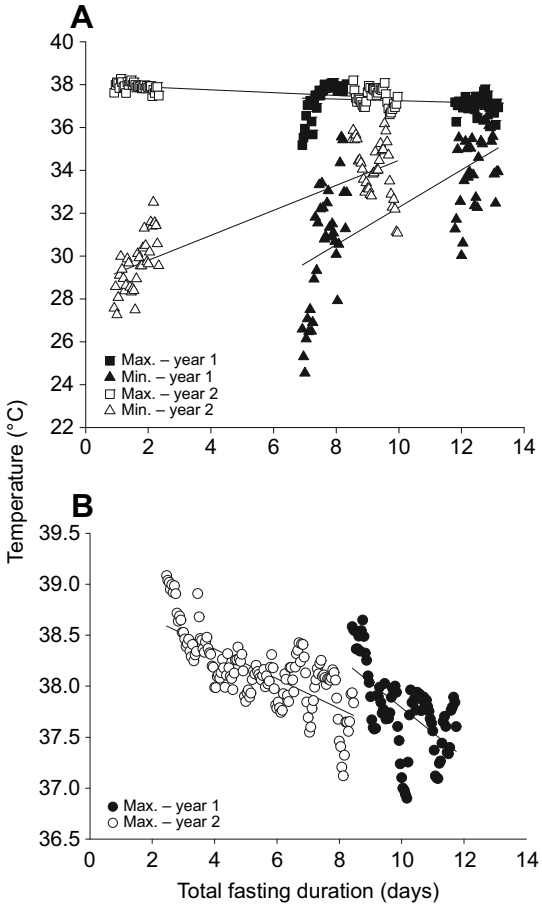


**Fig. 4. Maximum and minimum tissue temperatures of king penguins when fasting in water or in air.** Values are based on hourly means selected across tissues and are grand means taken from all birds plotted against total fasting duration (in air and water). (A) Temperatures when fasting in water (stages 2 and 4), whereas (B) shows the temperatures when fasting in air (stage 3). Values are shown separately for year 1 (*N*=6 birds, closed symbols) and year 2 (*N*=7 birds, open symbols). The solid lines indicate the relationships established from LMM analysis.

increased significantly throughout total fasting duration, while maximum tissue temperature declined slightly (Fig. 4A). This indicates that, contrary to our prediction, birds maintained perfusion to peripheral tissues not only during the 'short' fasting period (stage 2) but also during the 'long' fasting period in water (stage 4). Our respirometry results showed that the s$\dot{V}_{O_2}$ increased with both total fasting duration (in air and water) and fasting duration in water only (Fig. 5A). Similarly, a higher flank tissue temperature (indicating greater perfusion) was accompanied by a greater s $\dot{V}_{O_2}$ (Fig. 5B). Hence, fasting king penguins maintained peripheral perfusion when resting in water, despite the associated greater heat loss and, therefore, thermoregulatory costs. This is most likely explained by their need to access subcutaneous fat stores to fuel metabolism. By contrast, when fasting in air (stage 3), temperatures in all tissues slowly declined with total fasting duration, albeit from an elevated level, and overall temperature decline was small (Figs 2 and 4B).

### Tissue temperature variations

When king penguins were introduced to the water tank during the day, the temperatures of all tissues monitored declined initially (experimental days I and III in Fig. 2A and experimental day III in Fig. 2B; during experimental day I in the latter figure, birds had

