

## RESEARCH ARTICLE

# The dive performance of immature king penguins following their annual molt suggests physiological constraints

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## ABSTRACT

Like all birds, penguins undergo periodic molt, during which they replace old feathers. However, unlike other birds, penguins replace their entire plumage within a short period while fasting ashore. During molt, king penguins (*Aptenodytes patagonicus*) lose half of their initial body mass, most importantly their insulating subcutaneous fat and half of their pectoral muscle mass. The latter might challenge their capacity to generate and sustain a sufficient mechanical power output to swim to distant food sources and propel themselves to great depth for successful prey capture. To investigate the effects of the annual molt fast on their dive/foraging performance, we studied various dive/foraging parameters and peripheral temperature patterns in immature king penguins across two molt cycles, after birds had spent their first and second year at sea, using implanted data-loggers. We found that the dive/foraging performance of immature king penguins was significantly reduced during post-molt foraging trips. Dive and bottom duration for a given depth were shorter during post-molt and post-dive surface interval duration was longer, reducing overall dive efficiency and underwater foraging time. We attribute this decline to the severe physiological changes that birds undergo during their annual molt. Peripheral temperature patterns differed greatly between pre- and post-molt trips, indicating the loss of the insulating subcutaneous fat layer during molt. Peripheral perfusion, as inferred from peripheral temperature, was restricted to short periods at night during pre-molt but occurred throughout extended periods during post-molt, reflecting the need to rapidly deposit an insulating fat layer during the latter period.

**KEY WORDS:** Dive/foraging performance, *Aptenodytes patagonicus*, Body insulation, Thermoregulation, Peripheral temperature, Subcutaneous fat, Wiggles, Seabirds

## INTRODUCTION

To maintain the integrity and functionality of their plumage (e.g. thermoregulation, flight), all birds undergo a periodic molt, during which new feathers are synthesized and replace the previous generation (Payne, 1972). Patterns and complexities of this annual event differ between species and have evolved to fit with other major periodic events, such as breeding and migration (Payne, 1972). Whereas many birds undergo a sequential molt of their flight

feathers, others undergo a simultaneous wing molt (e.g. waterfowl), which leaves them flightless for extended periods. Penguins are an extreme case, as they undergo a complete molt, during which they replace their entire plumage within a short period (13–34 days) while fasting ashore (Adams and Brown, 1990). For penguins, the annual molt is the most stressful nutritional period of their annual cycle (Cherel et al., 1994). In addition to sustaining their resting metabolism, birds have to fuel the synthesis of new feathers and are susceptible to increased thermoregulatory costs, as the required perfusion of the integument and the loss of the old feathers elevate heat loss. Energy expenditure during molt has been estimated for a number of penguin species and is typically elevated by up to twice the resting level (Adams and Brown, 1990). Accordingly, in preparation for molt, penguins lay down a considerable energy store in form of subcutaneous and abdominal fat, while skeletal muscle mass, especially the pectoral muscle, hypertrophies (Cherel, 1995). Penguins reach the greatest body mass within their annual cycle just before molt and suffer the greatest body mass loss during their molt fast (Stonehouse, 1967). For example, Cherel et al. (1988) found that king penguins (*Aptenodytes patagonicus*) lost 58% of their initial body mass when experimentally fasted for 41 days during molt. In this species, the energy expenditure during the molt fast is 33% higher than during the breeding fast (Cherel et al., 1994). Insufficient initial storage and/or excessive use of nutrient reserves during the molt period reduce the survival of birds, and increased mortality during (ashore) or after the molt (at sea) has been reported for a number of penguin species (Dann et al., 1992; Boersma, 1978; van Heezik and Davis, 1990).

In king penguins, metabolism during molt is fueled predominately by lipids (85%), with the majority coming from subcutaneous fat (Cherel et al., 1994). Some fuel is also provided by proteins, especially from pectoral muscle. The latter supplies 57% of the ~1.3 kg total protein required (for fuel and feather synthesis), while the integument provides 20% (Cherel et al., 1994). Consequently, birds lose most of their subcutaneous fat during the molt (89%), while pectoral muscle mass and its protein content decline by 47% and 51%, respectively (Cherel et al., 1994). Such atrophy of the pectoral muscle is mostly a consequence of tissue catabolism to provide protein for feather synthesis and to fuel metabolism, but might be exacerbated by disuse (Alexander and Goldspink, 1977; Piersma, 1988; but see Portugal et al., 2009). In addition to a reduction in total muscle mass, pectoral muscle atrophy will likely lead to changes in muscle architecture (e.g. fiber type profile, cross-sectional area and fascicle length), which together will affect the forces and velocities that can be produced by muscles (Wickiewicz et al., 1983; Portugal et al., 2009). Hence, at the end of molt, when adult birds return to sea to forage and rebuild their body reserves in preparation for breeding, pectoral muscle atrophy might challenge their capacity to generate and sustain a sufficient mechanical power output to swim to distant food sources

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(Cooper, 1978) and to propel themselves to great depth for successful prey capture.

