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RESEARCH ARTICLE

High peripheral temperatures in king penguins while resting at sea: thermoregulation versus fat deposition

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ABSTRACT

Marine endotherms living in cold water face an energetically challenging situation. Unless properly insulated, these animals will lose heat rapidly. The field metabolic rate of king penguins at sea is about twice that on land. However, when at sea, their metabolic rate is higher during extended resting periods at the surface than during foraging, when birds descend to great depth in pursuit of their prey. This is most likely explained by differences in thermal status. During foraging, peripheral vasoconstriction leads to a hypothermic shell, which is rewarmed during extended resting bouts at the surface. Maintaining peripheral perfusion during rest in cold water, however, will greatly increase heat loss and, therefore, thermoregulatory costs. Two hypotheses have been proposed to explain the maintenance of a normothermic shell during surface rest: (1) to help the unloading of N_2 accumulated during diving; and (2) to allow the storage of fat in subcutaneous tissue, following the digestion of food. We tested the latter hypothesis by maintaining king penguins within a shallow seawater tank, while we recorded tissue temperature at four distinct sites. When king penguins were released into the tank during the day, their body temperature immediately declined. However, during the night, periodic rewarming of abdominal and peripheral tissues occurred, mimicking temperature patterns observed in the wild. Body temperatures, particularly in the flank, also depended on body condition and were higher in 'lean' birds (after 10 days of fasting) than in 'fat' birds. While not explicitly tested, our observation that nocturnal rewarming persists in the absence of diving activity during the day does not support the N_2 unloading hypothesis. Rather, differences in temperature changes throughout the day and night, and the effect of body condition/mass supports the hypothesis that tissue perfusion during rest is required for nutritional needs.

KEY WORDS: Seabirds, Heterothermia, Subcutaneous fat, Normothermia, Fat storage, Heat loss

INTRODUCTION

Marine endotherms living in cold water are confronted with potentially significant heat loss and high thermoregulatory costs, as the heat capacity and thermal conductivity of water are substantially higher than those of air (Kooyman et al., 1976; Dejours, 1987). Hence, effective insulation is of great importance. Most marine mammal species possess a substantial subdermal fat layer (blubber) to achieve such insulation. By contrast, many marine birds rely to a large extent on the air layer that is trapped within their plumage. While air is a better insulator than fat, problems arise during diving, when the increasing pressure with depth reduces the insulating air layer trapped within the plumage, so that heat loss increases (Kooyman et al., 1976; Wilson et al., 1992; De Vries and Van Erden, 1995; Ponganis et al., 2003). Hence, avian divers that rely exclusively on their plumage air layer for insulation (e.g. cormorants) face an enormous heat loss to the environment, burdening their energy budget (Grémillet et al., 2001; but see Enstipp et al., 2007). Penguins possess both (1) air that is trapped within their dense down layer and (2) a layer of adipose tissue, uniformly distributed under the skin (subcutaneous fat; Pond and Mattacks, 1985). The subcutaneous fat layer and the alteration of peripheral blood perfusion (vasoconstriction) enable these birds to build up a thermal gradient between the warmer body core and the cooler body shell, reducing heat flux to the environment and keeping thermoregulatory costs at bay (Kooyman et al., 1976; Ponganis et al., 2001, 2003). The effectiveness of such a response has been demonstrated experimentally in marine mammals (e.g. Kvadsheim and Folkow, 1997). Effective thermoregulatory capacity is a prerequisite for the extreme dive performance of the two largest penguin species, the king penguin (*Aptenodytes patagonicus*) and the emperor penguin (*Aptenodytes forsteri*). Current depth records for these species are 343 m versus 564 m, with maximum dive durations of 9.2 min versus 27.6 min, respectively (Wienecke et al., 2006; Sato et al., 2011; Pütz et al., 1998; Pütz and Cherel, 2005). Certainly, the hydrodynamic design and propulsive mode (wing propelled) of penguins enables these birds to move through the water column at low energetic costs (Culik et al., 1994, 1996a). However, exactly how these penguins achieve such extreme dive performance is still the subject of intense research. Recent studies on emperor penguins (mostly concerning birds diving from isolated ice holes) have started to unravel the physiological mechanisms behind such performance (Knower Stockard et al., 2005; Ponganis et al., 2007, 2009, 2011; Meir et al., 2008; Meir and Ponganis, 2009; Williams et al., 2011). Unfortunately, because of the lack of a comparable set-up, such studies have not been possible with king penguins.

However, a number of studies have investigated the diving energetics and thermoregulation of king penguins foraging at sea (Froget et al., 2004; Culik et al., 1996b; Handrich et al., 1997; Schmidt et al., 2006; Enstipp et al., 2017). These studies found substantial temperature declines in core (abdominal) and/or peripheral tissues (Culik et al., 1996b; Handrich et al., 1997; Schmidt et al., 2006; Enstipp et al., 2017) and this has led to the suggestion of a regional hypothermia as a mechanism to lower diving metabolism and increase breath-hold duration (Butler, 2004; Schmidt et al., 2006). For example, Handrich et al. (1997) recorded abdominal temperatures in foraging king penguins that fell as low as 11°C. While some of this extreme tissue cooling might have been caused by the ingestion of cold prey, abdominal temperature was

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10–20°C below the recorded stomach temperature (Handrich et al., 1997). Furthermore, Schmidt et al. (2006) found that pectoral muscle temperature of king penguins during foraging bouts dropped on average by ∼2°C, with maximal declines of up to 5.5°C. The temperature of the subcutaneous brood patch, a bare and highly vascularized skin area, decreased on average by ∼13°C, with maximal declines of up to 21.6°C (Schmidt et al., 2006). In king penguins swimming/diving within a shallow (30 m) static water channel for up to 3 h, Fahlman et al. (2005) also observed a temperature decline in subcutaneous tissues as well as in the lower, middle and upper abdomen that depended on the nutritional status of the birds. Lastly, Enstipp et al. (2017) reported peripheral temperature drops (subcutaneous flank) in juvenile king penguins foraging at sea to as low as 12.5°C.

Following these hypothermic periods, the birds rest for extended periods at the surface and rewarm both their deep and peripheral tissues to normothermic values (Schmidt et al., 2006). Reperfusion of peripheral tissues while resting (i.e. vasodilation), and the associated peripheral rewarming, might be important as it ensures the supply of oxygen and nutrients to these tissues, while it removes metabolic by-products (Kooyman, 1989). However, this also leads to an increase in heat loss during this inactive period and might explain why metabolic rate estimates for king penguins at sea are higher at night (when they are resting at the surface) than during the daytime, when they forage (Froget et al., 2004). The study by Froget et al. (2004) also reported metabolic rate estimates for king penguins at sea that were about twice those of birds ashore in the colony, illustrating the scope of metabolic costs associated with activity and thermoregulation at sea. Besides increased heat loss and heat production, the heat increment of feeding, associated with the digestion/assimilation of food, will also increase metabolism during these resting periods, while some of that heat might be used to substitute for regulatory thermogenesis (Green et al., 2006; Lovvorn, 2007; Enstipp et al., 2008). However, the reasons why king penguins maintain a peripheral normothermia when resting in cold water, despite the potentially high energetic costs associated with it, are still unclear. Two general hypotheses have been proposed: (1) the 'N_2 unloading hypothesis' and (2) the 'nutritional hypothesis'. In the N_2 unloading hypothesis, the maintenance of peripheral perfusion during extended surface breaks in king penguins is required to help unloading of N_2, accumulated during diving (Fahlman et al., 2007). N_2 accumulation was suggested to explain why king penguins terminate foraging bouts, especially during the day, when prey might be readily available (Fahlman et al., 2007). In general, N_2 is highly fat soluble and accumulates in subcutaneous fat during diving (Behnke et al., 1935). Hence, at the end of extended deep diving bouts, a poor peripheral circulation (vasoconstriction) might delay N_2 clearance and, thereby, increase the likelihood of bubble formation (Fahlman et al., 2007). Accordingly, perfusion of subcutaneous adipose tissues after diving might speed up the removal of N_2 and help to avoid decompression sickness (Fahlman et al., 2007). In the nutritional hypothesis, maintenance of peripheral perfusion during inactivity at night could be required for the assimilation of free fatty acids (FFA) following digestion of food (Schmidt et al., 2006). Subcutaneous adipose tissue is the most important tissue in penguins for FFA storage (Cherel et al., 1993). Accordingly, peripheral perfusion is required to allow fat deposition, and this might explain the observed general rewarming that occurs after foraging, when birds rest at the surface.

In this study, we explored the nutritional hypothesis by monitoring the temperature of various tissues of king penguins as a proxy of tissue perfusion, while birds underwent alimentary manipulation inside a water tank. Our objectives were as follows: (1) to investigate tissue temperatures of king penguins resting in a shallow seawater tank during the day (digestive period) and night (post-digestive period), in the absence of diving activity, and (2) to investigate the effect of body condition on observed tissue temperature variations ('fat' versus 'lean' birds).