**Table 2. Normothermic events recorded in the flank tissue of king penguins fasting in water during stages 2 and 4**

| | Number of events ≥36°C | | | | | | | | Mean maximum temperature during events ≥36°C | | | | | | | | Relative duration (%) of events ≥36°C | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experimental day | 1 | | 2 | | 3 | | 4 | | 1 | | 2 | | 3 | | 4 | | 1 | | 2 | | 3 | | 4 | |
| Day/Night | D | N | D | N | D | N | D | N | D | N | D | N | D | N | D | N | D | N | D | N | D | N | D | N |
| Individual ID | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | 2 | 2 | 2 | | 3 | 1 | 1 | | 37.3 | | 38.0 | 37.9 | 37.5 | | 38.5 | 38.4 | | | 22 | 56 | 41 | | 59 | 97 | 100 |
| 2 | 3 | 1 | 1 | | 2 | 4 | 2 | | 37.2 | | 37.9 | 38.3 | 37.5 | | 37.7 | 37.7 | | | 36 | 95 | 100 | | 99 | 84 | 81 |
| 3 | 1 | 3 | 2 | | 2 | 2 | 1 | | 37.3 | | 37.6 | 38.0 | 37.7 | | 37.8 | 37.5 | | | 21 | 80 | 96 | | 97 | 44 | 73 |
| 4 | 0 | 5 | 3 | | 4 | 3 | 2 | | | | 37.8 | 38.0 | 37.3 | | 37.5 | 36.9 | | | 0 | 79 | 88 | | 65 | 28 | 36 |
| 5 | 2 | 5 | 1 | | 1 | 4 | 1 | | 37.0 | | 37.5 | 37.9 | 38.0 | | 38.1 | 38.1 | | | 46 | 70 | 90 | | 100 | 86 | 100 |
| 6 | 1 | 5 | 1 | | 4 | 6 | 6 | | 37.5 | | 37.6 | 37.9 | 37.0 | | 37.5 | 36.8 | | | 17 | 71 | 100 | | 95 | 65 | 70 |
| 7 | 1 | 1 | 2 | | 1 | 1 | 2 | | 38.1 | | 38.0 | 37.8 | 38.1 | | 38.4 | 37.8 | | | 69 | 100 | 89 | | 100 | 100 | 92 |
| 8 | 3 | 3 | 2 | | 2 | 3 | 2 | | 37.6 | | 37.8 | 37.8 | 38.7 | | 38.3 | 38.4 | | | 28 | 19 | 24 | | 41 | 20 | 22 |
| 9 | 2 | 1 | 1 | | 1 | 6 | 3 | | 38.4 | | 38.8 | 38.7 | 38.6 | | 37.7 | 38.1 | | | 84 | 100 | 100 | | 100 | 79 | 96 |
| 10 | 1 | 3 | 2 | | 1 | 3 | 1 | | 37.2 | | 37.6 | 37.7 | 37.4 | | 37.1 | 37.5 | | | 13 | 94 | 76 | | 70 | 70 | 64 |
| 11 | 1 | 1 | 1 | | 2 | 2 | 1 | | 38.2 | | 38.0 | 38.2 | 37.4 | | 37.3 | 37.6 | | | 100 | 100 | 100 | | 25 | 22 | 19 |
| 12 | 0 | 1 | 2 | | 2 | 4 | 3 | | | | 37.3 | 38.0 | 37.2 | | 37.2 | 36.5 | | | 0 | 8 | 91 | | 99 | 86 | 59 |
| 13 | 2 | 2 | 3 | | 1 | 5 | 2 | | 38.0 | | 38.0 | 38.1 | 38.1 | | 37.0 | 37.1 | | | 26 | 18 | 64 | | 97 | 83 | 57 |
| Total | 19 | 33 | 23 | | 26 | 44 | 27 | Mean | 37.6±0.4 | | 37.8±0.3 | 38.0±0.2 | 37.7±0.4 | | 37.7±0.4 | 37.6±0.5 | Mean | | 36 | 68 | 81 | | 81 | 66 | 67 |

Indicated are the number of events per day/night period, the mean maximum temperature during these events and the relative time per day/night period spent in normothermia (%). Note that the initial day periods during experimental days I and III were excluded to avoid bias due to the disturbance associated with introduction to the pool.



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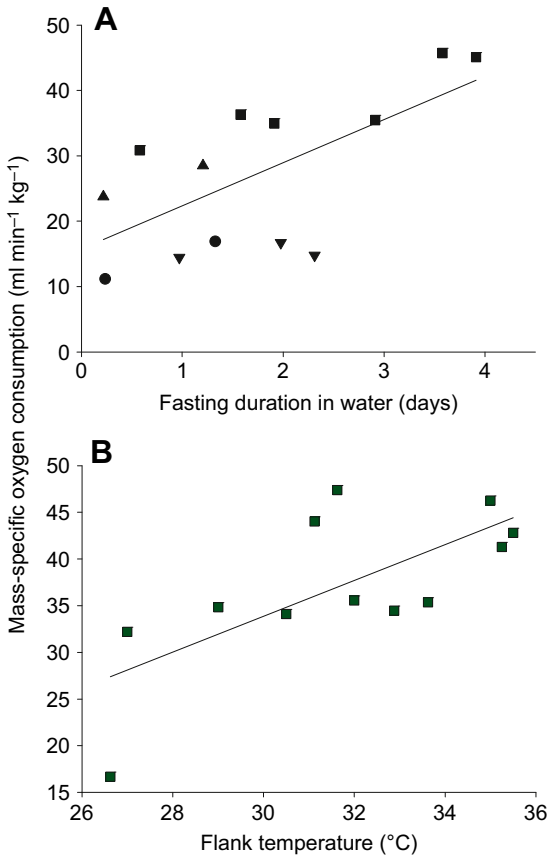


**Fig. 5. Mass-specific oxygen consumption rate ($s\dot{V}_{O_2}$) of king penguins when floating inside the water tank.** (A) $s\dot{V}_{O_2}$ (ml $min^{-1}$ $kg^{-1}$) versus fasting time in water (experimental days I–IV; *N*=4 birds, *n*=13 trials). Different symbols represent different birds. (B) $s\dot{V}_{O_2}$ during selected 5-min periods, when the bird floated calmly inside the water tank, and the corresponding flank temperature during the fasting period in water (*n*=12 observations in six trials, *N*=1 bird, green squares). The solid lines indicate the relationships established from LMM analysis.

