

RESEARCH ARTICLE

Apparent changes in body insulation of juvenile king penguins suggest an energetic challenge during their early life at sea

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ABSTRACT

Little is known about the early life at sea of marine top predators, like deep-diving king penguins (*Aptenodytes patagonicus*), although this dispersal phase is probably a critical phase in their life. Apart from finding favourable foraging sites, they have to develop effective prey search patterns as well as physiological capacities that enable them to capture sufficient prey to meet their energetic needs. To investigate the ontogeny of their thermoregulatory responses at sea, we implanted 30 juvenile king penguins and 8 adult breeders with a small data logger that recorded pressure and subcutaneous temperature continuously for up to 2.5 years. We found important changes in the development of peripheral temperature patterns of foraging juvenile king penguins throughout their first year at sea. Peripheral temperature during foraging bouts fell to increasingly lower levels during the first 6 months at sea, after which it stabilized. Most importantly, these changes re-occurred during their second year at sea, after birds had fasted for ~4 weeks on land during their second moult. Furthermore, similar peripheral temperature patterns were also present in adult birds during foraging trips throughout their breeding cycle. We suggest that rather than being a simple consequence of concurrent changes in dive effort or an indication of a physiological maturation process, these seasonal temperature changes mainly reflect differences in thermal insulation. Heat loss estimates for juveniles at sea were initially high but declined to approximately half after ~6 months at sea, suggesting that juvenile king penguins face a strong energetic challenge during their early oceanic existence.

KEY WORDS: Thermoregulation, Peripheral temperature, Subcutaneous fat, Vasoconstriction, Diving, Seabirds

INTRODUCTION

Little is known about the early life at sea of marine top predators, like deep-diving king penguins (*Aptenodytes patagonicus* Miller). Juvenile king penguins leave the colony for the first time when ~1 year old and disperse at sea for an extended period (1–3 years), interrupted only by an annual moult on land, after which most birds return to their natal colony and attempt to recruit into the breeding population (Weimerskirch et al., 1992; Saraux et al., 2011). This initial dispersal phase at sea is probably a critical phase in their life

(Orgeret et al., 2016), when mortality is high compared with that of adults (Le Bohec et al., 2007; Saraux et al., 2011), and foraging proficiency might play a key role in their survival (Wunderle, 1991; Daunt et al., 2007). Apart from finding favourable foraging sites, birds have to develop effective prey search patterns as well as physiological capacities (breath holding, locomotion, thermoregulation) that enable them to capture sufficient prey (predominately myctophid fishes; Cherel and Ridoux, 1992; Raclot et al., 1998; Bost et al., 2002), often at great depth (Charrassin and Bost, 2001), to meet their energetic needs. However, studies investigating the ontogeny of foraging behaviour in marine birds in the wild are still rare (Daunt et al., 2007; Thiebot et al., 2013; Orgeret et al., 2016; De Grissac et al., 2016), with most studies focusing on dispersive movements (Clarke et al., 2003; Kooyman and Ponganis, 2008; Wienecke et al., 2010; Pütz et al., 2014), and studies concerning physiological aspects are lacking (Ponganis et al., 1999).

For juvenile king penguins, the transition from their terrestrial existence in the colony to a life at sea must be a considerable physiological challenge with respect to the development of a sufficient dive capacity but also an effective thermoregulatory control (water temperature, $T_w \sim 1\text{--}6^\circ\text{C}$). Cold sea water is an enormous heat sink and unless birds are properly insulated and physiologically sufficiently developed (adapted vasomotor response), they will lose heat rapidly, burdening their energy balance with high thermoregulatory costs. In penguins, two important structures provide thermal insulation and prevent excessive heat loss to the environment. On the external side, air is trapped within the dense down layer of their plumage, which is covered by rigid contour feathers, forming an impenetrable waterproof layer (Williams et al., 2015). In air, this external insulation was estimated to provide between 56% and 87% of the total insulation (Jarman, 1973; Le Maho et al., 1976; Stahel and Nicol, 1982; Barré, 1984). However, during diving, the air layer is compressed as birds descend to depth, reducing insulation (Wilson et al., 1992; De Vries and Van Erden, 1995). In fully immersed juvenile king and macaroni penguins (*Eudyptes chrysolophus*), external insulation was estimated to contribute only 8% and 10% to total insulation, respectively, with the remainder provided by a layer of adipose tissue, uniformly distributed under the skin (Barré and Roussel, 1986; Pond and Mattacks, 1985). This 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 a cooler body shell, reducing heat flux to the environment and keeping thermoregulatory costs at bay (Kooyman et al., 1976; Ponganis et al., 2001, 2003).

When adult king penguins forage at sea, often for extended periods and to great depth (maximum depth: 343 m, maximum dive duration: 9.2 min; Pütz et al., 1998; Pütz and Cherel, 2005; Andrews and Enstipp, 2016), substantial temperature declines in their peripheral tissues (subcutaneous, brood patch) and in their

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List of symbols

M_b	body mass (kg)
T_{end}	temperature at the end of an initial temperature drop ($^{\circ}\text{C}$)
T_{max}	single highest temperature value recorded per day ($^{\circ}\text{C}$)
T_{mean}	average of all temperature values recorded per day ($^{\circ}\text{C}$)
T_{min}	single lowest temperature value recorded per day ($^{\circ}\text{C}$)
T_w	water temperature ($^{\circ}\text{C}$)
ΔT	temperature difference between the start and end of an initial temperature drop ($^{\circ}\text{C}$)

abdomen have been observed (Culik et al., 1996; Handrich et al., 1997; Schmidt et al., 2006; Schmidt, 2006). Temperature declines in abdominal and subcutaneous tissues have also been recorded in king penguins diving within a still water channel (Fahlman et al., 2005). Similarly, temperature declines in subcutaneous tissues have been observed in emperor penguins (*Aptenodytes forsteri*) diving from an ice hole (Ponganis et al., 2003) and in foraging Brünnich's guillemots (*Uria lomvia*) (Niizuma et al., 2007). These peripheral temperature declines during foraging/diving are the result of a combination of factors that affect the amount of heat loss via conduction/convection to the environment: (1) low T_w and the high thermal conductance and thermal capacity of water; (2) air loss and compression of the plumage air layer with acceleration and depth, reducing external insulation (see fig. 9 in Ponganis et al., 2003); (3) swim speed; (4) the possible temperature reduction in deep tissues; and (5) the extent of peripheral vasoconstriction. When fully vasoconstricted, heat transfer from the warmer core to the periphery will be slowed considerably, as the only remaining pathway is conduction through the tissues (Whittow, 1986).

However, while effective heat conservation mechanisms seem to be in place in adult king penguins and other avian divers, little is known about the development of these mechanisms in juvenile birds under natural conditions. During whole-body immersions in the laboratory, juvenile king and gentoo penguins (*Pygoscelis papua*) showed a gradual increase in internal insulation over time, suggesting that the peripheral vasoconstriction response was not fully developed upon first immersion (Barré and Roussel, 1986; Dumonteil et al., 1994). By contrast, Eichhorn et al. (2011) reported a remarkable heterothermy in the body core and in peripheral tissues of free-living king penguin chicks throughout the winter period, when they fast for up to 5 months, in air (Cherel and Le Maho, 1985). Subcutaneous temperatures as low as 19°C and a temperature gradient between the core and the shell of up to 15.7°C were measured, indicating the presence of a peripheral vasoconstrictive response, long before their first departure to sea (Eichhorn et al., 2011).

Furthermore, the subcutaneous fat layer in penguins serves a dual function: it acts as an insulator, particularly during diving (Kooyman et al., 1976) but also, as in most other birds, it is the main site of energy storage (Blem, 1990; Cherel et al., 1994) and, as such, undergoes seasonal changes. The consequences of these seasonal changes on penguin thermoregulation and energy expenditure at sea are largely unknown (Green et al., 2005).

The purpose of this study was to investigate the thermoregulatory responses of juvenile king penguins during their dispersal phase at sea. In particular, (1) we studied the development of peripheral temperature drops during foraging/diving, indicative of a vasomotor response, and (2) we explored the mechanism behind the observed changes in temperature drops over time, especially concurrent changes in dive behaviour. By constructing a heat loss model, (3) we investigated the energetic consequences of the observed thermal responses in juvenile king penguins. Finally, (4) we

compared the patterns observed in juvenile birds with the mature responses in adult breeders. A detailed analysis of changes in foraging behaviour throughout the dispersal phase of juvenile king penguins will be reported in a separate investigation.