We predicted that if peripheral reperfusion/rewarming is related to the requirements of fat deposition in subcutaneous tissues, we should see a general increase in peripheral tissue temperature during the post-digestive period (at night), when compared with the digestive period (during the day). Furthermore, peripheral tissue temperatures of birds should be affected by their body condition, as 'lean' birds should preferentially restore subcutaneous fat stores (to improve insulation) and, therefore, display higher peripheral temperatures, reflecting greater peripheral perfusion. By contrast, 'fat' birds might deposit fat preferentially in abdominal adipose tissue and display higher abdominal temperatures than 'lean' birds.

MATERIALS AND METHODS

Experiments were conducted on Possession Island, Crozet Archipelago (46°25′S, 51°51′E), in the Southern Indian Ocean during the Austral summer (November 2014 to March 2015). At the time of capture, king penguins were in the courtship phase of their breeding cycle at the colony 'La Baie du Marin'. We equipped eight adult males (identified by song; Jouventin, 1982) with temperature data loggers (see below). 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).

Experimental set-up

Surgical procedure

On the day of capture, body mass and a number of morphometric measurements were taken (e.g. length of flippers, feet, beak and head). Birds then underwent surgical implantation of temperature data loggers at four different sites (see below). The surgical procedure followed the protocol previously detailed in Froget et al. (2004) and Fahlman et al. (2005), with the following modifications concerning drug administration: in preparation for surgery, birds were injected with a mixture of Valium (1 ml; Virbac, Carros, France), glycopyrrolate (0.6 ml; Vétoquinol, Paris, France) and butorphanol (1 ml; Vétoquinol) into the pectoral muscle, 30 min before anaesthesia was induced with isoflurane, delivered in O_2. To avoid post-operative infection, birds were injected with marbofloxacin (Vétoquinol) into the brachial flipper vein. Injections of Rimadyl and cefalexin (Vétoquinol) were delivered during surgery and every 12 h post-surgery for up to 72 h, to reduce potential pain and inflammation.

Temperature loggers

Penguins were implanted with temperature loggers (iButton MXMDS1922L-F5, Memec, Avnet, Massy, France; resolution ±0.0625°C, range 0–50°C, diameter and height 17.35×5.89 mm, mass 2.9 g, recalibrated in a water bath with an absolute accuracy of ±0.1°C) at four distinct locations (Fig. 1), which recorded instant temperature every 11 min. Three of the four temperature loggers were implanted into the subcutaneous fat layer within the flank, back and brood patch, while the last logger was placed into the abdominal cavity, lateral to the brood patch (Fig. 1). 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

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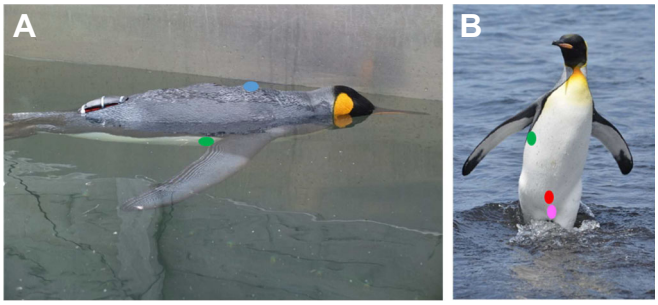

Fig. 1. Experimental set-up and the position of the loggers. (A) A king penguin resting in the seawater tank, showing the position of two of the four temperature loggers. When king penguins floated in the tank, the back logger (blue dot) remained above water, whereas loggers at all other positions were submerged. (B) The position of the loggers in the flank (green dot), the brood patch (pink dot) and the abdominal cavity (red dot). The external logger on the lower back was part of a different study.

recorded ambient air temperature in the enclosure and water temperature (T_w) within the tank (monitored with iButtons; those in water were submerged to 0.8 m).

To allow full recovery from surgery, birds were kept together in a wooden enclosure (in air; 3×3 m, no roof) without disturbance (apart from feeding) for an average of 6.1±0.1 days, before introduction into the seawater tank. Upon completion of experimentation, birds underwent the same procedure to remove the temperature loggers. Before release back into the colony, penguins were colour marked (using hair dye: Belle Color, L'Oreal, Clichy, France) so they could be followed throughout the breeding season.

Seawater tank

A water tank (2.5×1.3×1.2 m length×width×height, filled to a water level of 0.8 m) was built adjacent to lab facilities and the colony. Fresh seawater was pumped continuously from the adjacent bay. A surface skimmer at one end of the tank and a sufficiently high water turnover (complete turnover within 1 h) ensured a good water quality within the tank and maintained a fairly constant water temperature throughout experimentation (mean T_w 5.7±0.6°C). Birds inside the water tank were monitored continuously by video camera (DVR Eye, Pix Controller, Export, PA, USA).

Feeding and experimentation

Penguins did not ingest food voluntarily and were, consequently, force fed. For this, birds were captured within the wooden enclosure or from within the water tank and briefly restrained while being weighed (accuracy ±0.1 kg) and fed whole fish (sardines, *Sardina pilchardus*, stored at 5°C; Armement des Mascareignes, Le Port, France). Birds easily adapted to this procedure, which never exceeded 10 min (mean duration 6.9±3.7 min). During recovery from surgery (on average, 2.2±0.1 days after surgery) and before introduction into the water tank, birds were fed 3 times per day for 4 successive days (stage 1), to enable them to regain their original body mass at capture. Mean meal size was 0.3±0.1 kg, which amounted to a total of 3.3±0.4 kg of fish fed to each bird within 4 days. Consequently, body mass of birds at the time of introduction into the water tank did not differ from their initial mass at first capture (P=0.63).

To investigate the hypothesis that maintained peripheral perfusion at night is related to fat deposition, all birds underwent a specific protocol that included feeding and fasting periods inside a shallow seawater tank and/or in air (see Fig. 2 for an outline of the temporal organization of our experiment). The experimental protocol included four distinct periods, during which we recorded tissue temperature: (1) stage 1, during which birds rested for 4 days inside the wooden enclosure ('fat' birds in air); (2) stage 2, during which birds spent 2 consecutive days inside the shallow seawater tank ('fat' birds in water, body mass 13.6±0.4 kg; Table 1); (3) stage 3, during which birds fasted for 10 days and were alternated between the water tank and the wooden enclosure; (4) stage 4, during which birds spent another 3 consecutive days inside the water tank ('lean' birds in water; body mass 11.9±0.7 kg; Table 1). During stage 1 (in air), birds were fed 0.9±0.1 kg fish per day (see above). When inside the water tank (2 days during stage 2 and 3 days during stage 4; but not during the fasting period), birds were fed four meals per day, at 08:30 h, 11:30 h, 14:30 h and 17:30 h, and were fasted at night. Total meal size per day per bird at that point varied between 1.5±0.2 and 1.8±0.0 kg. Hence, we simulated conditions typically encountered by these birds at sea, albeit in the absence of diving activity.

Data analysis

All data of one bird were removed from the analysis because of its digestive problems and repeated food regurgitation. Furthermore, one logger in the back (bird ID2) and one logger in the abdominal cavity (ID5) failed to record data. Within the four distinct stages, days were split into daytime periods (08:00 h to 22:00 h; last feeding occurred at 17:30 h) and night-time periods (22:00 h to 08:00 h); during the latter, birds rested without disturbance. The lower critical temperature for adult king penguins in air has been estimated at −5°C (Froget et al., 2002). Air temperatures throughout experimentation ranged between 4.6 and 17.3°C. Hence, when birds rested in air (with the possibility of remaining in the shade on sunny days), they were within their thermoneutral zone. For our investigation, we considered the tissue temperatures of birds recorded during stage 1 (in air) to represent the normothermic condition for each respective tissue.

In our analysis, we first focused on the general temperature patterns recorded in our penguins within the seawater tank (stage 2 and stage 4; 5 days in total), to allow a comparison with temperature patterns observed in foraging birds at sea. We then concentrated our investigation on tissue temperatures recorded in penguins during stage 2 ('fat' birds in water). Specifically, for the flank tissue (permanently submerged in a penguin when resting in water, Fig. 1), we investigated normothermic periods (~37°C) that occurred during the night. We also compiled a frequency distribution for all four tissue temperature recordings obtained during the night in stage 2 and contrasted this with tissue temperatures obtained during stage 1 ('fat' birds in air). Lastly, we studied temperature changes during the two periods that birds spent in water with contrasting body conditions (stage 2 versus stage 4).