already been inside the water tank for 2 days and, hence, temperatures did not decline further). However, during the following night, all tissue temperatures recovered and often approached normothermic values during experimental day II (i.e. abdomen, flank and back). During the second 2-day fast in water (stage 4), the initial temperature drop upon introduction to the water tank was smaller, and temperatures recovered quickly and were maintained at near-normothermic levels (experimental days III–IV; Figs 2 and 3). The temperature development of the back tissue during fasting in water differed from all other tissues. The temperature of the back, in general, changed little and was maintained at a high level throughout fasting in water, although it declined slightly during stage 4 (Figs 2 and 3). This is most likely explained by the observation that the back tissue was above water almost the entire period the birds spent in water, while all other tissues, in which temperature was monitored, were permanently submerged (see fig. 1 in Lewden et al., 2017). Accordingly, the heat conservation scenario must have differed substantially between the back and all other tissues. It is, therefore, not surprising that temperatures of the back tissue, when birds were inside the water tank, followed a trajectory similar to that of all other tissues during fasting in air (Fig. 2). This illustrates the different responses of birds to fasting, depending on context (air versus water) and underlines the great importance of regional heterothermia to lower energetic costs (Handrich et al., 1997; Eichhorn et al., 2011).

While temperatures in the flank, abdomen and brood patch on average increased during stage 2 and were then maintained during stage 4, when penguins fasted in water, such description ignores the frequent temperature oscillations (warming and cooling cycles) we observed in all of these tissues (but not in the back), which were especially pronounced in the flank (Fig. 2B). Such temperature oscillations might reflect complex changes in underlying neurophysiological adjustments (Tattersall et al., 2016). A study investigating the thermoregulatory responses of Muscovy ducklings to cold and fasting found that, in cold-acclimated birds, the frequency of short-term body temperature fluctuations was reduced during fasting (Tattersall et al., 2016). This was interpreted by the authors as a novel energy-saving mechanism: a reduction in short-term body temperature fluctuations was suggested to lead to a profound reduction in the estimated energetic costs of warming, independent of the metabolic savings associated with diurnal body temperature changes. While we did not investigate short-term temperature fluctuations in our penguins, we observed a significant increase in minimum tissue temperature during fasting (when birds were inside the water tank), which was accompanied by a small decrease in maximum tissue temperature (Fig. 4). Such temperature development might indicate a change in the amplitude of temperature fluctuations.

Frequent temperature oscillations, especially in the flank tissue, are also illustrated in Table 2, which indicates the number, relative duration and mean maximum temperature during events, when temperature was equal to or exceeded 36°C in the flank (normothermic events). The number of these events increased in birds throughout the fasting period in water during both day and night periods, while the relative time spent in normothermia also increased up to experimental day III, after which it declined (Table 2). Similar to what has been observed in free-ranging king penguins during their resting periods between foraging bouts at the sea surface, the normothermic periods observed in the flank tissue of our inactive captive birds often lasted for extended periods (i.e. the entire night or day in some individuals; Table 2; Schmidt et al., 2006; Enstipp et al., 2017). The mean maximum flank temperature during normothermic events was similar throughout the fasting period in water (Table 2), indicating that the higher mean hourly tissue temperatures during stage 4 (‘long’ fast), when compared with stage 2 (‘short’ fast), are not merely a consequence of differences in body condition and insulation during these two stages (see Enstipp et al., 2017).

A number of studies has investigated the physiological mechanisms underlying the great fasting capacity of penguins in air (Dewasmes et al., 1980; Le Maho et al., 1976; Cherel et al., 1988, 1994; Eichhorn et al., 2011). When fasting in air, the deep temperature of king penguins is typically maintained or decreases only to a small extent (Fahlman et al., 2005; Viblanc et al., 2014; but see Eichhorn et al., 2011 for fasting chicks), while a reduction in metabolism is mostly achieved through a decline in thermal conductance (e.g. regional heterothermia) and a general decline in activity (Eichhorn et al., 2011; Viblanc et al., 2014). In our study, we also observed a small but persistent decline in all tissue temperatures when birds fasted in air (stage 3, Fig. 2), so that the maximum temperature recorded in any of the tissues monitored declined by ∼1°C over the course of 12 days (Fig. 4B). By contrast, few studies have investigated the physiological responses of fasting penguins when floating on water (Fahlman et al., 2004, 2005). When king penguins were introduced to a shallow water channel, abdominal and subcutaneous temperatures declined immediately (Fahlman et al., 2005). That temperature decline was more pronounced in fasted than in fed or re-fed birds, and the higher