MATERIALS AND METHODS**Bird capture and logger implantation**

In November/December 2013, 30 juvenile king penguins of both sexes (~ 1 year old; Table 1) were captured from the 'Baie du Marin' colony on Possession Island, Crozet Archipelago, in the Southern Indian Ocean ($46^{\circ}25'34''\text{S}$, $51^{\circ}51'36''\text{E}$). At the time of capture, all juveniles were near the end of their first moult from down to waterproof plumage, after which birds typically leave the colony and disperse at sea for an extended period. This study focused on the juvenile stage of king penguins (1–2 years old) but also extended to their immature stage (2–3 year old birds). In addition, in late December 2014, 8 adult male king penguins (>3 years old; Table 1; sex determined by song and breeding patterns; Jouventin, 1982; Descamps et al., 2002) were captured from the same colony during the incubation phase of their breeding cycle (after egg exchange with their partner). Following capture, birds underwent surgery for the implantation of a data logger. The logger (Light Ultralight Logger, LuL, MIBE, IPHC, Strasbourg, France) was encapsulated either directly in wax only (15 juveniles) or first placed in an additional pouch of heat shrink tubing filled with silicon oil, before application of a thin wax layer (15 juveniles and all adults). The dimensions of the implanted loggers were on average $32 \times 26 \times 15$ mm (length, width, height) with a mean mass of 8.9 ± 0.5 g (range: 6.3–11.4 g, depending on casing). Loggers were programmed to record pressure (resolution: 0.5 mbar, accuracy: ± 30 mbar, calibrated range: linear to 40 bar; where 1 bar is 100 kPa) and temperature (resolution: 0.02°C , accuracy: $\pm 0.5^{\circ}\text{C}$, range: -35 to 65°C , time constant: 70–220 s depending on logger encapsulation) every 5 s (internal sensors), so that the recording ideally would cover the birds' entire dispersal phase.

The anaesthesia/surgical procedure followed the protocol previously detailed in Froget et al. (2004) and Fahlman et al. (2005). During surgery, a maximum of ~ 6 –8 contour feathers (and associated down) were removed from two parallel rows of feathers on the right flank of the bird (midaxillary line, halfway between the leg and flipper articulations, a position permanently submerged in a bird at sea). After feather removal, the area was cleaned and disinfected with Betadine, before an incision of ~ 3 cm was made. A blunt probe was used to create a pouch within the subcutaneous fat, just underneath the skin, ~ 2 –3 cm caudal to the incision site. The pouch was flushed with saline, before and after logger introduction. It was then closed with a single suture in the cranial–caudal axis, to ensure logger fixture within the pouch. The subcutaneous fat at the incision site and, subsequently, the overlaying skin were both closed with sutures. Positioning of the logger in this way (Fig. 1) ensured that temperature measurements of the logger were not compromised by healing processes of the skin and/or potential interference with plumage insulation associated with feather removal during surgery. Removal of the loggers 1–3 years after implantation showed that loggers were encapsulated by a thin layer of reactive connective tissue and were located within the distal part of the subcutaneous fat tissue, unchanged from the original position. Juvenile and adult birds also received a passive transponder tag (32 mm low-frequency glass transponder, weighing 0.8 g; Texas Instruments, Dallas, TX, USA), which was placed subcutaneously between their leg and tail, and allowed detection upon return to the colony through an RFID system (radio-frequency identification; Gendner et al., 1992; Gendner et al.,

Table 1. Summary of logger deployments

Bird	M_b (kg)	Total recording duration (days)	Time at sea (days)	No. of trips	Start trip 1	Trip 1 duration (days)
Juveniles						
LuL04	9.8	413.7	380.9	3	08/12/2013	330.9
LuL05	12.3	204.9	194.5	1	08/12/2013	
LuL08	10.4	15.0	6.5	1	10/12/2013	
LuL09	12.9	642.8	593.8	3	15/12/2013	337.1
LuL11	8.6	432.5	391.8	2	08/12/2013	363.5
LuL13	8.5	788.7	704.5	7	15/12/2013	337.3
LuL14	10.8	375.1	359.7	2	17/12/2013	329.1
LuL15	11.5	837.7	738.5	9	17/12/2013	338.3
LuL18	11.5	19.0	10.5	1	18/12/2013	
LuL22	10.2	101.0	89.2	1	05/01/2014	
LuL23	10.8	268.1	255.5	1	06/01/2014	
LuL25	11.1	44.4	35.8	1	04/01/2014	
LuL29	11.3	439.3	394.2	3	07/01/2014	300.1
Mean	10.7±0.4	352.5±77.6	319.6±69.9	2.7±0.7	20/12/2013 (±3.3 days)	333.7±7.1
Adults						
LuL31	9.7	263.1	173.5	13	29/12/2014	16.7
LuL32	9.7	120.3	72.4	5	31/12/2014	18.8
LuL33	10.1	43.0	23.7	2	01/01/2015	12.3
LuL36		216.5	139.6	7	03/01/2015	13.2
LuL37	11.7	305.5	257.9	17	05/01/2015	7.5
Mean	10.3±0.5	189.7±47.9	133.4±40.6	8.8±2.7	01/01/2015 (±1.2 days)	13.7±1.9

Mean indicates grand means±s.e.m. Trip 1 duration for juvenile birds refers to their first trip upon moult completion, when they leave the colony for the first time in their life; only complete trips were included. For adults, the first trip recorded occurred during the incubation period.

2005). All birds were additionally marked with hair dye on both legs (L'Oréal, Clichy, France), to enable visual observations in the colony before departure and potential detection upon return. Finally, birds were weighed (accuracy: ±20 g) and morphological measurements were taken (length of flippers, feet and beak).

For recovery from surgery, birds were kept inside a wooden enclosure for up to 2 days, during which time they received antibiotic treatment as well as anti-inflammatory/pain relieving drugs. Upon recovery, birds were released at the capture site. On average, juvenile birds remained in the colony for 8.5±0.7 days following release (range: 2.4–12.1 days), before they initiated their first trip to sea. During that period, juveniles were occasionally observed to enter the bay for brief periods to swim, dive and preen, before returning to the colony (see Corbel et al., 2009). Adult birds remained in the colony on average for 3.0±0.5 days after their release (range: 1.6–4.6 days), before initiating their foraging trip.

Bird return and recapture

In the period up to March 2017, 19 juvenile/immature birds and 5 adult birds were successfully recaptured and underwent surgery for logger removal, which followed the same surgical protocol as detailed above. All loggers were opened and data were downloaded onto a PC.

All experimental procedures were approved by the French ethics committee (APAFIS, permit no. 02015041411414001) and the French Polar Environmental Committee (permit nos 2013-76, 2014-121, 2015-145; TAAF) and conducted in accordance with its guidelines.

Data analysis

Logger data

Of the 19 loggers retrieved from young birds, three failed to record, one stopped recording before the bird went to sea and two loggers

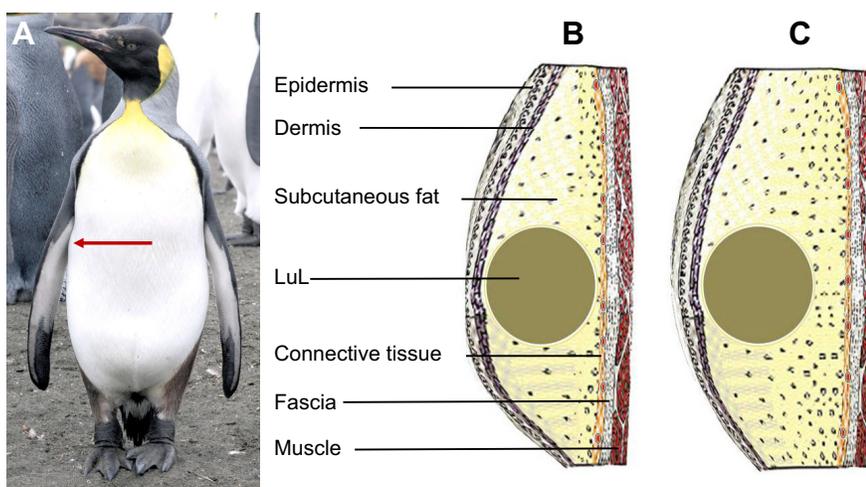


Fig. 1. Juvenile king penguin before its departure to sea and logger position within the subcutaneous fat. (A) The position of the implanted logger (LuL) is visible on the right flank of the bird, as indicated by the arrow. At this position, the logger was permanently submerged when the bird was at sea. (B,C) The logger position within the subcutaneous fat upon implantation (B) and after several months at sea (C). Note the thin layer of reactive connective tissue that encapsulated the logger and the suggested different thickness of the subcutaneous fat layer between the body core and the logger in B and C.

are currently in transit from the remote field site. The remaining 13 loggers recorded data for periods ranging between 2 weeks and 2.5 years before battery failure (Table 1). The total number of recorded days spent at sea ranged from 7 to 739 days across juveniles, with recording durations exceeding 1 year in 7 out of 13 birds (Table 1). All loggers from 5 adult birds recorded data over periods of 6 weeks to 10 months, which corresponds to a total of 24–258 days spent at sea (Table 1).