We considered birds to be in a digestive state throughout the daytime and in a post-digestive state for most of the night. Frequent defecation throughout the daytime was observed in all birds (with the exception of the bird excluded from the analysis), indicating that birds digested the provided food. During digestive activity, blood flow to the digestive organs within the body core will increase (Gallavan and Chou, 1985; Bottje and Harrison, 1986, 1987), while peripheral perfusion will be reduced. Hence, if a peripheral reperfusion/rewarming is related to the requirements of fat deposition in subcutaneous tissues, we would expect to see an increase in peripheral tissue temperature during the post-digestive period (at night), when compared with the digestive period (daytime). Furthermore, we would also expect that the body condition of birds will affect peripheral tissue temperature, for the

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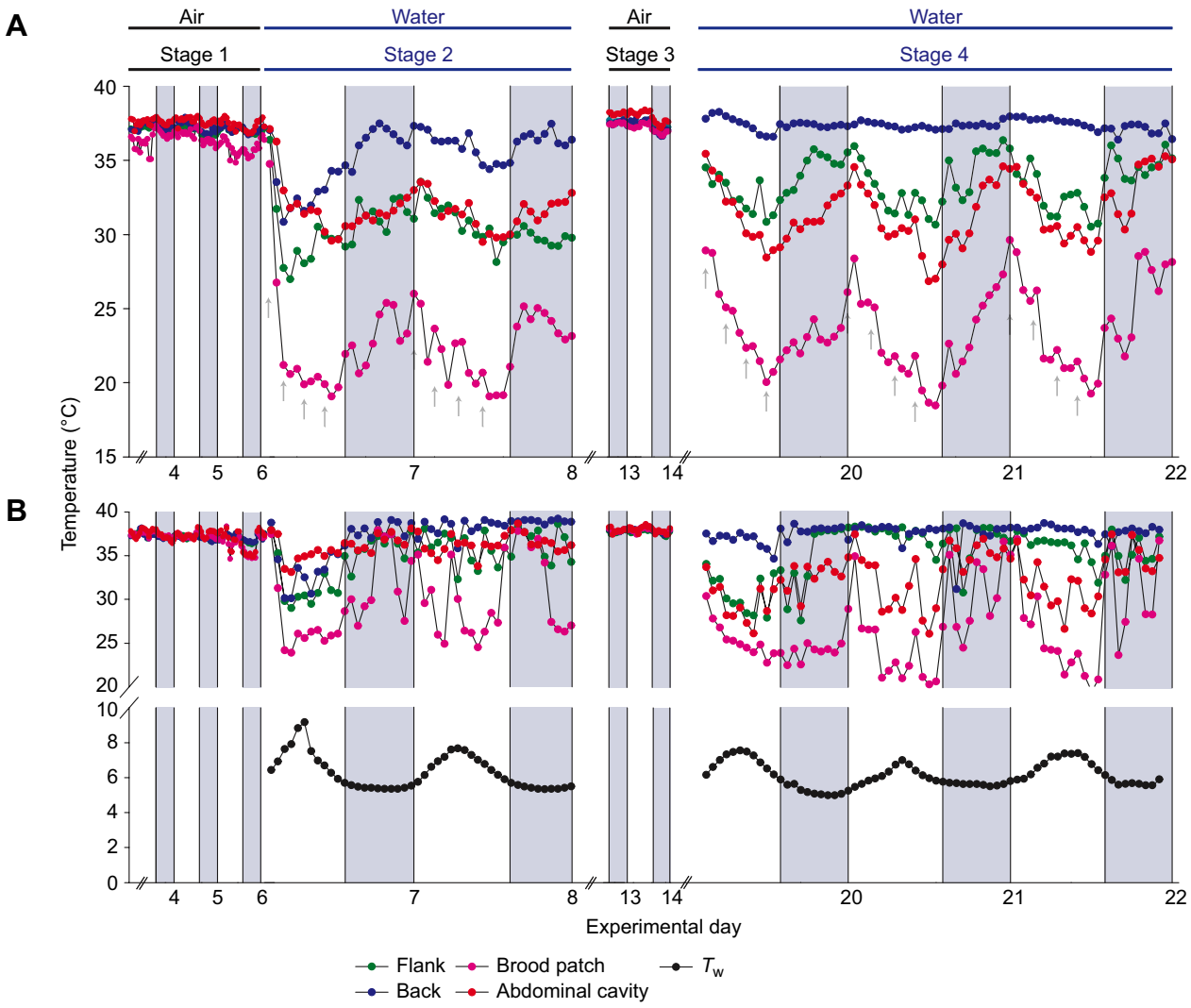

Fig. 2. Temperature recordings from four tissues when king penguins rested in air and in water. (A) Hourly mean temperature from 7 birds throughout the experiment. The initial part of the experiment (stage 1, 4 days in air) was followed by 2 days in water (stage 2, 'fat' birds), after which birds were fasted for 10 days in air/water (stage 3), before they spent another 3 days in water (stage 4, 'lean' birds). Feeding events occurred during the day, as indicated by the arrows. (B) Temperature recordings from a single bird (ID 3) for comparison, plotted with water temperature (T_w). Night periods are indicated by the grey bars at the top. Only 3 days are displayed for stage 1, and only a period of 34 h, when birds rested in air, is shown for stage 3. Tissue temperature oscillations between day and night are visible in water, while they are absent in air.

following reasons. While the exact time course and preferential site of fat deposition/mobilization in birds is not clear, Blem (1990) suggested that 'subcutaneous fat may tend to be the first deposited and the last to be used', while intraperitoneal fat 'may be the first to be mobilized during stress'. By contrast, experiments conducted with rats suggest that abdominal adipose tissue is important for the short-term (meal-to-meal) dynamics of both fat mobilization and storage, while subcutaneous adipose tissue is more important for the long-term management of energy reserves (West et al., 1989; Sugden et al., 1994; Bertile et al., 2003). From studies concerning fuel mobilization during fasting, it seems clear that subcutaneous fat provides most fuel during these periods in penguins and seals (Cherel and Le Maho, 1985; Nordøy and Blix, 1985; Nordøy et al., 1990; Markussen and Ryg, 1992; Cherel et al., 1994), while seal pups might increase protein catabolism, to retain a critical blubber layer for insulation (Øritsland et al., 1985). Clearly, the benefit of the time course with respect to subcutaneous fat suggested by Blem (1990) is obvious for avian divers like penguins, where subcutaneous fat provides most insulation. Accordingly, after a fasting period, birds should preferentially restore their subcutaneous fat layer to provide insulation in cold water. If so, then peripheral tissue temperatures during rest (reflecting perfusion) should be higher in fasted (hereafter 'lean') birds, when compared with non-fasted (hereafter 'fat') birds. However, higher peripheral temperatures in lean birds might also be a consequence of their reduced insulation, when compared with fat birds, without any differences in perfusion. Hence, there are two potential explanations for the elevated peripheral temperatures in lean birds: (1) active regulation of peripheral perfusion, presumably to deposit subcutaneous fat; and (2) reduced subcutaneous insulation leading to greater heat flux from the core to the skin. To distinguish between these two potential causes, we focused on temperatures recorded within the flank. We determined (1) the total amount of time that flank temperature was above 36°C (i.e. near normothermia) during the night in fat and lean birds and (2) the mean temperature during these near-normothermic episodes (birds did not maintain a constant normothermic level and temperature oscillations were common, so that birds showed multiple near-normothermic periods

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Table 1. Tissue temperatures of king penguins resting during the night in air (stage 1) or in water (stage 2 and stage 4) and corresponding body masses