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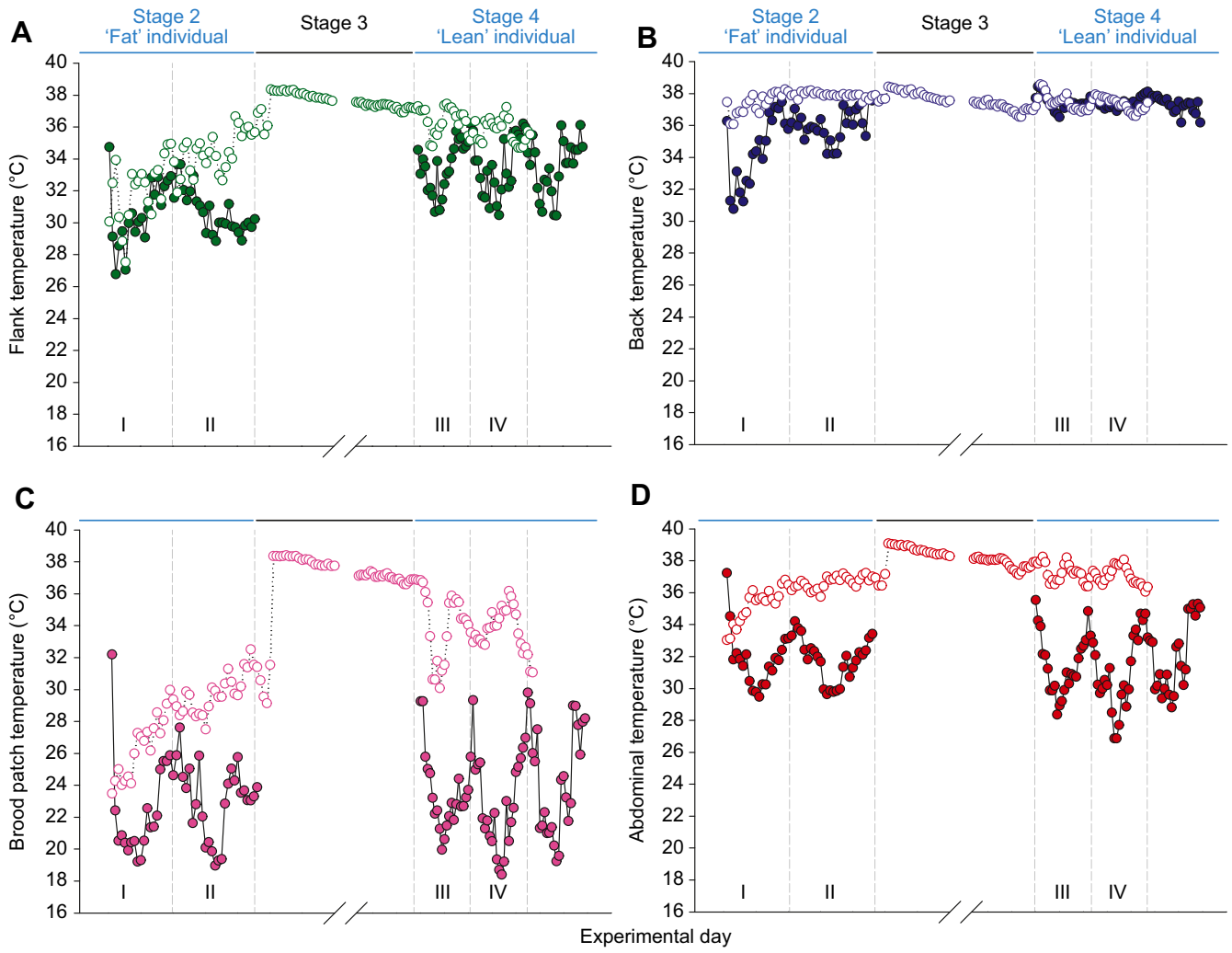


**Fig. 6. Tissue temperatures of king penguins during fasting and feeding trials.** Values are hourly means and show temperatures from the four tissues when birds either fasted (open symbols; *N*=13 birds) or were being fed (closed symbols; *N*=7 birds). Values for the fed condition are from Lewden et al. (2017). 'Fat' and 'lean' individuals during stages 2 and 4 (in water) refer to birds with a higher and lower body mass, respectively, which corresponds to a short and long fasting duration before treatment (fasting/fed), respectively. Note that during stage 3 (in air) birds were fasted in both investigations and only values for the present investigation are shown.

$\dot{V}_{O_2}$ of fasted birds under these conditions, when compared with the others, were attributed to their lower body condition/body insulation (Fahlman et al., 2005). However, birds were only maintained inside the water channel for ∼3 h, so that no tissue rewarming could be observed (Fahlman et al., 2005). Furthermore, total fasting duration of penguins (in air) had little effect on their RMR in water (Fahlman et al., 2004). Our results are in general agreement with the findings of these latter two studies. For example, our study also showed that the effect of total fasting duration (in air and water) on penguin temperature development was negligible, when compared with fasting duration in water only. However, because we maintained king penguins inside a water tank for a prolonged period (4 days in total), we were able to investigate the thermal responses of king penguins to fasting in water in greater detail than previous studies.