Analysis

All data were analysed using custom-written programs in Matlab (version R2015b; MathWorks, Natick, MA, USA). The considerable temperature differences within the subcutaneous tissues of birds (range: 39.7–9.6°C), affected the response of the pressure sensor, requiring a zero offset correction of depth data. This was accomplished through a series of smoothing and filtering procedures of the original data. In accordance with the resolution/accuracy of the pressure sensor and the zero offset correction, only dives exceeding 1 m depth were included in the analysis.

All juvenile birds conducted extended trips at sea before returning to their natal colony (Table 1). In some birds, we observed days without diving activity in association with high peripheral temperatures, suggesting that birds had exited the water and stayed on land or ice. These periods were usually short (range: 1–11 days) and measurements from these periods were excluded from the analysis. In 6 of these birds, the recordings were sufficiently long to include moult periods, when birds spent up to 28 days on land (second moult of their life; entering the immature stage). This moult period after the first year at sea was followed by further extended trips. In the current analysis, we focused on the first year spent at sea ('juvenile stage'), which started with the departure from the colony and ended with the second moult, which began on average 380.8 ± 7.7 days after departure (mean for 6 birds). For 3 young birds, the recording also included their second year spent at sea ('immature stage'), which started with the departure after their second moult and ended with the start of their third moult, ~2 years after their initial departure.

Adult birds conducted multiple foraging trips that started during the incubation period (5 birds), continued throughout the winter (June–September; 3 birds) and extended into the following spring (September–December, 2 birds; Table 1). As with the juveniles, days without diving activity in association with high peripheral temperatures were excluded from the analysis, as birds must have been on land, presumably within the colony. For the current analysis, we focused on the first two short foraging trips conducted during incubation (mean trip duration: 13.0 ± 1.5 days) and the long winter trips (64.8 ± 13.9 days).

Dive classification

After zero offset correction of depth data and the cutting of all recordings into appropriate sections, dive parameters were extracted for all dives >1 m, using a custom-written Matlab program that classified dive behaviour as described in detail in Halsey et al. (2007). In brief, a trip was divided into distinct periods of dive bouts (when birds conducted dives in quick succession) and periods between dive bouts (inter-bout periods; when birds rested at the surface). Within both types of period, further classification according to dive depth, time of day and other parameters was made (sub-bouts). Most importantly, a dive bout or sub-bout contained a minimum of three dives and ended when the duration spent at the surface after a dive within a bout or sub-bout exceeded 15 min (Halsey et al., 2007).

Treatment of dive and temperature parameters

In the next step, we calculated daily means for all relevant dive parameters (e.g. dive depth, dive duration) and temperature (T_{mean} , T_{max} and T_{min}). T_{mean} is the average of all temperature values recorded per day, while T_{max} and T_{min} are the single highest and lowest temperatures recorded per day, respectively. From the daily means, weekly or monthly averages were calculated for all birds. In the first analysis, investigating the temporal development of dive parameters and peripheral temperature in juveniles, we used these weekly averages and computed grand means, established from bird means throughout the first year at sea. Dive effort was calculated as the product of the total time spent submerged per day and mean depth during submergence and was chosen to best represent the foraging effort of birds, as it integrates both temporal and spatial aspects of foraging. For 3 young birds, the analysis was also applied to their second year at sea. The same analysis was conducted for adult birds. However, as foraging trips of adults were of much shorter duration than the trips conducted by juveniles, values were averaged for the first and last week of a trip, excluding the first and last day of a trip (when birds conducted shallow travelling dives).

In the second analysis, we focused on individual temperature drop events that occurred in connection with foraging (Fig. 2). Typically, at the start of a dive bout, peripheral temperature dropped rapidly (initial temperature drop), presumably reflecting changes in the perfusion of the subcutaneous tissue (vasoconstriction). We selected these rapid initial temperature drops (phase 2, Fig. 2) across the recording periods of all juvenile birds based on (1) the temperature preceding the drop (rejecting events that started at a temperature of less than 90% of the previous normothermic plateau), (2) the duration of the following hypothermic plateau (excluding temperature declines <1 h in duration, typically associated with isolated dives) and (3) the slope of the temperature decline (rejecting events when temperature reversals occurred). The initial temperature drop was defined as ending when the temperature reached 90% of the maximum temperature decline during the event. For our analysis, we selected 2564 temperature drops from 11 juveniles that occurred during 3163 days at sea. On average, therefore, we selected one temperature drop event every ~1.2 days. For each temperature drop event, we extracted the temperature at the end of the initial drop (phase 2, Fig. 2; T_{end}) and the temperature difference between the start and end of the initial drop (phase 2; ΔT).

Heat loss model

To illustrate the consequences of peripheral temperature changes during foraging, we estimated the heat loss experienced by juvenile king penguins during their first year at sea. This was achieved by adapting the heat loss model described in detail by Grémillet et al. (1998) to the conditions for king penguins. This model calculates the heat flux from a bird body without internal temperature gradients, surrounded by an insulating air and/or fat layer, to the water (for details and derivation of model equations, see Grémillet et al., 1998). Input values for this model were taken from individual measurements (beak length and body mass, M_b), our recordings (peripheral temperature and depth, which also provided time activity data) or were assumed. For example, we assumed a swim speed of 2 m s^{-1} during diving and an air volume in the plumage at the surface of 0.25 l kg^{-1} (Le Vaillant et al., 2012; Wilson et al., 1992). We assumed the logger to be embedded within subcutaneous fat tissue (Fig. 1) and estimated heat loss from the site of the logger (using the temperatures recorded at this site), with an assumed constant fat layer of 7.5 mm between the logger and the dermis

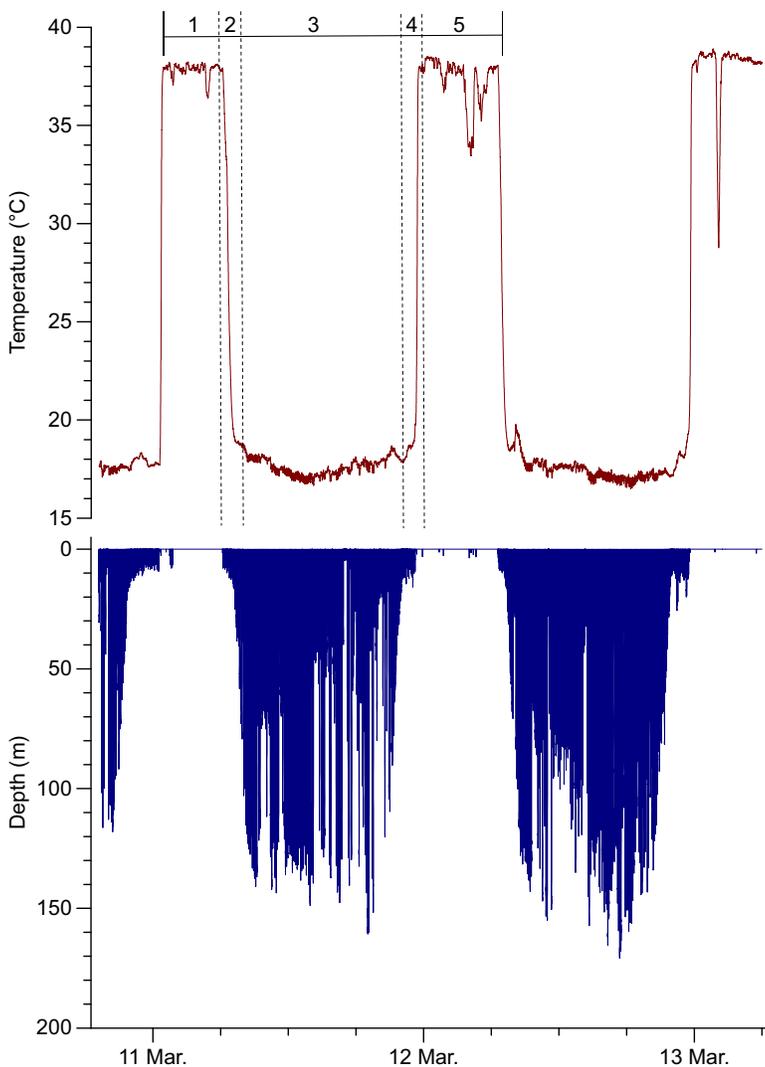


Fig. 2. Peripheral temperature (top) and dive depth (bottom) in a juvenile king penguin (LuL09). This sequence of ~2 days (11–13 March 2014) illustrates the typical diel activity pattern, with birds foraging during the day and resting at the surface and/or conducting shallow ‘travelling’ dives during the night. Peripheral temperature declined strongly during diving but remained at normothermia during most of the night. The typical sequence during a temperature drop event is indicated at the top. Each event was characterized by five distinct phases: (1) the preceding normothermic plateau, (2) the initial temperature drop, (3) the hypothermic plateau, (4) the rewarming phase and (5) the subsequent normothermic plateau. Dashed lines indicate the start and end (T_{end}) of phases 2 and 4.