	Body mass (kg)		Flank temperature (°C)			Back temperature (°C)			Brood patch temperature (°C)			Abdominal cavity temperature (°C)		
Bird ID	Stage 2	Stage 4	Stage 1	Stage 2	Stage 4	Stage 1	Stage 2	Stage 4	Stage 1	Stage 2	Stage 4	Stage 1	Stage 2	Stage 4
ID1	14.2	12.6	37.5±0.2	34.3±5.0	36.7±2.6	37.2±0.2	37.2±2.0	37.2±0.5	37.5±0.3	29.7±5.1	23.2±6.7	37.9±0.3	37.7±0.4	35.9±1.1
Max.				37.9	38.4		38.4	38.3		36.7	37.1		38.4	38.2
Min.				23.3	27.2		29.4	36.3		20.2	16.1		36.4	33.2
ID2	13.2	11.2	37.4±0.2	27.4±4.1	33.0±4.7	–	–	–	37.0±0.4	21.6±1.7	25.04±4.4	38.1±0.2	36.0±0.7	35.8±1.4
Max.				36.5	38.7	–	–	–		30.4	36.8		37.9	39.1
Min.				22.9	24.0	–	–	–		18.8	18.4		34.4	33.8
ID3	13.6	12.0	37.0±0.3	35.4±2.2	35.4±3.3	37.0±0.3	37.6±1.0	37.6±1.5	36.9±0.8	31.6±4.8	31.6±5.4	36.9±0.9	35.8±1.1	35.8±2.1
Max.				37.9	38.5		38.6	38.6		37.4	38.0		38.3	38.3
Min.				29.3	27.4		33.5	29.2		24.7	22.2		32.6	28.0
ID4	13.7	11.8	36.7±0.6	30.7±5.8	35.7±1.8	36.8±0.6	35.9±1.1	36.8±0.5	36.5±1.0	25.2±5.8	25.9±3.0	37.5±0.5	32.8±3.3	32.6±2.9
Max.				37.6	38.0		37.6	37.8		36.8	32.5		38.2	37.2
Min.				24.4	31.2		32.2	35.3		17.9	20.4		27.0	26.6
ID5	13.2	11.8	36.9±0.3	37.4±0.6	36.2±1.0	37.2±0.3	37.6±0.5	37.27±0.4	36.5±0.9	29.4±5.3	25.2±3.3		–	–
Max.				38.2	37.7		38.3	38.0		37.7	36.0		–	–
Min.				34.0	33.2		35.3	34.7		20.3	20.1		–	–
ID6	13.6	11.8	36.7±0.4	23.9±2.0	32.3±2.9	37.2±0.4	32.7±3.8	37.5±1.3	35.2±2.1	15.6±1.3	19.9±2.2	37.4±0.6	25.7±3.5	27.5±3.2
Max.				34.9	38.5		38.0	38.9		18.6	24.9		33.0	35.5
Min.				22.0	29.2		25.1	29.5		14.0	16.3		21.7	22.3
ID7	13.7	11.8	36.7±0.4	27.0±4.1	33.4±3.3	37.0±0.4	37.6±1.1	37.4±0.9	36.2±0.5	16.0±1.3	23.9±5.0	37.6±0.3	24.0±2.1	24.0±5.7
Max.				37.8	37.5		39.0	38.4		19.3	36.8		29.4	36.8
Min.				23.9	27.5		32.2	33.2		14.2	18.8		20.9	18.8
Mean±s.d.	13.6±0.4	11.9±0.7	36.9±0.3	30.1±4.8	34.5±3.0	37.0±0.2	36.6±1.3	37.4±0.9	37.1±0.3	23.5±5.8	24.8±4.5	37.5±0.4	31.7±4.8	31.8±2.7

Mean temperature values (±s.d.), as well as maximum and minimum temperatures are shown. *N*=7 birds for flank and brood patch tissues and *N*=6 birds for back tissue and abdominal cavity.

of varying duration; Fig. 2B). We reasoned that if mean temperature levels during these near-normothermic periods were similar in the two conditions, while lean birds spent a greater part of the night at elevated flank temperatures, this would lend support to the nutritional hypothesis.

Statistical analyses

All statistical analyses were performed in JMP® (SAS Institute Inc., Cary, NC, USA). Body mass differences of birds during different stages and differences in the mean temperature of tissues between the day and night were tested using a paired *t*-test. Temperature variations of tissues between the first and last hour of day and night periods during stage 2 and stage 4 were investigated using linear mixed model analysis (LMM). We included hour (first versus last), period (day versus night), body condition (fat versus lean) and the interaction term hour×period as fixed effects, while bird ID was included as a random effect. Similarly, mean flank temperatures during near-normothermic periods and the total time per night spent above 36°C (stage 2 versus stage 4) were investigated using LMM analysis. For tissues where body condition significantly affected temperature variations between day and night, LMM was also used to investigate the effect of body mass and meal size on tissue temperatures at night. If interactions within a model were significant, we conducted a *post hoc* Tukey HSD test. Significance was accepted at $P<0.05$. All values are presented as means±s.d., unless indicated otherwise.

RESULTS

Upon first introduction into the seawater tank, birds explored the tank and even briefly submerged for a few seconds. However, they quickly adapted to the set-up and, apart from grooming, rested calmly at the surface throughout experimentation (Fig. 1A). When left undisturbed, activity levels of birds did not differ between day and night and no antagonistic behaviour between birds was observed. Recorded temperatures in all tissues declined strongly when birds entered the water (T_w=5.7±0.6°C; mean temperature declines: back 5.5±1.0°C, flank 10.2±1.9°C, brood patch 15.8±2.6°C, abdominal cavity 5.5±1.7°C; Fig. 2). Feeding events throughout the day caused additional temperature declines in all tissues, sometimes preceded by temperature reversals (Fig. 2). During the night inside the seawater tank, periodic rewarming was observed in all tissues and we found a considerable overlap of tissue temperatures when birds rested in water (stage 2) or in air (stage 1; Fig. 3).

Diel temperature patterns

Overall, tissue temperatures of our birds during the 5 day period they spent in the seawater tank (stage 2 and stage 4) did not differ significantly between day and night, and this was true for all tissues investigated (flank: 31.7±3.3 versus 33.1±4.4°C; back: 36.3±2.3 versus 37.0±1.3; brood patch: 22.5±2.9 versus 24.3±4.9°C; abdominal cavity: 31.3±4.2 versus 32.1±4.6°C; $P>0.05$ for all tissues). As birds could not dive/forage within our set-up (apart from occasional brief/shallow submersions), this is not surprising. However, while overall temperature values did not differ significantly between day and night, temperature variation during these periods did (LMM; interaction: hour×period; Table 2). During the day, the temperature of all tissues decreased significantly between the first and last hour (08:00–09:00 h versus 21:00–22:00 h; see Fig. 4). At night, temperatures increased between the first and last hour (22:00–23:00 h versus 07:00–08:00 h). However, given the considerable individual variation, this temperature increase was only significant for the brood patch and the abdominal cavity (Fig. 4).

Temperatures during the night (post-digestive)

As conditions in the tank during the night resembled the situation during the night at sea (i.e. birds in the post-digestive condition, resting at the surface), which was clearly not the case for the day period, we focused our following investigations on night periods only. When resting in air during stage 1, birds maintained stable

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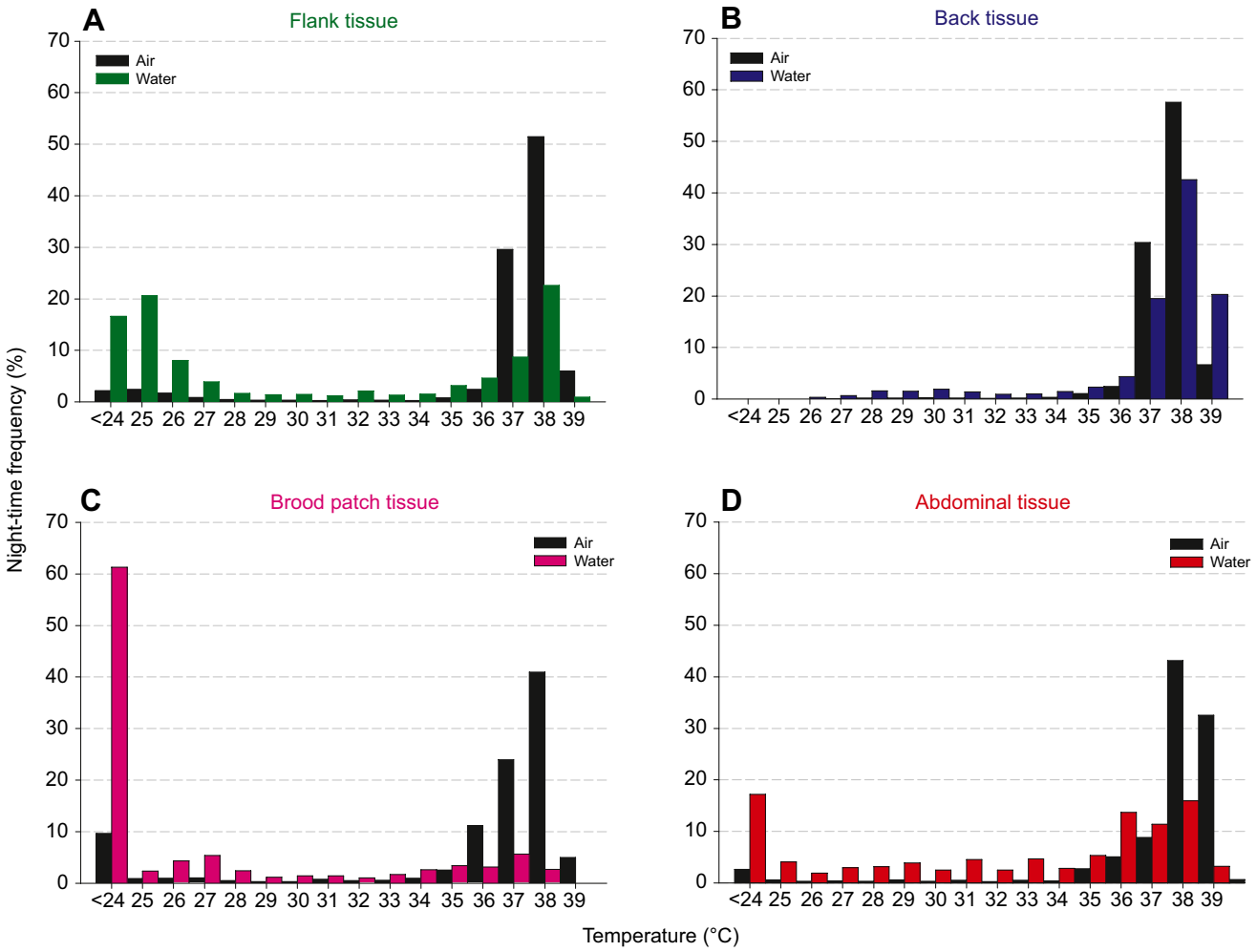