Our study found a significant increase in deep and peripheral tissue temperatures of king penguins when fasting in water over time, indicating the maintenance of peripheral perfusion. The increase in deep tissue temperature (abdominal cavity) is likely to be a consequence of the increase in metabolic rate with fasting duration and not linked with changes in perfusion. The higher tissue temperatures during experimental days II–IV, when compared with experimental day I, suggest that birds were in a greater need to access subcutaneous fat stores to fuel metabolism during the later part of their fasting period in water. We predicted that birds will maintain peripheral perfusion during the short fast (first 2-day fasting period in water), while we expected that birds might reduce peripheral perfusion during the long fast (second 2-day fasting period) to reduce heat loss. Hence, our results are in agreement with our first prediction, whereas we did not observe a reduction in peripheral perfusion (as indicated by tissue temperatures) during the later fasting period. The latter might be explained by an overall too short fasting duration in our experiment, so that the body condition/insulation of birds (critical fat layer thickness; Øritsland et al., 1985) was still sufficient to balance the heat loss associated with peripheral perfusion. This is supported by the $M_b$ of our birds at the end of experimentation (11.0±0.2 kg), which was still above the critical $M_b$ of ∼10 kg. At this latter point, birds enter into phase III of fasting, which is associated with a switch from a predominant lipid metabolism to protein catabolism (Cherel et al., 1988). The decline in the relative duration of normothermic events observed in the flank tissue during experimental day IV (on average 67%), when compared with experimental day III (on average 81%), might

indicate the beginning of a switch in thermal strategy (i.e. peripheral vasoconstriction to avoid excessive heat loss).

### Metabolism

Our respirometry trials showed two important points: (1) the $s\dot{V}_{O_2}$ of birds when floating in the water tank increased with the time they spent fasting in water (Fig. 5A); and (2) when fasting in water, higher flank temperatures of birds were associated with a greater $s\dot{V}_{O_2}$ (Fig. 5B). The increase in peripheral temperatures we observed during stage 2 (experimental days I–II) and their maintenance at an elevated level during stage 4 (experimental days III–IV) clearly suggest that birds maintained peripheral perfusion during these periods. Accordingly, heat loss must have been increased during this time, so that thermoregulatory costs must have also been elevated. This is illustrated by the positive relationship between flank temperature and $s\dot{V}_{O_2}$; a higher flank temperature (reflecting a greater perfusion level) was associated with a greater $s\dot{V}_{O_2}$ (Fig. 5B). Hence, the increase in $s\dot{V}_{O_2}$ with fasting duration in water is most likely explained by the increase/maintenance of peripheral perfusion but also by a decline in body condition and, therefore, body insulation, as birds presumably reduced their subcutaneous fat layer to fuel metabolism (Cherel et al., 1994; Fahlman et al., 2005; Enstipp et al., 2017).

$s\dot{V}_{O_2}$ of king penguins measured in our study during their first hours of fasting in water (17.5 ml $min^{-1}$ $kg^{-1}$; mean $T_w$ ~8°C) was similar to previously reported measurements under comparable conditions (Culik et al., 1996a; Fahlman et al., 2004, 2005). However, while we observed a significant increase in $s\dot{V}_{O_2}$ throughout the fasting period, when birds floated on water, this was not the case in previous studies. For example, Fahlman et al. (2004) reported an $s\dot{V}_{O_2}$ of 14.0 ml $min^{-1}$ $kg^{-1}$ in king penguins floating inside a water channel ($T_w$ 4°C; $M_b$ 12.1 kg) for ~3 h, after they had fasted for 5–12 days in air. After a total fasting duration of 11–25 days (in air), the $M_b$ of penguins in that study was reduced to 10.4 kg but $s\dot{V}_{O_2}$ was only increased to 16.9 ml $min^{-1}$ $kg^{-1}$ during a further 3 h respirometry trial in the water channel (Fahlman et al., 2004). This difference in response found in our study, when compared with previous studies, might be related to differences in the experimental set-up. While previous studies recorded $\dot{V}_{O_2}$ in trials when birds remained on water for relatively short periods (i.e. a few hours), our birds were maintained in the seawater tank for two consecutive days during each introduction to the tank (stages 2 and 4). For example, the $\dot{V}_{O_2}$ recorded by Fahlman et al. (2005) coincided with a strong temperature decline in abdominal and peripheral tissues of birds, similar to the temperature declines observed upon first introduction to the seawater tank in our study (Fig. 2A, experimental day I). In our study, birds recovered from this initial temperature decline and maintained elevated temperatures in all tissues throughout their fasting periods in water (Figs 2 and 3). However, this was clearly not the case in previous studies and might explain the relatively low $s\dot{V}_{O_2}$ values of birds in water after extensive fasting in air (Fahlman et al., 2004, 2005). A direct comparison between studies is further complicated by the fact that resting measurements within the water channel in Fahlman et al. (2004, 2005) were taken opportunistically, when birds rested at the water surface between dives for periods of at least 20 min. As birds will generate substantial amounts of heat during swimming, some of this heat might have been available even during resting periods, delaying the need for heat production and, therefore, reducing thermoregulatory costs and $\dot{V}_{O_2}$ (Kaseloo and Lovvorn, 2005, 2006; Lovvorn, 2007).