(Y.H., unpublished observation). Depth and temperature data from all birds were averaged for each day, from which weekly means were computed. These weekly means were used to estimate heat loss for each bird throughout the recording period. Heat loss was estimated separately for underwater (at mean dive depth) and surface periods, and a composite estimate was established based on the time spent underwater versus at the surface. Finally, we used heat loss estimates for the first month at sea as a reference point, to which all other estimates were compared. Heat loss for all subsequent months of the first year at sea was then computed as the percentage change relative to the first month.

Statistical analysis

All statistical analyses were conducted in JMP (version Pro 11.2.0, SAS Institute Inc., Cary, NC, USA) and R (version 3.2.4; www.R-project.org). The breakpoint for T_{min} and T_{mean} in Fig. 3 was identified from segmented regression analysis using the ‘strucchange’ package in R (Zeileis et al., 2002). Separate linear mixed-effects models were then used for pre- and post-breakpoint analyses to test for the effects of time at sea and dive effort on temperature parameters (T_{min} , T_{mean} , T_{max} , T_{end} and ΔT) and heat loss. Similarly, linear mixed-effects model analysis was also used to test for the effects of time at sea on dive effort and for the effects of trip number and trip phase of adult birds (first versus last week of a

trip, excluding the first and last day of a trip) on temperature parameters and dive effort. Where appropriate, interaction terms were first included in the respective model and then removed if not significant. Time at sea, dive effort, trip number and trip phase were included as fixed effects, while bird ID was included as a random effect. To investigate the relative importance of time at sea versus dive effort on temperature parameters, we standardized the values for these two factors before running the model, so that estimates were directly comparable. Significance for all statistical tests was accepted at $P < 0.05$. All mean values are presented with s.e.m.

RESULTS

The activity of both juvenile and adult king penguins followed a diel pattern, whereby birds foraged during the day, often for extended periods, while at night birds either conducted shallow dives (probably in association with travelling) or rested at the surface (Fig. 2). Peripheral temperature followed accordingly, with lower temperatures during foraging/diving (hypothermia) and higher temperatures when resting at the surface (normothermia; Fig. 2). The typical sequence during a temperature drop event observed in association with foraging/diving bouts in juvenile king penguins is illustrated in Fig. 2. These temperature drop events can be divided into five distinct phases: (1) the normothermic plateau preceding the temperature drop, when peripheral temperature was ~38°C; (2) the

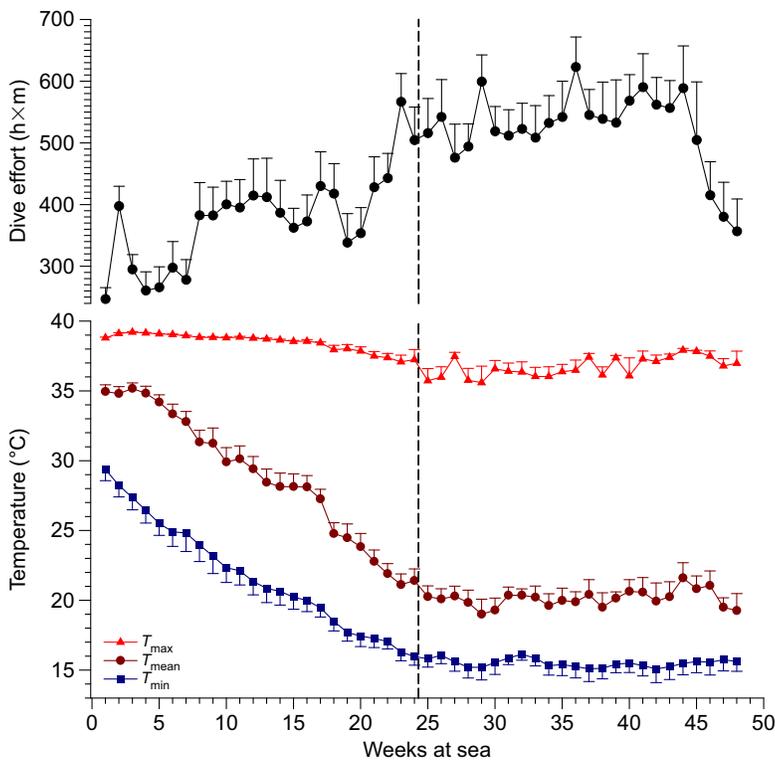


Fig. 3. Dive effort (top) and peripheral temperature (bottom) of juvenile king penguins throughout their first year at sea.

Values are weekly grand means (\pm s.e.m.) based on the daily means of individuals ($N=5-13$). Dive effort oscillated but generally increased over time and reached a high plateau at about week 23, which was maintained for ~ 5 months, before it decreased considerably during the last month. T_{mean} (the average of all temperature values recorded per day) and T_{min} (the single lowest temperature recorded per day) decreased steeply during the first ~ 24 weeks before reaching a stable level. The dashed line indicates the break point between these two periods. T_{max} (the single highest temperature recorded per day) decreased slightly over time; its larger oscillations during the second period indicate weeks with extended diving days, when birds maintained low peripheral temperatures (Fig. 6B).

initial temperature drop, when temperature declined rapidly; (3) the hypothermic plateau, when temperature stabilized and small oscillations in connection with individual dive cycles occurred; (4) the rewarming phase, when temperature rose rapidly; and (5) the normothermic plateau following the rewarming phase, when peripheral temperatures stabilized at $\sim 38^\circ\text{C}$. Peripheral temperature patterns changed significantly over time in both juvenile and adult birds, and below we investigated the underlying mechanisms and potential consequences for juvenile birds during their first months at sea.

Weekly means of T_{min} , T_{mean} , T_{max} and dive effort in juveniles, immature birds and adults

All temperature parameters and dive effort of juvenile birds changed significantly throughout their first year at sea (Fig. 3). While all temperature parameters declined over time, dive effort increased. However, there was a significant breakpoint in the development of peripheral temperature over time, at 24.3 ± 1.0 weeks at sea. The slopes before and after this breakpoint differed significantly, with T_{min} declining on average by $0.55 \pm 0.02^\circ\text{C}$ per week up to week 24 ($P < 0.0001$), after which it stabilized ($0.03 \pm 0.01^\circ\text{C}$ per week; $P = 0.02$; Fig. 3). T_{max} declined slightly during the first 24 weeks (a total of 1.6°C ; $P < 0.0001$). During the same period, dive effort increased significantly ($P < 0.0001$) and significantly affected T_{min} ($P = 0.005$) and T_{mean} ($P < 0.0001$) but not T_{max} ($P = 0.78$). However, the effect of dive effort on T_{min} and T_{mean} was considerably less than the effect of time at sea per se. Running the model after standardizing these factors revealed that the effect of time at sea on T_{min} and T_{mean} was 16 and 8 times stronger, respectively, than the effect of dive effort. After week 24, neither T_{min} nor T_{mean} changed significantly ($P = 0.06$ and $P = 0.67$, respectively) and despite the considerable changes in dive effort, it had no effect on these parameters ($P = 0.51$ and $P = 0.98$, respectively). During the same period, T_{max} increased slightly (for a total of $1.2 \pm 0.5^\circ\text{C}$; $P = 0.01$) without any effect of dive effort ($P = 0.73$). Dive effort remained at a

high level during this second period, before declining significantly during the last month at sea ($P = 0.0003$; Fig. 3).

Investigating the temperature patterns during the second year at sea (3 immature birds) showed that peripheral temperature developed similarly in the two years. In these 3 birds, T_{min} and T_{mean} declined significantly during the first 5 months at sea in both years, though the slope of that decline was less steep during the second year (T_{min} : -0.69 ± 0.03 versus $-0.37 \pm 0.02^\circ\text{C}$ per week for first and second year, respectively; T_{mean} : -0.58 ± 0.03 versus $-0.52 \pm 0.03^\circ\text{C}$ per week for the first and second year, respectively). However, during the second year, peripheral temperatures did not stabilize, as during the first year, but continued to decline (with some fluctuations), so that temperatures at the end of the trip were similar in the two years. Temperature parameters for these 3 birds during their first and last month at sea did not differ between years (T_{min} , $P = 0.20$; T_{mean} , $P = 0.28$; T_{max} , $P = 0.06$). T_{min} declined from $29.1 \pm 2.4^\circ\text{C}$ to $14.9 \pm 1.2^\circ\text{C}$ during the first year, while it declined from $26.8 \pm 1.1^\circ\text{C}$ to $16.9 \pm 0.6^\circ\text{C}$ during the second year. Similarly, T_{mean} declined from $35.4 \pm 1.1^\circ\text{C}$ to $19.2 \pm 1.2^\circ\text{C}$ during the first year, while it declined from $34.0 \pm 0.9^\circ\text{C}$ to $21.7 \pm 0.3^\circ\text{C}$ during the second year. Dive effort during the second year also fluctuated but generally increased over time and was significantly higher than during the first year ($P < 0.0001$). As during the first year, its effect on temperature parameters was weak in comparison with time at sea per se.