Fig. 3. Frequency distribution of tissue temperature from four sites recorded during the night, when birds rested in air or in water (stage 2). A large overlap between the black and coloured bars (as in the back tissue) indicates that recorded tissue temperatures were similar when birds rested in air and in water, with temperatures being normothermic in air (~37°C).

temperatures in all tissues throughout the night, with temperatures varying by less than 1°C (Table 1, Fig. 2). We chose mean temperatures from this period to represent the normothermic condition for each tissue investigated. Accordingly, normothermic temperature was defined as 36.9±0.3°C for the flank, 37.0±0.2°C for the back, 37.1±0.3 for the brood patch, and 37.5±0.4°C for the abdominal cavity, respectively (Table 1, Fig. 2A). In contrast to the situation in air, tissue temperatures of birds in water varied greatly during the night in stage 2 (Table 1). The greatest temperature difference was found in the brood patch, with a difference of 16.5°C between the maximum and minimum temperature recorded in one bird (Fig. 2B). Similar temperature differences for birds in water during the night (stage 2) were also found for the flank (14.6°C), back (12.9°C) and abdominal tissues (11.3°C; Table 1). Despite a considerable variation, tissue temperatures generally increased throughout the night (Fig. 2) and in some cases normothermic values were reached (see maximum values in Table 1 and individual temperature traces in Fig. 2B). This was especially true for the back and the flank tissues, where some birds (e.g. ID1 and ID5) maintained peripheral tissues at normothermic values for extended periods (e.g. for an entire night, ~10 h), while such periods were rather short-lived in other birds (e.g. ID3 and ID7) or completely absent (ID2, ID4, ID6). There was also a considerable overlap in observed temperatures at night between birds resting in air (normothermic temperatures) and birds resting in water (stage 2; Fig. 3). Temperatures within the back tissue overlapped by 74% in the two conditions and were mostly above or slightly below the established normothermic threshold when birds rested in air (Fig. 3). Similarly, temperature overlaps of 45% and 42% were observed for the flank tissue and the abdominal cavity, respectively. Furthermore, for 32% of the night, peripheral flank tissue reached temperatures in excess of 37°C. The least overlap was found in the brood patch (30%), where temperatures for birds resting in water were frequently well below the normothermic threshold (Fig. 3).

The effect of body condition (lean versus fat birds)

After fasting, mean body mass of birds during stage 4 (lean birds; 11.9±0.7 kg) was significantly lower than that during stage 2 (fat birds; 13.6±0.4 kg; $P<0.0001$). Temperatures of the flank and back tissues during the night in lean birds (stage 4) were significantly higher than those in fat birds (stage 2), indicating that body condition (body mass) affected these tissue temperatures (LMM; flank: $P<0.0001$, back: $P=0.04$; Table 2, Fig. 5). However, as birds did not maintain temperatures at a stable level and multiple temperature oscillations typically occurred throughout the night, so

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Table 2. Results from linear mixed model analysis concerning the effects of time of day and body condition on tissue temperature

	Flank			Back			Brood patch			Abdominal cavity		
	t	*P*	Estimate±s.e.	*t*	*P*	Estimate±s.e.	*t*	*P*	Estimate±s.e.	*t*	*P*	Estimate±s.e.
Hour (last)	−1.74	0.0841	−0.54±0.80	−1.30	0.1961	–	−4.39	<0.0001***	−1.51±0.34	−1.96	0.0529	−0.52±0.27
Period (night)	0.10	0.9188	–	−0.13	0.8958	–	−1.45	0.1484		−0.07	0.9408	–
Hour (last)×period (night)	5.26	<0.0001***	1.63±0.31	3.76	0.0003**	0.58±0.15	9.5	<0.0001***	3.28±0.34	8.71	<0.0001***	2.32±0.27
Body condition (fat)	−4.24	<0.0001***	−1.36±0.32	−5.40	<0.0001***	−0.85±0.16	−0.3	0.7416	–	0.4	0.69	–

General linear mixed model estimates for the effects of time (hour: first versus last; period: day versus night; and the interaction term hour×period) and body condition (fat versus lean) on tissue temperature variations in king penguins. The model was run separately for all tissues; bird ID was included as a random effect. Flank: R^2=0.41, *N*=137; back: R^2=0.39, *N*=117; brood patch: R^2=0.59, *N*=137; abdominal cavity: R^2=0.74, *N*=117. **P*<0.005; ***P*<0.001.

that normothermic periods alternated with hypothermic periods (Fig. 6), considering only mean temperatures might be misleading. For the flank temperature, we therefore calculated the total amount of time birds spent per night in near-normothermic conditions (>36°C) and the mean temperature during these near-normothermic periods. This showed that in the fat condition, flank temperature was on average elevated above 36°C for 37.1±9.9% of the night, while in the lean condition this was the case for 51.7±8.9% of the night. However, given the considerable individual variation, this difference failed to reach statistical significance (LMM, P=0.13). During the daytime, the respective values were 16.8±9.7% and 18.2±8.9% for the fat and lean condition, respectively. Mean flank temperature during near-normothermic periods throughout the night did not differ between conditions (37.5±0.3 and 37.6±0.6°C in the fat and lean condition, respectively; LMM, P=0.39). Lastly, mean abdominal temperature during the night did not differ significantly between stage 2 and stage 4 (Table 1). However, while mean abdominal temperature (31.8±5.3°C) was higher than mean flank temperature (30.3±4.7°C) in fat birds (stage 2), the reverse was true for lean birds (stage 4; 31.7±3.8 versus 33.7±2.7°C; Table 1, Fig. 2) but differences failed to reach significance, given the considerable individual variation.

DISCUSSION

We found that king penguins, fed and released for the first time into a seawater tank during the day, immediately reduced their body temperature (Fig. 2). When maintained inside this tank for 2–3 days, episodic rewarming of abdominal and peripheral tissues to normothermic values occurred during the night, when birds rested. While the temperature patterns varied greatly between tissues and individual birds, and temperatures underwent rapid changes rather than being maintained at a stable level, patterns nevertheless mimicked those observed in the wild, where tissue rewarming occurs during nightly resting at the surface (Schmidt et al., 2006). Differences in temperature changes throughout the day (when feeding occurred) and the night (when birds rested undisturbed), and the negative relationship between tissue temperature (flank and back) and body condition/body mass are in agreement with our predictions and support the hypothesis that tissue perfusion during rest (as reflected in recorded tissue temperatures) is required for nutritional needs.

Diel temperature patterns

A number of studies have investigated the foraging behaviour of adult king penguins at sea during various phases of their breeding cycle (Pütz and Bost, 1994; Pütz et al., 1998; Charrassin et al., 1998, 2001, 2002). At sea, their activity follows a diel pattern: during daylight hours, birds forage for extended periods, often diving to great depth in pursuit of their prey; at night, birds conduct shallow travelling dives and/or rest at the surface (Pütz et al., 1998; Moore et al., 1999; Pütz and Cherel, 2005). Typically, body temperature also follows a diel pattern: when foraging during the day, abdominal and peripheral tissue temperatures fall well below normothermic values and even in the hard-working pectoral muscle, temperature declines of up to 5.5°C have been observed (Culik et al., 1996b; Handrich et al., 1997; Schmidt et al., 2006). When resting at the surface during the night, tissue temperatures return to normothermic values (Culik et al., 1996b; Handrich et al., 1997; Schmidt et al., 2006).