In our study, the $s\dot{V}_{O_2}$ of penguins after fasting for 4 days within the water tank was substantially higher than at the start of their fasting period in water, reaching values in excess of 40 ml $min^{-1}$ $kg^{-1}$ (Fig. 5A). To put this into perspective, Froget et al. (2004) estimated the $\dot{V}_{O_2}$ of king penguins during their foraging trips based on the recording of heart rate. They found that $s\dot{V}_{O_2}$ reached its highest level during the first hour after the end of a foraging bout, when birds floated at the surface, re-perfusing peripheral tissues and digesting food (Handrich et al., 1997; Schmidt et al., 2006). $s\dot{V}_{O_2}$ during this period was estimated to average 25.8 ml $min^{-1}$ $kg^{-1}$ in birds with a mean $M_b$ of 12.8 kg (Froget et al., 2004). The higher $s\dot{V}_{O_2}$ recorded in our study, after 4 days of fasting, might be accounted for by differences in body insulation (i.e. a $M_b$ of 11.1 kg in our study versus 12.8 kg in Froget et al., 2004), increasing heat loss (Enstipp et al., 2017) and, therefore, thermoregulatory costs in our birds. Furthermore, as the $\dot{V}_{O_2}$–heart rate relationship used to estimate $\dot{V}_{O_2}$ from the recording of heart rate in free-ranging king penguins (Froget et al., 2004) was derived from treadmill exercise in air (Fahlman et al., 2004), it might also be possible that the $\dot{V}_{O_2}$ estimate for the first hour after foraging is underestimating the true costs. Halsey et al. (2008) determined the costs associated with recovery from hypothermia in king penguins after they had been swimming inside a shallow water channel for 2 h. During these trials in water, the abdominal temperature of fasted birds ($M_b$ 10.6 kg) declined by 1–6°C (maximum; depending on thermistor position within the abdomen). When recovering in air, the $s\dot{V}_{O_2}$ of fasted birds reached values ~32 ml $min^{-1}$ $kg^{-1}$ (Halsey et al., 2008; their fig. 1A).

### Fasting versus feeding

Contrasting our investigation into the physiological responses of king penguins to prolonged fasting in water with that of feeding in water, we found that in both cases tissue temperatures increased during their periods in water (Fig. 6). During feeding conditions, temperature increases occurred mostly during the night, when birds were left undisturbed (Lewden et al., 2017), while during fasting temperature increases (or the maintenance at an elevated level) occurred during the day and night. Similarly, normothermic periods in the flank tissue (temperatures ≥36°C) occurred predominately during the night in the fed condition, while they extended throughout 24 h periods in the fasted condition (Table 2). These periods also occurred much more frequently during fasting and their duration was considerably longer than during feeding. For example, in fed birds ('lean' condition), the flank temperature was ≥36°C for 52% of the night period, while in fasted birds this was the case for 81% and 67% during the last two nights in the water tank (Lewden et al., 2017; Table 2). In general, when in water, tissue temperatures, reflecting perfusion, were considerably higher during the fasting condition, when compared with the fed condition (Fig. 6). The higher tissue temperatures of king penguins observed in the fasting condition in water (reflecting perfusion level) when compared with the feeding condition, might be explained by a greater need to access subcutaneous fat stores during the former to fuel metabolism. When fed inside the water tank, following digestion, birds will need to deposit any surplus free fatty acids (FFA) in the abdomen and/or subcutaneous tissue, requiring perfusion of these tissues. However, the required extent of tissue perfusion will depend on the surplus of FFA. It might be possible that the amount of fish fed to penguins in our feeding investigation (Lewden et al., 2017) did not require a tissue perfusion comparable to that of the current study, when birds were fasting.

In the wild, the temperature of deep and peripheral tissues declines during foraging bouts of king penguins, following a reduction in tissue perfusion (vasoconstriction; Culik et al., 1996b; Handrich et al., 1997; Schmidt et al., 2006; Enstipp et al., 2017).

Upon completion of their daily foraging activity, when birds typically rest at the surface, the temperature of deep and peripheral tissues increases to normothermic values. This enlarges the body volume of penguins, which is maintained at a normothermic level and increases the temperature gradient between the penguin body and the surrounding cold water, elevating heat loss. While such a response might seem paradoxical at first, it might be required for a number of reasons. Firstly, peripheral tissue perfusion might be required for the supply of oxygen and nutrients to these tissues, while it will also allow the removal of metabolic by-products (Kooyman, 1989). Importantly though, peripheral perfusion will also be required to allow deposition of FFA in subcutaneous tissues after profitable foraging (Lewden et al., 2017), while after less profitable foraging, when foraging success is insufficient to cover energy expenditure, this will allow fat stores to be accessed to fuel metabolism, as suggested by Fahlman et al. (2005). The findings of the current study lend support to such a scenario. The strategy chosen by king penguins to manage the apparent conflict between nutritional requirements and thermoregulatory demands might seem non adaptive at first. However, delaying metabolic processes that require peripheral tissue perfusion until after the end of a foraging bout has two major advantages: (1) it keeps the metabolic rate during diving low, increasing apnea duration; and (2) it keeps heat loss at bay, which is considerably smaller at the surface than at depth.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: A.L., Y.H.; Methodology: A.L., Y.H.; Formal analysis: A.L., M.R.E.; Investigation: A.L., B.B., C.B., Y.H.; Resources: J.-Y.G.; Writing - original draft: A.L., M.R.E.; Writing - review & editing: A.L., M.R.E., Y.H.; Supervision: M.R.E., Y.H.