In adult birds, peripheral temperature (T_{mean} and T_{min}) declined significantly over the course of a trip, so that temperature was always lower at the end of a trip compared with the start (Fig. 4; $P = 0.01$ and $P < 0.0001$ for T_{mean} and T_{min} , respectively). Peripheral temperature also declined significantly over the course of the breeding season, between the first incubation trip and the longer winter trips (Fig. 4; $P < 0.0001$ for T_{mean} and T_{min}). T_{max} , by contrast, did not change over the course of a trip ($P = 0.8$) but was lower during the longer winter trips when compared with the shorter incubation trips ($P < 0.0001$). The dive effort of adult birds increased significantly during incubation ($P = 0.02$) but not thereafter ($P = 0.9$; Fig. 4). Over the

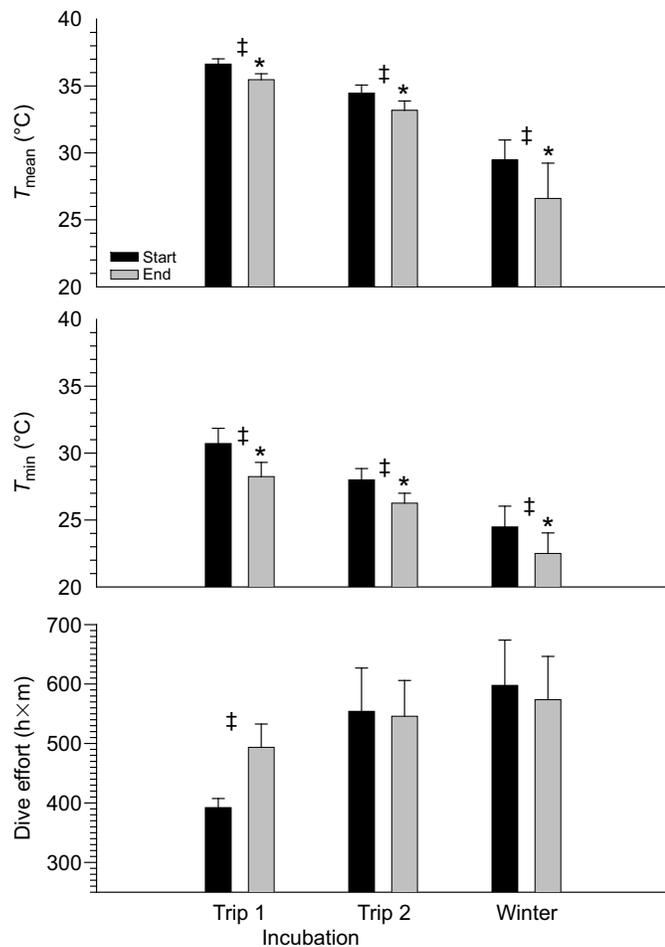


Fig. 4. Peripheral temperature (T_{mean} , T_{min} ; top) and dive effort (bottom) of adult king penguins during foraging trips. Shown are values for the start and end of the first two incubation trips (trip 1 and 2 in January and February 2015, respectively) and the later winter trip (June to October 2015). Values are grand means (\pm s.e.m.) established from individual bird means ($N=3-5$). Values were averaged during the first and last week of each trip, excluding the first and last day of a trip. Trip duration during incubation was on average 13.7 ± 1.9 days for trip 1 and 12.3 ± 1.0 days for trip 2, while winter trips lasted on average 64.8 ± 13.9 days. Peripheral temperature decreased throughout the breeding season and temperatures at the end of a trip were always lower than those at the start of a trip. By contrast, dive effort increased between the first and second incubation trip but not thereafter and did also not change during a trip. *Significantly different from start of trip. ‡Significantly different from other trips.

course of a trip, dive effort did not change ($P=0.4$). Similarly, there was no significant effect of dive effort on any temperature variable ($P=0.9$ for T_{mean} and T_{min} ; $P=0.07$ for T_{max}).

Initial temperature drops during foraging in juveniles

Our second analysis focused on individual temperature drop events that occurred in association with foraging/diving. The amplitude of the initial temperature drops during these events (phase 2; Fig. 2) increased significantly over time in all juveniles and was independent of the dive effort during these events (Fig. 5). Substantial initial temperature drops occurred during bouts of short/shallow dives (Fig. 5C) as well as during bouts of long/deep dives (Fig. 5B) and even in the absence of diving (Fig. 6A). Typically, peripheral temperature during the initial drop fell rapidly, and this decline coincided with the start of a foraging bout but also

occurred beforehand, during extended rest phases at the surface (Fig. 6A). Depending on diving activity, the hypothermic plateau that followed the initial temperature drop extended over many hours and occasionally spanned periods of up to 48 h, when a foraging day was followed by travelling dives throughout the night, before the start of a new foraging day (Fig. 6B).

Overall, peripheral temperature fell from the start of these events (initial temperature drop, phase 2; Fig. 2) to increasingly lower values during the first 6–7 months at sea, after which it stabilized (Figs 5 and 7 A,B). During the first 6–7 months, T_{end} decreased progressively, so that during the first month at sea, T_{end} reached on average $28.7\pm 0.5^\circ\text{C}$, while after 7 months, it fell to $18.1\pm 0.7^\circ\text{C}$ (minimum: 12.5°C ; $P<0.0001$; Fig. 7B). Accordingly, ΔT during the first month at sea was on average $8.1\pm 0.6^\circ\text{C}$, while after 7 months it was $18.2\pm 0.7^\circ\text{C}$ (maximum: 24.1°C ; Fig. 7C; $P<0.0001$). During that same period, birds significantly increased their overall dive effort ($P<0.0001$; Fig. 3). However, there was no significant relationship between overall dive effort and the initial temperature drop during foraging bouts ($P=0.3$ for both T_{end} and ΔT). After ~ 7 months at sea, temperature parameters stabilized and did not change significantly for the remainder of the first year at sea (Fig. 7B,C; $P=0.2$ and $P=0.07$ for T_{end} and ΔT , respectively). The overall dive effort of birds remained at a high level for another 3 months (from August to October), and even slightly increased before it fell significantly after ~ 10 months ($P<0.001$; Fig. 3). As before, there was no significant relationship between overall dive effort and any temperature parameter during this period ($P=0.3$ and $P=0.4$ for T_{end} and ΔT , respectively).

Heat loss in juveniles during their first year at sea

Heat loss estimates for juvenile birds were highest during their first month at sea. Using the first month as a reference point showed that heat loss declined significantly during the subsequent 6 months at sea, on average by more than 40% (Fig. 8; $P<0.0001$). After this period, heat loss stabilized ($P=0.5$).

DISCUSSION

We found important changes in the development of peripheral temperature patterns of foraging juvenile king penguins throughout their first year at sea. Most importantly, similar changes re-occurred during their second year at sea, after birds had fasted for ~ 4 weeks on land during their second moult. Furthermore, similar peripheral temperature patterns were also present in adult birds during foraging trips throughout their breeding cycle. While dive effort gradually increased during the first year at sea, to reach the highest level after ~ 5 months, our results suggest that the observed seasonal temperature changes mainly reflect differences in thermal insulation and are not the simple consequence of concurrent changes in dive effort or an indication of a physiological maturation process. Leaving the colony in a lean state, just after their first moult, juveniles probably increased their subcutaneous fat progressively, as they foraged successfully. During diving, this fat layer enables birds to build a strong thermal gradient between the core and periphery that reduces heat loss and, hence, thermoregulatory costs. Accordingly, estimated heat loss of juveniles was initially high and declined to approximately half after 6 months at sea. It is likely that foraging proficiency in juvenile birds is lower during the early phase at sea and develops over time (Wunderle, 1991; Daunt et al., 2007). Hence, such behavioural challenges in combination with thermal challenges, as indicated by peripheral temperature changes in our study, suggest that juvenile king penguins face adverse energetic conditions during their early oceanic existence.