In our set-up, birds could not dive (apart from brief/shallow submersions) and, consequently, we did not observe such a clear diel temperature pattern. Overall tissue temperatures of our birds did

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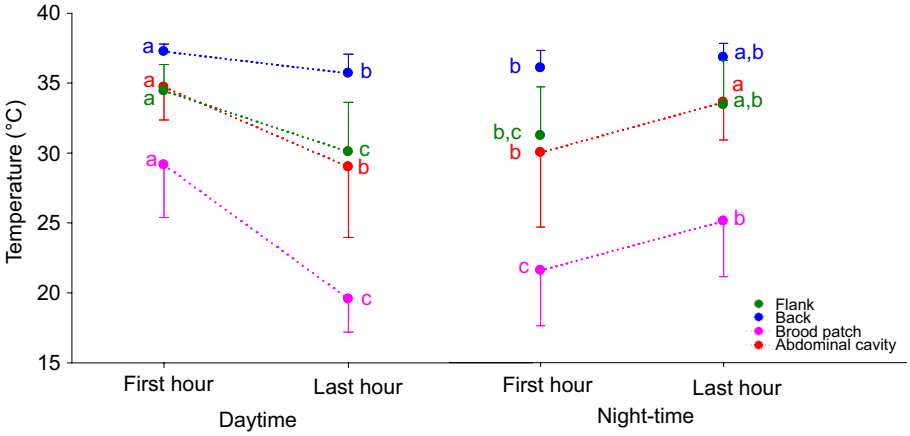

Fig. 4. Tissue temperature changes of king penguins during the day and night in water. Marginal means (±s.e.) for 6–7 birds (see Materials and methods) were estimated for different tissues, using separate linear mixed models (LMMs) during the first/last hour of the day and night. Dotted lines indicate a significant difference between the first and last hour of each period (*post hoc* Tukey HSD, $P<0.05$) for all tissues. Data points that do not share the same letters are significantly different from each other ($P<0.05$).

not differ significantly between day and night periods. However, despite individual variation, there was a general pattern of declining temperatures during the day and increasing temperatures during the night (Figs 2 and 4), mimicking patterns observed at sea. The decrease of abdominal temperature during the day was probably caused by the ingestion of cold fish (Culik et al., 1996b; Eichhorn et al., 2011; Fig. 2B), while the decrease of peripheral temperatures (flank, back, brood patch) may be due to a redistribution of blood flow associated with digestive processes (Gallavan and Chou, 1985; Bottje and Harrison, 1986, 1987; Fahlman et al., 2005). However, some of the recorded temperature changes, especially during the day, could also be a consequence of stress associated with the force-feeding procedure, which might have altered blood flow (Cabanac and Aizawa, 2000; Edgar et al., 2013; Herborn et al., 2015).

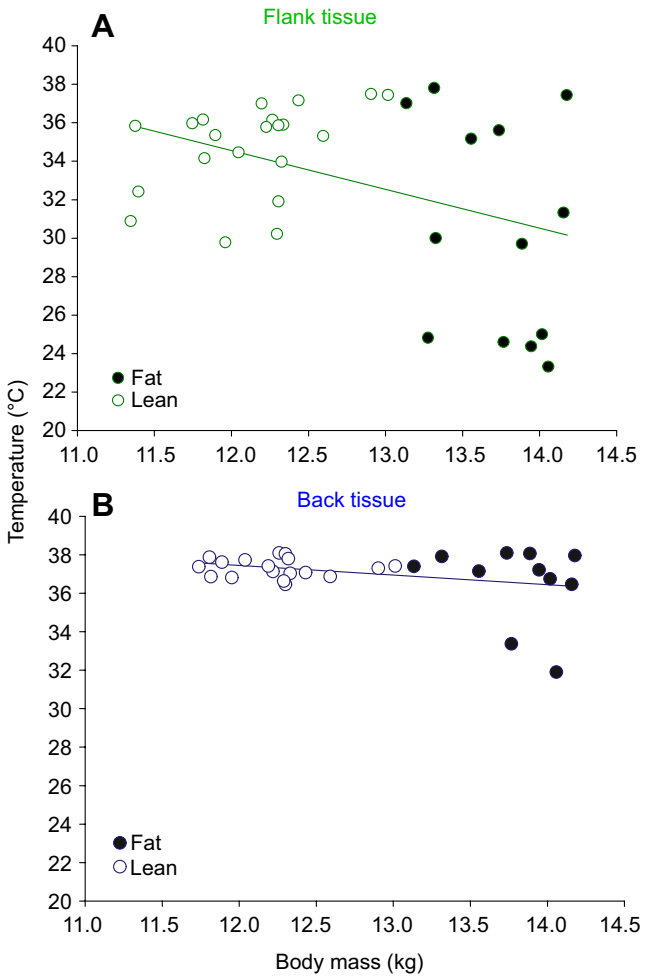

Fig. 5. Flank and back temperature of 7 king penguins resting in water during the night in the lean (stage 2) and fat (stage 4) condition. Values for flank (A) and back (B) temperature were averaged for each night spent in water. Body condition (mass) significantly affected flank temperature, such that temperatures were higher in lean birds than in fat birds (LMM, $P<0.0001$, $r^2=0.68$, $n=34$ nights). This was also true for the back tissue, albeit the relationship was driven by one individual (LMM, $P=0.04$, $r^2=37$, $n=29$ nights).

Temperatures during the night (post-digestive)

Given the limitations of our experimental set-up with respect to replicating daytime conditions typical for king penguins at sea, we focused our analysis on the night-time, where conditions were similar to the wild (i.e. fed birds resting at the surface). Here, we found that all birds displayed normothermic periods in all tissues investigated (Table 1, Figs 2B and 3). While such normothermia was maintained in some birds and tissues for extended periods (e.g. during the entire night in the flank of ID5), tissue temperatures in most birds oscillated over shorter periods. Consequently, normothermic periods in our birds were of shorter duration than those observed in the wild (see Schmidt et al., 2006). Given the shorter duration of normothermic periods within our set-up, temperature averages of peripheral tissues in king penguins resting for extended periods at the sea surface (brood patch 37.3±0.7°C and flank 37.9±0.2°C; Schmidt, 2006; Schmidt et al., 2006) were considerably higher than the temperatures in our birds, averaged throughout the night (brood patch 23.5±5.8°C and flank 30.1±4.8°C; Table 1). Nevertheless, during normothermic periods, our birds reached temperatures comparable to those of king penguins at sea in all tissues investigated (see maximum values in Table 1). The shorter normothermic periods and the great inter-individual variability observed in our birds could be related to the experimental set-up imposed on the birds (short-term captivity, frequent force-feeding during the day) and/or their known capacity to slow down/delay digestive activity (Thouzeau et al., 2003, 2004). In fact we observed one bird (excluded from the analysis) that regurgitated complete meals in an undigested state 12 h after force-feeding. Clearly, if birds did not digest their provided meals or slowed digestion considerably, perfusion and temperature patterns different from the wild situation are to be expected. However, frequent defecation was observed during the daytime, indicating that birds digested food. This is further supported by the observed tissue temperature increases during the night in all birds after being fed during the day.

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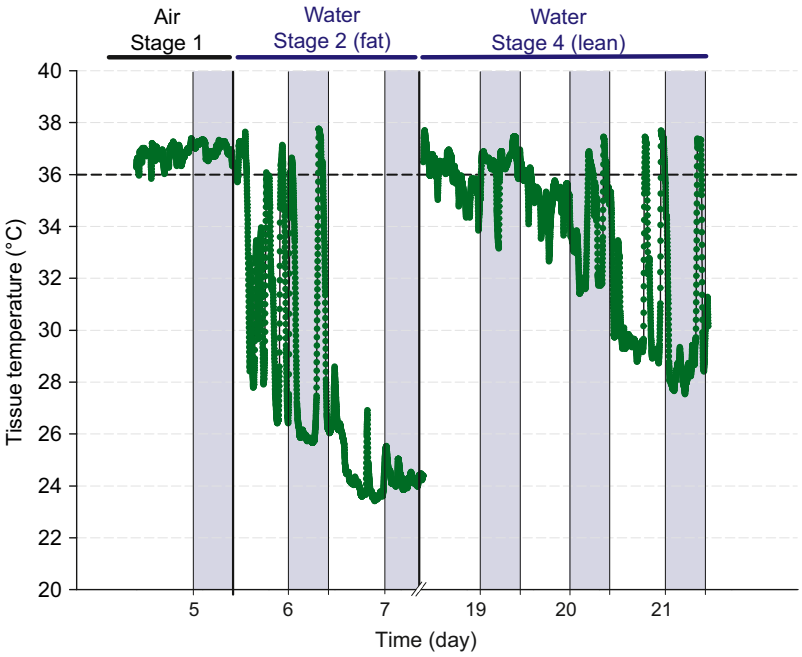

Fig. 6. Continuous temperature recording for the flank tissue of a king penguin (ID1) resting in air (stage 1) and in water (stage 2 and stage 4). Stage 2 and 4 were separated by a 10 day fasting period, not shown here. Night periods are indicated by the grey bars at the top. The dotted line indicates the temperature threshold above which temperatures were considered to be near-normothermic (36°C). Periodic tissue rewarming above the threshold occurred during the night in both the fat and lean condition. However, this occurred more frequently in the lean condition, while the mean temperature during these near-normothermic periods did not differ between conditions.