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#### References

**Bost, C.-A., Zorn, T., Le Maho, Y. and Duhamel, G.** (2002). Feeding of diving predators and diel vertical migration of prey: king penguins' diet versus trawl sampling at Kerguelen Islands. *Mar. Ecol. Prog. Ser.* **227**, 51-61.

**Bost, C. A., Cotté, C., Terray, P., Barbraud, C., Bon, C., Delord, K., Gimenez, O., Hanrich, Y., Naito, Y., Guinet, C. et al.** (2015). Large-scale climatic anomalies affect marine predator foraging behaviour and demography. *Nat. Commun.* **6**, 8220.

**Butler, P. J. Woakes, A. J.** (2001). Seasonal hypothermia in a large migrating bird: saving energy for fat deposition? *J. Exp. Biol.* **204**, 1361-1367.

**Charrassin, J.-B. and Bost, C.-A.** (2001). Utilization of the oceanic habitat by king penguins over the annual cycle. *Mar. Ecol. Prog. Ser.* **221**, 285-297.

**Charrassin, J.-B., Le Maho, Y. and Bost, C.-A.** (2002). Seasonal changes in the diving parameters of king penguins (*Aptenodytes patagonicus*). *Mar. Biol.* **141**, 581-589.

**Cherel, Y. and Ridoux, V.** (1992). Prey species and nutritive value of food fed during summer to king penguin *Aptenodytes patagonica* chicks at Possession Island, Crozet Archipelago. *Ibis* **134**, 118-127.

**Cherel, Y., Robin, J.-P. and Le Maho, Y.** (1988). Physiology and biochemistry of long-term fasting in birds. *Can. J. Zool.* **66**, 159-166.

**Cherel, Y., Gilles, J., Handrich, Y. and Le Maho, Y.** (1994). Nutrient reserve dynamics and energetics during long-term fasting in the king penguin *(Aptenodytes patagonicus). J. Zool. Lond.* **234**, 1-12.

**Chossat, C.** (1843). *Recherches Expérimentales Sur l'inanition*. Paris, France: Impremerie Royale.

**Culik, B. M., Pütz, K., Wilson, R. P., Allers, D., Lage, J., Bost, C.-A. and Le Maho, Y.** (1996a). Diving energetics in king penguins (*Aptenodytes patagonicus*). *J. Exp. Biol.* **199**, 973-983.

**Culik, B. M., Pütz, K., Wilson, R. P., Bost, C.-A., Le Maho, Y. and Verselin, J.-L.** (1996b). Core temperature variability in diving king penguins (*Aptenodytes patagonicus*): a preliminary analysis. *Polar Biol.* **16**, 371-378.

**Dejours, P.** (1987). Water and air physical characteristics and their physiological consequences. In *Comparative Physiology: Life in Water and on Land* (ed. P. Dejours, L. Bolis, C. R. Taylor and E. R. Weibel), pp. 3-11. Berlin: Springer Verlag.

**Delord, K., Barbraud, C. and Weimerskirch, H.** (2004). Long-term trends in the population size of king penguins at Crozet archipelago: environmental variability and density dependence? *Polar Biol.* **27**, 793-800.

**Dewasmes, G., Le Maho, Y., Cornet, A. and Groscolas, R.** (1980). Resting metabolic rate and cost of locomotion in long-term fasting emperor penguins. *J. Appl. Physiol.* **49**, 888-896.

**Drent, R. H. and Daan, S.** (1980). The prudent parent: energetic adjustments in avian breeding. *Ardea* **68**, 225-252.

**Eichhorn, G., Groscolas, R., Le Glaunec, G., Parisel, C., Arnold, L., Medina, P. and Handrich, Y.** (2011). Heterothermy in growing king penguins. *Nat. Commun.* **2**, 435.

**Enstipp, M. R., Bost, C.-A., Le Bohec, C., Bost, C., Le Maho, Y., Weimerskirch, H. and Handrich, Y.** (2017). Apparent changes in body insulation of juvenile king penguins suggest an energetic challenge during their early life at sea. *J. Exp. Biol.* **220**, 2666-2678.

**Fahlman, A., Handrich, Y., Woakes, A. J., Bost, C.-A., Holder, R., Duchamp, C. and Butler, P. J.** (2004). Effect of fasting on the VO2-fh relationship in king penguins, *Aptenodytes patagonicus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, 870-877.

**Fahlman, A., Schmidt, A., Handrich, Y., Woakes, A. J. and Butler, P. J.** (2005). Metabolism and thermoregulation during fasting in king penguins, *Aptenodytes patagonicus*, in air and water. *Am. J. Physiol.* **289**, R670-R679.

**Froget, G., Butler, P. J., Woakes, A. J., Fahlman, A., Kuntz, G., Le Maho, Y. and Handrich, Y.** (2004). Heart rate and energetics of free-ranging king penguins (*Aptenodytes patagonicus*). *J. Exp. Biol.* **207**, 3917-3926.

**Gilbert, C., Blanc, S., Le Maho, Y. and Ancel, A.** (2008). Energy saving processes in huddling emperor penguins: from experiments to theory. *J. Exp. Biol.* **211**, 1-8.