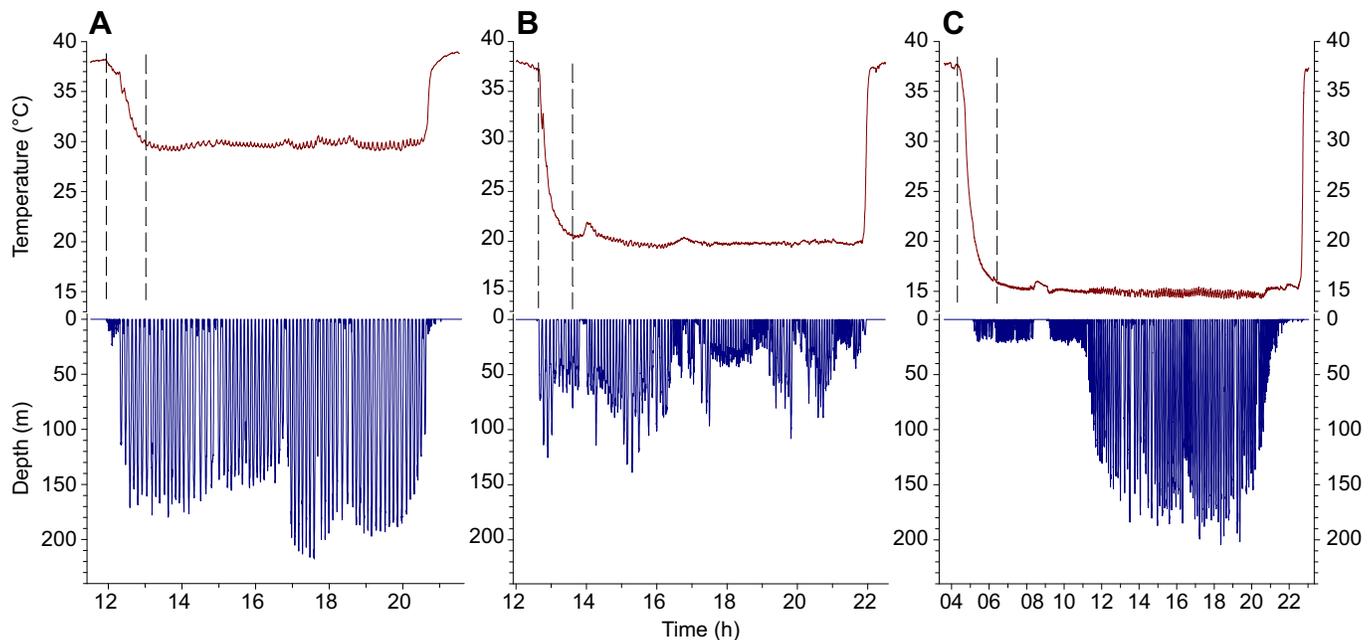


Fig. 5. Peripheral temperature drops associated with foraging/diving activity in one juvenile king penguin (LuL13) during its first year at sea. Peripheral temperature (top) and dive depth (bottom) are shown during a typical foraging/dive bout after the bird had been at sea for ~1.5 months (1 February 2014; A), ~4 months (24 April 2014; B) and ~5 months (21 May 2014; C). The initial temperature drop (indicated by dashed lines) increased in amplitude over time and was unrelated to the diving effort during the initial drop.

Different mechanisms might explain the observed changes in peripheral temperatures of foraging juvenile king penguins over time and we will discuss them in detail below.

Concurrent changes in dive effort and/or changes in T_w

It is conceivable that the peripheral temperature of birds during foraging solely followed their dive effort and that the observed decline in peripheral temperature parameters during the first ~6 months at sea resulted from changes in heat loss associated with changes in dive effort and T_w . If this was the case, we should have observed considerable daily fluctuations in peripheral temperature patterns throughout the year, as dive effort fluctuated strongly between bouts, days and even months. Figs 3 and 7 clearly illustrate that this was not the case. When computing weekly averages, we found that dive effort of juvenile king penguins increased over the course of their first year at sea (Fig. 3). However, there were at least three distinct phases: (i) during the first ~5 months at sea, dive effort underwent considerable fluctuation but generally increased; (ii) dive effort then jumped to its highest level and this was maintained for ~5 months, before (iii) declining during the last month at sea (Fig. 3). By contrast, T_{mean} and especially T_{min} declined continuously during the first period, when dive effort fluctuated considerably. Both T_{mean} and T_{min} stabilized at the time when dive effort reached its peak level and also remained stable when dive effort declined strongly during the last month at sea (Fig. 3). Hence, there is a clear disconnection between the development of dive effort and peripheral temperature changes. Our analysis also showed that, while significant, the effect of dive effort on T_{min} and T_{mean} was weak when compared with the effect of time at sea alone. Furthermore, when investigating peripheral temperature patterns associated with individual temperature drop events, we found that the amplitude of the initial temperature drops observed during these events increased strongly in all juveniles during their first 6–7 months at sea, after which it stabilized and this

occurred independent of the concurrent dive effort during these events (Figs 5 and 6). Accordingly, dive effort alone does not explain the observed changes in peripheral temperature sufficiently.

As our study used implanted data loggers, we were unable to record external T_w . Orgeret et al. (2016) showed that juvenile king penguins from the same colony move over considerable distances during their first year at sea, with probable changes in T_w . In our calculations concerning heat loss, we assumed a T_w of 4°C (Guinet et al., 1997; Charrassin and Bost, 2001), while birds might have foraged in somewhat colder water. Nevertheless, while such potential differences in T_w might be sufficient to explain the relatively small changes in T_{max} we observed over time (~1.6°C), they would clearly be insufficient to explain the enormous differences we observed in T_{min} and T_{mean} during the first 6 months at sea (Fig. 3).

Potential physiological maturation of the thermoregulatory response

Temperature declines in subcutaneous tissues during diving have been observed in a number of avian divers (Ponganis et al., 2003; Fahlman et al., 2005; Schmidt, 2006; Schmidt et al., 2006; Niizuma et al., 2007) and are believed to reflect peripheral vasoconstriction, reducing the temperature gradient between the periphery and the environment and, thereby, heat loss. The effectiveness of such a response has been demonstrated experimentally in marine mammals (e.g. Kvadsheim and Folkow, 1997). It is conceivable that such a response requires some physiological maturation and that the observed changes in peripheral temperature patterns of juvenile king penguins reflect this maturation. Clearly, our juvenile king penguins had never spent any substantial amount of time in water, apart from some occasional ‘baths’, before leaving the colony. Peripheral vasoconstriction is part of the dive reflex whereby, upon submergence, neural and hormonal pathways elicit a response (decrease in heart rate, redistribution of blood flow

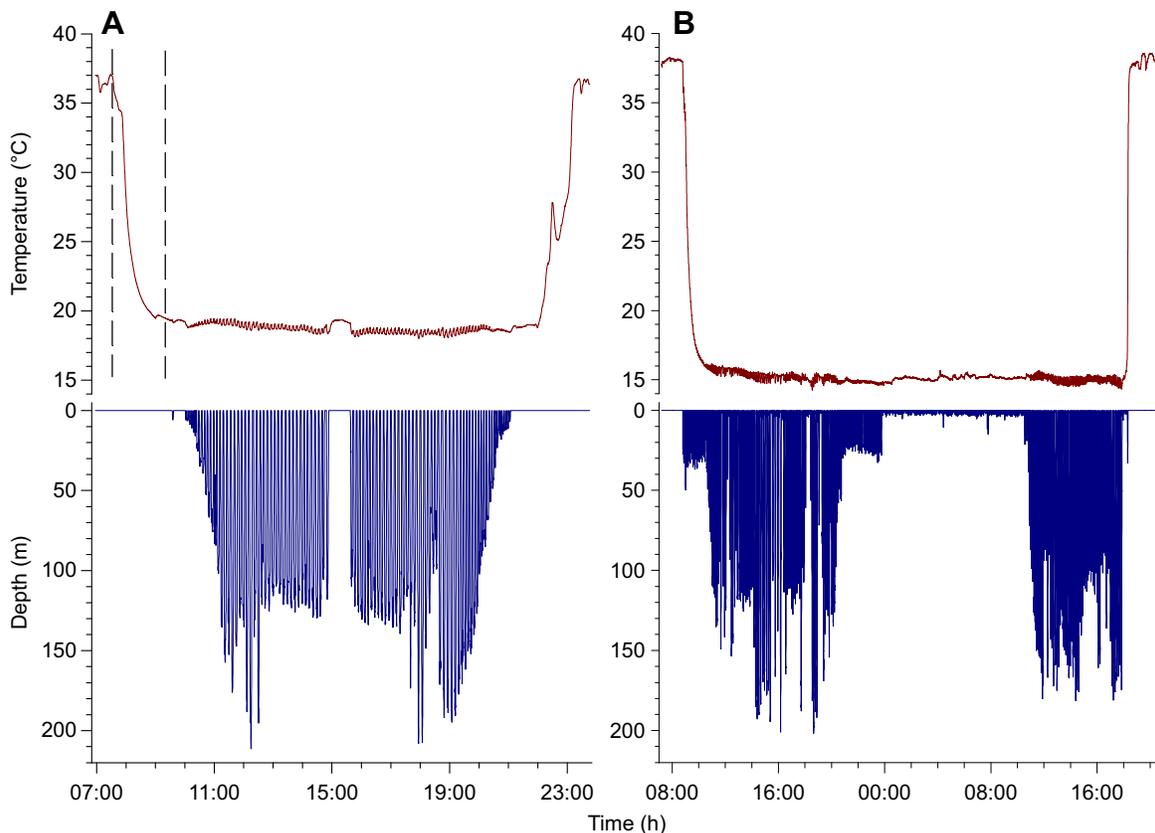


Fig. 6. Initial temperature drop preceding diving/foraging activity and extended drop event in juvenile king penguins. (A) The initial temperature drop (indicated by dashed lines) of a juvenile king penguin (LuL04) clearly precedes diving (20 July 2014). (B) An extended peripheral temperature drop event (~34 h) in association with diving activity (LuL13) during the austral winter (15 May 2014). The initial temperature drop occurs in connection with the first foraging bout and the ensuing hypothermic plateau is extended across the first foraging bout (~14 h), throughout a bout of nocturnal shallow travelling dives (~11 h) and the foraging bout of the following day (~7 h). Note the small temperature oscillations during the hypothermic plateau, associated with individual dive cycles.