Tissue temperatures in penguins resting in water during the night differed considerably between sites (Table 1, Figs 2 and 3), with the highest and lowest temperatures recorded from the back and the brood patch, respectively. Temperature was consistently at the highest level in the back, which was the only site typically not submerged (apart from periods of short/shallow submersions) and, hence, exposed to air rather than water (Fig. 1A). Clearly, the heat conservation scenario at this site must have differed considerably from that at all other sites, so that birds could maintain perfusion and high tissue temperatures in the back without being subjected to increased heat loss associated with cold water exposure. By contrast, the lowest temperatures were recorded at the brood patch (Table 1, Figs 2 and 3). This bare and highly vascularized skin fold facilitates the rapid transfer of heat from parent to egg during incubation (Midtgård, 1984, 1985), while it might be a prime site for heat loss when birds are at sea, especially during foraging. Schmidt et al. (2006) suggested that the brood patch might be important in regulating pectoral muscle temperature during diving by allowing a controlled loss of heat via an adjustment of its perfusion ('peripheral perfusion adjustment'). While the recorded brood patch temperatures in our birds resting in water at night were considerably lower than the temperatures observed in birds at sea (Schmidt et al., 2006), they oscillated strongly between day and night (Fig. 2), suggesting some important changes in perfusion status.

Effect of body condition and the nutritional hypothesis

Based on the temperature recordings in various tissues of king penguins foraging at sea (pectoral muscle, brood patch, flank), Schmidt and colleagues (Schmidt, 2006; Schmidt et al., 2006) suggested that the high temperatures observed at night, presumably associated with high heat loss and thermoregulatory costs, are linked with the requirements of maintaining peripheral perfusion to facilitate the deposition of subcutaneous fat after successful foraging. Our experiments recording tissue temperatures in fat and lean birds showed a trend of increasing tissue temperatures during the night (Figs 2 and 4, Table 2), which fits well with our prediction in support of the nutritional hypothesis. Furthermore, night temperatures in the flank were significantly affected by body condition (Tables 1 and 2). As predicted, flank temperatures of penguins during the night were significantly higher in the lean condition than in the fat condition. These higher flank temperatures recorded in lean birds could be the consequence of either (1) an active regulation of peripheral perfusion, presumably to deposit subcutaneous fat, or (2) a reduced subcutaneous insulation leading to greater heat flux from the core to the skin, without changes in perfusion. As near-normothermic periods that occurred throughout the night reached similar temperature levels in both fat (stage 2) and lean birds (stage 4) but the overall duration of these periods per night was longer in lean birds, our results argue against simple changes in body insulation explaining the observed patterns (Fig. 6). Rather, our results suggest that these higher mean temperatures in the flank of lean birds are the consequence of an active vasomotor regulation, presumably to allow deposition of subcutaneous fat. Such preferential deposition of subcutaneous fat in lean birds would be of great benefit for their insulation in cold water and would keep heat loss at bay (see Enstipp et al., 2017). It also agrees with the suggestion by Blem (1990) that subcutaneous fat in birds tends to be the first deposited. By contrast, fat birds are well insulated; hence, the need to perfuse subcutaneous tissues for fat deposition should be reduced. These birds might instead store any surplus FFA in abdominal adipose tissue and reduce peripheral perfusion to avoid additional heat loss. Our finding that flank temperatures in lean birds remain consistently above abdominal temperatures, while the reverse tends to be true for fat birds, supports such a scenario (Table 1, Fig. 2).

Hence, our results are in general agreement with the suggestion that the apparent paradox of high peripheral temperatures in king penguins during extended resting phases at the surface (reflecting sustained peripheral perfusion) is associated with perfusion requirements for subcutaneous fat deposition. The high thermoregulatory costs associated with such maintained peripheral perfusion are illustrated by our observation that some birds with high peripheral temperatures during the night lost body mass during experimentation (stage 2), despite feeding.

There has been evidence for a delay of digestive processes in king penguins and seals (Gauthier-Clerc et al., 2000; Sparling et al., 2007). By contrast, Svärd et al. (2009) found that food digestion

began during foraging in fasted Steller sea lions (*Eumetopias jubatus*) and was not deferred until afterwards, suggesting that access to the ingested energy was important in these nutritionally stressed animals. Our penguins defecated frequently throughout the day in both conditions (fat and lean) and, hence, it seems unlikely that they deferred digestion. Accordingly, our results support the hypothesis that nutritionally stressed lean birds had a greater need to deposit any surplus FFA in the subcutaneous fat for insulation, when compared with fat birds. That a sufficient subcutaneous fat layer is of great importance is illustrated by the observation that fasting grey seal pups (*Halichoerus grypus*) increase protein catabolism to retain a critical subcutaneous fat layer for thermoregulatory reasons (Øritsland et al., 1985). Similarly, Enstipp et al. (2017) found a substantial decline in peripheral temperatures reached during foraging bouts of juvenile and adult king penguins over time and attributed this to changes in body insulation (i.e. subcutaneous fat).

As an alternative to the nutritional hypothesis explaining peripheral normothermia during extended surface breaks in king penguins, Fahlman et al. (2007) suggested that such perfusion is required to support the unloading of N_2, accumulated during diving. Hence, they suggested that N_2 accumulation might force king penguins to terminate foraging bouts, even when prey might be readily available (Fahlman et al., 2007). While such a scenario seems plausible, it is unclear how birds might detect critical levels of N_2 that could trigger peripheral perfusion to flush out N_2. Furthermore, although not explicitly investigated in our experiment, peripheral rewarming observed in our birds during the night (reflecting peripheral perfusion) in the absence of diving activity during the day does not lend support to the N_2 unloading hypothesis. Accordingly, we suggest that increased peripheral temperatures observed during the night in our birds reflect tissue perfusion required for the deposition of fat in subcutaneous adipose tissue. Higher peripheral temperatures in the lean condition reflect the preferential deposition of subcutaneous fat under these circumstances.

Finally, Fahlman et al. (2005) suggested that the metabolic and regional temperature changes they observed in fasting (non-fed) king penguins in water (trials of ∼3 h) were associated with the need to mobilize fuel from subcutaneous adipose tissue. This is of course the flip side of the coin and it would be most interesting to study the temperature changes associated with both feeding (fat deposition) and fasting (fat mobilization) periods. Such a study should shed more light on the apparent trade-off between nutritional dynamics and thermoregulation.

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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.; Software: B.P.; Formal analysis: A.L., B.P.; Investigation: A.L., T.v.W.; Writing - original draft: A.L., M.R.E.; Writing - review & editing: A.L., M.R.E.; Supervision: Y.H.

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References
Behnke, A. R., Thomson, R. M. and Shaw, L. A. (1935). The rate of elimination of dissolved nitrogen in man in relation to the fat and water content of the body. *Am. J. Physiol.* **114**, 137-146.
Bertile, F., Criscuolo, F., Oudart, H., Le Maho, Y. and Raclot, T. (2003). Differences in the expression of lipolytic-related genes in rat white adipose tissues. *Biochem. Biophys. Res. Commun.* **307**, 540-546.
Blem, C. R. (1990). Avian energy storage. *Curr. Ornithol.* **7**, 59-113.
Bottje, W. G. and Harrison, P. C. (1986). The effect of high ambient temperature and hypercapnia on postprandial intestinal hyperemia in domestic cockerels. *Poult. Sci.* **65**, 1606-1614.
Bottje, W. G. and Harrison, P. C. (1987). Celiac cyclic blood flow pattern response to feeding and heat exposure. *Poult. Sci.* **66**, 2039-2042.
Butler, P. J. (2004). Metabolic regulation in diving birds and mammals. *Respir. Physiol. Neurobiol.* **141**, 297-315.
Cabanac, M. and Aizawa, S. (2000). Fever and tachycardia in a bird (*Gallus domesticus*) after simple handling. *Physiol. Behav.* **69**, 541-545.
Charrassin, J.-B., Bost, C. A., Pütz, K., Lage, J., Dahier, T., Zorn, T. and Le Maho, Y. (1998). Foraging strategies of incubating and brooding king penguins *Aptenodytes patagonicus*. *Oecologia* **114**, 194-201.
Charrassin, J.-B., Kato, A., Handrich, Y., Sato, K., Naito, Y., Ancel, A., Bost, C.-A., Gauthier-Clerc, M., Ropert-Coudert, Y. and Le Maho, Y. (2001). Feeding behaviour of free-ranging penguins determined by oesophageal temperature. *Proc. Biol. Sci.* **268**, 151-157.
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 Le Maho, Y. (1985). Five months of fasting in king penguin chicks: body mass loss and fuel metabolism. *Am. J. Physiol.* **249**, R387-R392.
Cherel, Y., Charrassin, J.-B. and Handrich, Y. (1993). Comparison of body reserve buildup in prefasting chicks and adults of king penguins (*Aptenodytes patagonicus*). *Physiol. Zool.* **66**, 750-770.
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.* **234**, 1-12.
Culik, B. M., Wilson, R. and Bannasch, R. (1994). Underwater swimming at low energetic cost by pygoscelid penguins. *J. Exp. Biol.* **197**, 65-78.
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.
De Vries, J. and van Eerden, M. R. (1995). Thermal conductance in aquatic birds in relation to the degree of water contact, body mass, and body fat: energetic implications of living in a strong cooling environment. *Physiol. Zool.* **68**, 1143-1163.
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.
Edgar, J. L., Nicol, C. J., Pugh, C. A. and Paul, E. S. (2013). Surface temperature changes in response to handling in domestic chickens. *Physiol. Behav.* **119**, 195-200.
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., Jones, D. R., Lorentsen, S.-H. and Grémillet, D. (2007). Energetic costs of diving and prey-capture capabilities in cormorants and shags (*Phalacrocoracidae*) underline their unique adaptation to the aquatic environment. *J. Ornithol.* **148** Suppl. 2, S593-S600.
Enstipp, M. R., Grémillet, D. and Jones, D. R. (2008). Heat increment of feeding in double-crested cormorants (*Phalacrocorax auritus*) and its potential for thermal substitution. *J. Exp. Biol.* **211**, 49-57.
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., 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.
Fahlman, A., Schmidt, A., Jones, D. R., Bostrom, B. L. and Handrich, Y. (2007). To what extent might N2 limit dive performance in king penguins? *J. Exp. Biol.* **210**, 3344-3355.
Froget, G., Handrich, Y., Le Maho, Y., Rouanet, J.-L., Woakes, A. J. and Butler, P. J. (2002). The heart rate/oxygen consumption relationship during cold exposure of the king penguin: a comparison with that during exercise. *J. Exp. Biol.* **205**, 2511-2517.
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.