**Groscolas, R.** (1986). Changes in body mass, body temperature and plasma fuel levels during the natural breeding fast in male and female emperor penguins *Aptenodytes forsteri*. *J. Comp. Physiol. B* **156**, 521-527.

**Halsey, L. G., Handrich, Y., Rey, B., Fahlman, A., Woaker, A. J. and Butler, P. J.** (2008). Recovery from swimming-induced hypothermia in king penguins: effects of nutritional condition. *Physiol. Biochem. Zool.* **81**, 434-441.

**Handrich, Y., Bevan, R. M., Charrassin, J.-B., Butler, P. J., Putz, K., Woakes, A. J., Lage, J. and Le Maho, Y.** (1997). Hypothermia in foraging king penguins. *Nature* **388**, 64-67.

**Hohtola, E.** (2012). Thermoregulatory adaptations to starvation in birds. In *Comparative Physiology of Fasting, Starvation, and Food Limitation* (ed. M. D. McCue), pp. 155-170. Berlin, Germany: Springer Verlag.

**Jouventin, P.** (1982). *Visual and Vocal Signals in Penguins, their Evolution and Adaptive Characters*. Berlin: Verlag Paul Parey.

**Kaseloo, P. A. and Lovvorn, J. R.** (2005). Effects of surface activity patterns and dive depth on thermal substitution in fasted and fed lesser scaup (*Aythya affinis*) ducks. *Can. J. Zool.* **83**, 301-311.

**Kaseloo, P. A. and Lovvorn, J. R.** (2006). Substitution of heat from exercise and digestion by ducks diving for mussels at varying depths and temperatures. *J. Comp. Physiol. B* **176**, 265-275.

**Kooyman, G. L.** (1989). *Diverse Divers*. Berlin: Springer Verlag.

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

**Kozlov, A. N., Shust, K. V. and Zemsky, A. V.** (1991). Seasonal and interannual variability in the distribution of *Electrona carlsbergi* in the southern polar front area. Selected Scientific Papers, Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR); SC-CAMLR-SSP/7, pp. 337-367.

**Le Maho, Y., Delclitte, P. and Chatonnet, J.** (1976). Thermoregulation in fasting emperor penguins under natural conditions. *Am. J. Physiol.* **231**, 913-922.

**Le Maho, Y., Vu Van Kha, H., Koubi, H., Dewasmes, G., Girard, J., Ferre, P. and Cagnard, M.** (1981). Body composition, energy expenditure, and plasma metabolites in long-term fasting geese. *Am. J. Physiol.* **241**, E342-E354.

**Lewden, A., Enstipp, M. R., Picard, B., van Walsum, T. and Handrich, Y.** (2017). High peripheral temperatures in king penguins while resting at sea: thermoregulation versus fat deposition. *J. Exp. Biol.* **220**, 3084-3094 (in press).

**Lighton, J. R. B.** (2008). *Measuring Metabolic Rates–a Manual for Scientists*. Oxford: Oxford Univ. Press.

**Lovvorn, J. R.** (2007). Thermal substitution and aerobic efficiency: measuring and predicting effects of heat balance on endotherm diving energetics. *Phil. Trans. R. Soc. B* **362**, 2079-2093.



Journal of Experimental Biology

**McCue, M. D.** (2010). Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. *Comp. Biochem. Physiol. -A Mol. Integr. Physiol.* **156**, 1-18.
**McKechnie, A. E. and Lovegrove, B. G.** (2002). Avian facultative hypothermic responses: a review. *Condor* **104**, 705-724.
**Orgeret, F., Weimerskirch, H. and Bost, C.-A.** (2016). Early diving behaviour in juvenile penguins: improvement or selection process. *Biol. Lett.* **12**, 20160490.
**Øritsland, N. A., Påsche, A. J., Markussen, N. H. and Ronald, K.** (1985). Weight loss and catabolic adaptations to starvation in grey seal pups. *Comp. Biochem. Physiol. A* **82**, 931-933.
**Raclot, T., Groscolas, R. and Cherel, Y.** (1998). Fatty acid evidence for the importance of myctophid fishes in the diet of king penguins, *Aptenodytes patagonicus*. *Mar. Biol.* **132**, 523-533.
**Schmidt, A., Alard, F. and Handrich, Y.** (2006). Changes in body temperature in king penguins at sea: the result of fine adjustments in peripheral heat loss? *Am. J. Physiol.* **291**, R608-R618.
**Stonehouse, B.** (1953). *The Emperor Penguin. I. Breeding behaviour and development. Falkland Islands Dependencies Survey Scientific Reports* **6**, 1. London: HMSO.
**Tattersall, G. J., Roussel, D., Voituron, Y. and Teulier, L.** (2016). Novel energy-saving strategies to multiple stressors in birds: the ultradian regulation of body temperature. *Proc. R. Soc. B* **283**, 20161551.
**Viblanc, V. A., Saraux, C., Malosse, N. and Groscolas, R.** (2014). Energetic adjustments in freely breeding-fasting king penguins: does colony density matter? *Funct. Ecol.* **28**, 621-631.