through selective vasoconstriction) believed to facilitate the economic utilization of the finite oxygen stores (Butler and Jones, 1997; Butler, 2004). Little is known about the ontogeny of the dive reflex, particularly vasomotor control in avian divers. However, Keijer et al. (1988) and Enstipp et al. (1999) found that the cardiac response (heart rate decline) was present during the first dives of tufted ducklings (*Aythya fuligula*) and double-crested cormorant fledglings (*Phalacrocorax auritus*), respectively. Such a decline in heart rate should indicate the presence of a developed dive reflex (including a vasomotor response; Butler and Jones, 1997). The strong decline in peripheral temperature observed in king penguin chicks during their winter fast also indicates the presence of an effective vasomotor control, months before they go to sea (Eichhorn et al., 2011).

Furthermore, if the observed peripheral temperature patterns in our study (i.e. a progressive decrease of T_{\min} and T_{mean} during the first 6 months) reflect physiological maturation of the vasomotor response, we would expect such a pattern not to be present in young birds during their second year at sea or in adult birds. Once established, effective vasomotor control seems unlikely to be lost again. However, we found that these peripheral temperature patterns re-occurred in immature birds after their second moult and were also present in adult breeders (Fig. 4). Hence, as with dive effort, physiological maturation of the vasomotor response alone is unlikely to explain the observed temperature patterns in peripheral tissues.

Seasonal changes in body condition and insulation status

When juvenile birds left the colony, they were in a relatively lean body condition. Although they are fed up to the start of their first moult, juvenile king penguins typically fast afterwards and are in phase II of fasting before departing to sea (Cherel et al., 2004; Corbel et al., 2009). During fasting, king penguin metabolism is fuelled predominately by lipids, especially from subcutaneous fat stores (Cherel and Le Maho, 1985; Cherel et al., 1994). Accordingly, the subcutaneous fat layer of juveniles leaving the colony must have been relatively thin, as illustrated in Fig. 1B. In our analysis concerning weekly averages, T_{mean} integrates all temperature recordings during a particular period and is, therefore, affected by dive effort. As peripheral temperature is lower during diving than when resting at the surface, a day with a short foraging bout will result in a higher T_{mean} than a day with extensive foraging. By contrast, T_{\min} and T_{\max} are the single lowest and highest temperatures recorded during the same period, respectively, and typically indicate the temperature during diving and resting, respectively. They should therefore best reflect changes in the state of blood perfusion and insulation status, so that T_{\max} and T_{\min} should represent maximal and minimal perfusion, respectively. Both T_{\min} and T_{\max} declined significantly during the first ~6 months at sea (Fig. 3), probably as a result of changes in body insulation. We suggest that as birds foraged successfully, they deposited fat in their subcutaneous layer, which must have increased considerably over time. In turn, the data logger recording

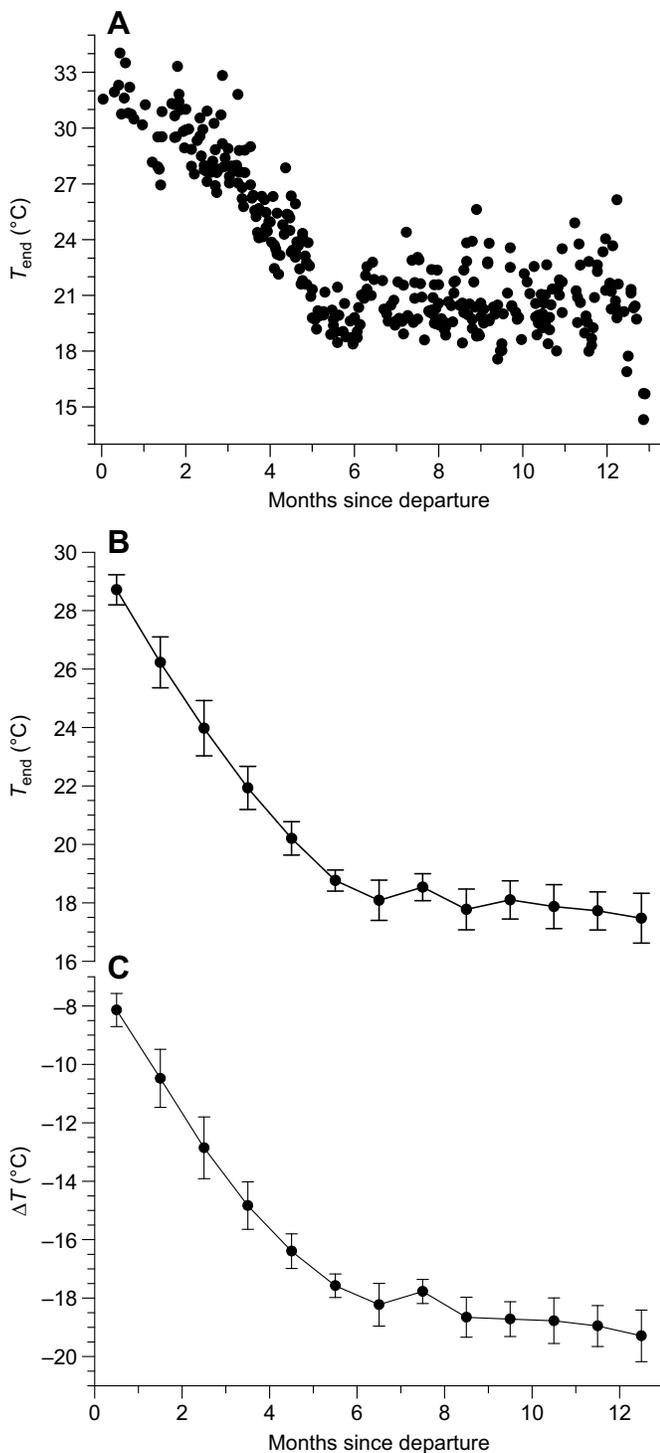


Fig. 7. Initial temperature drops associated with foraging bouts of juvenile king penguins during their first year at sea. (A) T_{end} (temperature at the end of the initial drop) during 344 peripheral temperature drop events of one juvenile bird (LuL04) during its 380 days spent at sea. (B) T_{end} and (C) ΔT (the temperature difference between the start and end of the initial drop) during initial temperature drops of all juvenile birds throughout their first year at sea. Values for B and C are monthly grand means (\pm s.e.m.) established from the daily means of individuals ($N=6-11$, 2564 events during 3163 days at sea). Increasingly lower T_{end} values were reached during the first 6–7 months, after which T_{end} stabilized. Accordingly, as T_{end} values declined during the first period at sea, ΔT increased in magnitude, with mean temperature differences of $\sim 18^{\circ}\text{C}$ reached after 6–7 months at sea.

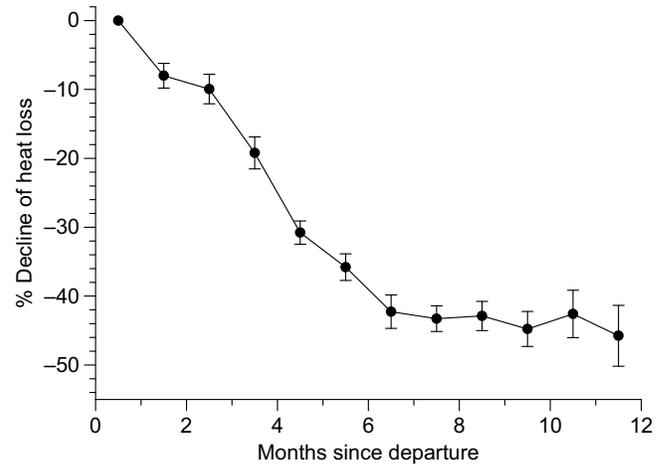


Fig. 8. Development of heat loss for juvenile king penguins during their first year at sea. Shown is the percentage decline in heat loss over time in comparison with that during the first month at sea, which was used as a reference. Values are monthly grand means (\pm s.e.m.) established from individual bird means ($N=6-13$). Heat loss was estimated by adapting the model of Grémillet et al. (1998) to king penguins and using data from the current recordings (peripheral temperatures and depth, which also provided time activity data). Heat loss was initially high and declined on average by more than 40% during the first 7 months at sea. After this period, heat loss stabilized.

temperature became increasingly thermally isolated with respect to the warmer body core (Fig. 1C). During this phase, all peripheral temperature parameters declined significantly, especially T_{min} and T_{mean} . After ~ 6 months, the build-up of the subcutaneous fat layer was probably sufficient, so that peripheral temperature parameters stabilized. Small fluctuations in these parameters throughout the remainder of the first year at sea, clearly visible in individual traces, illustrate the dynamic nature of this interplay between foraging success, subcutaneous fat layer thickness and peripheral temperature patterns.