Gallavan, R. H. and Chou, C. C. (1985). Possible mechanisms for the initiation and maintenance of postprandial intestinal hyperemia. *Am. J. Physiol.* **249**, G301-G308.
Gauthier-Clerc, M., Le Maho, Y., Clerquin, Y., Drault, S. and Handrich, Y. (2000). Penguin fathers preserve food for their chicks. *Nature* **408**, 928-929.
Green, J. A., Frappel, P. B., Clark, T. D. and Butler, P. J. (2006). Physiological response to feeding in little penguins. *Physiol. Biochem. Zool.* **79**, 1088-1097.
Grémillet, D., Wanless, S., Carss, D. N., Linton, D., Harris, M. P., Speakman, J. R. and Le Maho, Y. (2001). Foraging energetics of arctic cormorants and the evolution of diving birds. *Ecol. Lett.* **4**, 180-184.
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.
Herborn, K. A., Graves, J. L., Jerem, P., Evans, N. P., Nager, R., McCafferty, D. J. and McKeegan, D. E. F. (2015). Skin temperature reveals the intensity of acute stress. *Physiol. Behav.* **152**, 225-230.
Jouventin, P. (1982). *Visual and Vocal Signals in Penguins, Their Evolution and Adaptive Characters*. Berlin: Verlag Paul Parey.
Knower Stockard, T., Heil, J., Meir, J. U., Sato, K., Ponganis, K. V. and Ponganis, P. J. (2005). Air sac PO_2 and oxygen depletion during dives of emperor penguins. *J. Exp. Biol.* **208**, 2973-2980.
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. A Physiol.* **54**, 75-80.
Kvadsheim, P. H. and Folkow, L. P. (1997). Blubber and flipper heat transfer in harp seals. *Acta Physiol. Scand.* **161**, 385-395.
Lovvorn, J. R. (2007). Thermal substitution and aerobic efficiency: measuring and predicting effects of heat balance on endotherm diving energetics. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **362**, 2079-2093.
Markussen, N. H. and Ryg, M. (1992). Metabolic rate and body composition of harbour seals, *Phoca vitulina*, during starvation and refeeding. *Can. J. Zool.* **70**, 220-224.
Meir, J. U. and Ponganis, P. J. (2009). High-affinity hemoglobin and blood oxygen saturation in diving emperor penguins. *J. Exp. Biol.* **212**, 3330-3338.
Meir, J. U., Stockard, T. K., Williams, C. L., Ponganis, K. V. and Ponganis, P. J. (2008). Heart rate regulation and extreme bradycardia in diving emperor penguins. *J. Exp. Biol.* **211**, 1169-1179.
Midtgård, U. (1984). Density of arteriovenous anastomoses in some skin areas of the domestic fowl (*Gallus domesticus*). *Anat. Rec.* **209**, 455-459.
Midtgård, U. (1985). Arteriovenous anastomoses in the incubation patch of herring gulls. *Condor* **87**, 549-551.
Moore, G. J., Wienecke, B. and Robertson, G. (1999). Seasonal change in foraging areas and dive depths of breeding king penguins at Heard Island. *Polar Biol.* **21**, 376-384.
Nordøy, E. S. and Blix, A. S. (1985). Energy sources in fasting grey seal pups evaluated with computed tomography. *Am. J. Physiol.* **249**, R471-R476.
Nordøy, E. S., Ingebretsen, O. C. and Blix, A. S. (1990). Depressed metabolism and low protein catabolism in fasting grey seal pups. *Acta Physiol. Scand.* **139**, 361-369.
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.
Pond, C. M. and Mattacks, C. A. (1985). Cellular structure of adipose tissue in birds. *J. Morphol.* **185**, 195-202.
Ponganis, P. J., van Dam, R. P., Knower, T. and Levenson, D. H. (2001). Temperature regulation in emperor penguins foraging under sea ice. *Comp. Biochem. Physiol. A* **129**, 811-820.
Ponganis, P. J., van Dam, R. P., Levenson, D. H., Knower, T., Ponganis, K. V. and Marshall, G. (2003). Regional heterothermy and conservation of core temperature in emperor penguins diving under sea ice. *Comp. Biochem. Physiol. A* **135**, 477-487.
Ponganis, P. J., Stockard, T. K., Meir, J. U., Williams, C. L., Ponganis, K. V., van Dam, R. P. and Howard, R. (2007). Returning on empty: extreme blood O_2 depletion underlies dive capacity of emperor penguins. *J. Exp. Biol.* **210**, 4279-4285.
Ponganis, P. J., Stockard, T. K., Meir, J. U., Williams, C. L., Ponganis, K. V. and Howard, R. (2009). O_2 store management in diving emperor penguins. *J. Exp. Biol.* **212**, 217-224.
Ponganis, P. J., Meir, J. U. and Williams, C. L. (2011). In pursuit of Irving and Scholander: a review of oxygen store management in seals and penguins. *J. Exp. Biol.* **214**, 3325-3339.
Pütz, K. and Bost, C.-A. (1994). Feeding behavior of free-ranging king penguins (*Aptenodytes Patagonicus*). *Ecology* **75**, 489-497.
Pütz, K. and Cherel, Y. (2005). The diving behaviour of brooding king penguins (*Aptenodytes patagonicus*) from the Falkland Islands: variation in dive profiles and synchronous underwater swimming provide new insights into their foraging strategies. *Mar. Biol.* **147**, 281-290.
Pütz, K., Wilson, R. P., Charrassin, J.-B., Raclot, T., Lage, T., Le Maho, Y., Kierspel, M. A. M., Culik, B. M. and Adelung, D. (1998). Foraging strategy of king penguins (*Aptenodytes patagonicus*) during summer at the Crozet Islands. *Ecology* **79**, 1905-1921.
Sato, K., Shiomi, K., Marshall, G., Kooyman, G. L. and Ponganis, P. J. (2011). Stroke rates and diving air volumes of emperor penguins: implications for dive performance. *J. Exp. Biol.* **214**, 2854-2863.
Schmidt, A. (2006). Etude de la thermorégulation en mer chez le manchot royal: mécanismes et conséquences énergétiques. PhD thesis, Université de Strasbourg, France.
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.
Sparling, C. E., Fedak, M. A. and Thompson, D. (2007). Eat now, pay later? Evidence of deferred food-processing costs in diving seals. *Biol. Lett.* **3**, 94-98.
Sugden, M. C., Grimshaw, R. M., Lall, H. and Holness, M. J. (1994). Regional variations in metabolic responses of white adipose tissue to food restriction. *Am. J. Physiol.* **267**, E892-E899.
Svärd, C., Fahlman, A., Rosen, D. A. S., Joy, R. and Trites, A. W. (2009). Fasting affects the surface and diving metabolic rates of Steller sea lions *Eumetopias jubatus*. *Aquat. Biol.* **8**, 71-82.
West, D. B., Prinz, W. A. and Greenwood, M. R. (1989). Regional changes in adipose tissue blood flow and metabolism in rats after a meal. *Am. J. Physiol.* **257**, R711-R716.
Wienecke, B., Robertson, G., Kirkwood, R. and Lawton, K. (2006). Extreme dives by free-ranging emperor penguins. *Polar Biol.* **30**, 133-142.
Williams, C. L., Meir, J. U. and Ponganis, P. J. (2011). What triggers the aerobic dive limit? Patterns of muscle oxygen depletion during dives of emperor penguins. *J. Exp. Biol.* **214**, 1802-1812.
Wilson, R. P., Hustler, K., Ryan, P. G., Burger, A. E. and Noldeke, E. C. (1992). Diving birds in cold water: do Archimedes and Boyle determine energetic costs? *Am. Nat.* **140**, 179-200.