Hence, we suggest that while concurrent changes in dive effort and some physiological maturation (especially during the first weeks) might have contributed to the observed peripheral temperature patterns of juvenile king penguins during their first year at sea, they mainly reflect changes in body condition and thermal insulation. Such a scenario is supported by the observations of Fahlman et al. (2005), who found that the whole-animal resting metabolic rate of fasted king penguins (lower M_b) in a still water canal was significantly higher than that in fed birds (returning from a foraging trip; higher M_b) or re-fed birds and attributed this to differences in subcutaneous insulation and body fuel mobilization. Similarly, Green et al. (2005) reported that abdominal temperature and the basal rate of metabolism of adult macaroni penguins (estimated from heart rate) decreased during winter, supposedly facilitating the concurrent increase in dive capacity. The authors attributed this seasonal decrease in basal metabolism mostly to changes in the birds' conductance via a progressive improvement in body condition and insulation (Green et al., 2005). Nevertheless, the seasonal dynamics of fat storage and utilization in penguins and the potential conflict with its role as body insulator are largely unknown and require further study (Kooyman et al., 1976; Green et al., 2005).

The observed peripheral temperature patterns in juvenile king penguins have important implications for their energy balance during their first months at sea and this is illustrated in the resulting heat loss estimates, which are initially high and decline substantially during the first 6–7 months at sea (Fig. 8). Our heat loss estimates

are based on a model and, consequently, the actual heat loss experienced by juvenile king penguins at sea might vary from our estimates. For example, in our model we maintained constant values for a number of input parameters (T_w , fat layer thickness), while in reality they might have changed concurrently. Nevertheless, by including the recorded dive behaviour and peripheral temperature values, our aim was to achieve a conservative but realistic estimate of penguin heat loss in relative terms, to illustrate the energetic consequences of changes in peripheral temperature patterns over time.

In adult birds, peripheral temperature (T_{mean} and T_{min}) declined during foraging trips throughout the breeding season, from the first incubation shift to the longer winter trips (Fig. 4), suggesting a substantial change in body insulation (subcutaneous fat) over time. This fits well with the observation by Handrich et al. (1995) that body mass in breeding king penguins progressively increased throughout the first 7 months of their breeding cycle, reflecting a rise in body fuel reserves (Le Maho et al., 1993). At the start of their first incubation trip, T_{min} in adult birds ($30.7 \pm 1.1^\circ\text{C}$) was slightly higher than that in juvenile birds at the beginning of their trip ($29.4 \pm 0.8^\circ\text{C}$), potentially indicating a leaner body condition for adults at this phase (Fig. 4). The decline in T_{min} during the following ~ 2 weeks, however, was comparable in the two groups ($2.5 \pm 0.4^\circ\text{C}$ for adults versus $1.8 \pm 0.4^\circ\text{C}$ for juveniles). Winter trip duration differed considerably between the 3 adult birds (6, 9 and 13 weeks) but the decline in T_{min} between the start and end of a trip was similar in all birds (2.2, 2.0 and 1.7°C , respectively) and only about half that of juvenile birds for a comparable period, when at a similar T_{min} (from ~ 7 weeks at sea; Fig. 3). As prey availability for king penguins decreases considerably after the summer (Kozlov et al., 1991), adult birds increase their foraging range and dive to greater depth (Charrassin and Bost, 2001; Charrassin et al., 2002). Hence, the slower decline in T_{min} observed during the winter trips of adult birds, when compared with that of juveniles, might reflect a slower deposition of subcutaneous fat, as a result of a less positive energy balance. However, a direct comparison of peripheral temperature parameters between adult and juvenile birds is difficult, as the winter trips of adult birds occurred at a period when peripheral temperatures had stabilized in juvenile birds (after ~ 24 weeks at sea, around mid-June). A comparison between the two groups is further complicated by the different life history stages of the birds and the temporal organization of their trips. While juvenile birds remained at sea for their entire first year (apart from brief periods spent on land or ice), adult breeders repeatedly switched between foraging at sea and fasting in the colony, changing the overall dynamics of their body condition/insulation and, consequently, peripheral temperatures at sea.

Orgeret et al. (2016), who deployed satellite tags on 17 juvenile king penguins from the same Crozet colony during their first year at sea, found that dive performance (dive depth and duration) in 5 of these birds started to decline significantly after ~ 3 months and satellite transmission stopped after ~ 4 months, when these birds presumably died. The authors attributed this outcome to the inability of these birds to increase their dive capacity (as surviving birds did) when conditions became difficult, resulting in early mortality due to starvation (Orgeret et al., 2016). This supports our hypothesis of an energetic challenge in these juvenile king penguins during their first months at sea. Clearly, elevated heat loss during diving, as suggested by our recordings of peripheral temperature, will greatly enhance thermoregulatory costs and therefore tip the energy budget, if not balanced by sufficient food intake. Despite these energetic challenges, survival rates of juvenile king penguins

from the Crozet population are surprisingly high. Based on 10 years of automated transponder-based monitoring, Saraux et al. (2011) found an overall post-fledging return rate of 77%. In most juvenile seabirds, considerable mortality might occur during their first months of independence, especially during their first winter at sea (Daunt et al., 2007; Hunt, 2010). Hence, Saraux et al. (2011) suggested that selective mechanisms for juvenile king penguins must operate mainly before fledging, especially during the long winter fast and the subsequent spring, when up to $\sim 50\%$ of chicks might die of starvation (Cherel et al., 1987). Nevertheless, juvenile survival at sea is still considerably lower than that of adults (average annual survival: 90.6%; Le Bohec et al., 2007), suggesting a selection process during the first months at sea (Orgeret et al., 2016). One should keep in mind that, unlike the study by Orgeret et al. (2016), our current study focused on surviving birds, as we have no data from birds that failed to return.

In the current study, we investigated the thermoregulatory responses of juvenile king penguins during their dispersal phase at sea, focusing on the long-term development. Our findings suggest that a thermoregulatory response is in place by the time juveniles initiate their first trip. Yet, important thermal changes occur over time, especially during the initial period at sea (Fig. 5). Previous studies showed that the thermogenic capacity of juvenile king penguins increases after repeated experimental submersion in cold water (Barré and Roussel, 1986; Talbot et al., 2004; Teulier et al., 2016) and is considerably higher in immature birds (after ~ 1 year at sea) than in juveniles before their first departure to sea (Teulier et al., 2012). Hence, the relatively high peripheral temperatures during diving that we observed in juveniles during their initial period at sea could potentially also be a consequence of insufficient thermogenic capacity, so that birds avoid a deeper temperature drop by maintaining some peripheral perfusion. However, as thermogenic capacity in birds develops rapidly during cold exposure (Barré and Roussel, 1986; Talbot et al., 2004; Teulier et al., 2014), such a mechanism should not extend beyond the first few weeks at sea. Similarly, in adult king penguins, Handrich et al. (1997) found a progressive decrease in abdominal tissue temperatures during foraging trips of ~ 1 week. This phenomenon could also be associated with changes in thermogenic capacity after birds fasted/were inactive on land. However, the relevance of a decline in abdominal temperature during diving in adult penguins for the development of peripheral temperature patterns is not clear. Hence, a fine-scale investigation of the thermal changes during the first days/weeks at sea in conjunction with an investigation of concurrent changes in foraging behaviour will allow a better understanding of the regulation, fine control and development of peripheral temperature during diving (Figs 5 and 6).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.-A.B., H.W., Y.H.; Methodology: M.R.E., C.-A.B., C.L.B., C.B., H.W., Y.H.; Software: M.R.E., Y.H.; Formal analysis: M.R.E., Y.H.; Investigation: C.-A.B., C.L.B., C.B., Y.H.; Resources: C.-A.B., C.L.B., Y.L.M., H.W., Y.H.; Writing - original draft: M.R.E.; Writing - review & editing: M.R.E., C.-A.B., C.L.B., C.B., Y.L.M., H.W., Y.H.; Supervision: C.-A.B., H.W., Y.H.; Project administration: C.-A.B., H.W., Y.H.; Funding acquisition: C.-A.B., Y.L.M., H.W., Y.H.

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