The pectoral muscles in penguins also play an important role for oxygen storage (myoglobin, Mb) and, hence, dive capacity. In adult emperor penguins (*Aptenodytes forsteri*), the muscle O<sub>2</sub> store was estimated to account for ~47% of the total estimated O<sub>2</sub> store (Kooyman and Ponganis, 1998) and this seems also to be the case for king penguins (Ponganis et al., 1999a; but see Ponganis et al., 2015). Juvenile emperor penguins, which possess ~30% of the Mb concentration of adults at the time of fledging, conduct shallower and shorter dives during their first 2.5 months at sea than adults during foraging trips (Ponganis et al., 1999b, 2010). This suggests that a fully developed and sufficiently large muscle O<sub>2</sub> store is of great importance for these birds to facilitate their extreme dive performance. Because blood perfusion to the locomotor (pectoral) muscles of emperor penguins is greatly reduced during diving or even completely cut off (Meir et al., 2008; Williams et al., 2011), muscles rely greatly on the O<sub>2</sub> bound to myoglobin (Mb–O<sub>2</sub>).

Hence, at the end of the molt period ashore, penguins face a challenging situation. Firstly, they lost most of their insulative subcutaneous fat during the preceding molt and are susceptible to increased heat loss, especially during diving, burdening their energy budget (Enstipp et al., 2017). Secondly, they lost about half of their pectoral muscle mass, potentially compromising their locomotor ability and substantially reducing their capacity for heat generation (thermogenesis). Such overall reduction in pectoral muscle mass and the likely remodeling of its architecture also means a substantial reduction in pectoral muscle O<sub>2</sub> storage, limiting the capacity of the muscle for aerobic ATP production to provide propulsion. However, a switch to anaerobic metabolism will increase recovery times at the surface between dives and reduce dive efficiency (Kooyman et al., 1980; Ponganis et al., 1997a,b,c), and might, therefore, be restricted to extended dives and short swimming bursts (Baldwin et al., 1984; Baldwin, 1988; Ponganis et al., 1997d). In addition, the anaerobic energy store of the pectoral muscle (phosphocreatine and glycogen; Williams et al., 2012) will also be curtailed after substantial muscle mass loss during molt, further reducing the potential for ATP production.

Although penguins undergo severe physiological changes during their annual molt, it is unclear how these changes affect their dive/foraging performance during the trip following molt. A considerable reduction in dive performance parameters (e.g. dive duration, post-dive surface interval duration) in comparison to the trip preceding molt might be expected.

As part of an investigation to study the development of dive capacity, foraging behavior and thermoregulation in juvenile king penguins, we instrumented 30 birds (~1 year old) with an implantable data logger at the end of their first molt, just before departing from their natal colony for the first time in their life (Enstipp et al., 2017). Here, we report on the changes in foraging/dive behavior and thermoregulation that occurred across the molt cycles of immature king penguins, especially contrasting dive performance parameters between the pre-molt and post-molt trips. In particular, the aims of our investigation were to: (1) investigate how the molt fast ashore affected the dive/foraging performance during post-molt foraging trips, potentially revealing physiological constraints; (2) examine differences in dive/foraging effort between pre- and post-molt trips; (3) study the effects of extensive changes in body insulation on peripheral temperature patterns across the molt cycle; and (4) explore the effects of demands associated with feather synthesis (pre-molt) and subcutaneous fat deposition (post-molt) on peripheral perfusion patterns, as inferred from peripheral

temperature. Although our investigation focused on immature birds, the observed changes during the annual molt cycle likely occur throughout the life of king penguins.

## MATERIALS AND METHODS

### Bird capture, logger implantation and recapture

Our study was conducted at the king penguin (*Aptenodytes patagonicus* Miller 1778) colony of 'Baie du Marin' on Possession Island, Crozet Archipelago, in the Southern Indian Ocean (46°25'34"S, 51°51'36"E). All procedures concerning bird capture, logger implantation, recovery from surgery and bird release were detailed in a previous paper (Enstipp et al., 2017). In brief, in November–December 2013, 30 juvenile king penguins of both sexes were captured when they were ~1 year old, near the end of their first molt from down to waterproof plumage; hence, just before these juveniles left the colony for the first time and dispersed at sea for an extended period (1–3 years). Following capture, birds underwent surgery for the subcutaneous implantation of a LUL data logger (Light Ultralight Logger, MIBE, IPHC, Strasbourg, France). The logger was positioned on the right flank of a bird and placed within the subcutaneous fat layer, sitting between the dermis on the outside and the connective tissue/fascia and muscle layer on the inside (see fig. 1 in Enstipp et al., 2017). Loggers were coated in two ways, either with wax only (15 juveniles; 'wax only') or they were first placed into a pouch of heat shrink tubing filled with silicon oil, before application of a thin wax layer (15 juveniles; 'oil and wax'). All loggers (average dimensions after encapsulation: 32×26×15 mm, length×width×height, with a mean mass of 8.9±0.5 g) were programmed to record pressure (resolution: 0.5 mbar, accuracy: ±30 mbar, calibrated range: linear to 40 bar; 1 bar=10<sup>5</sup> Pa) and temperature (resolution: 0.02°C, accuracy: ±0.5°C, range: –35 to 65°C, time constant: 70–220 s, depending on coating) every 5 s (internal sensors), so that the recording ideally would cover the birds' entire dispersal phase. Birds also received a passive transponder tag that allowed detection upon return to the colony through a radio-frequency identification (RFID) system (Gendner et al., 1992, 2005; Le Bohec et al., 2007).

Until March 2017, 19 of the 30 instrumented juvenile king penguins were recaptured, by now immatures, and loggers were retrieved. Two additional birds were detected during this period but could not be recaptured.

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

#### Recording periods and molt cycles

Of the 19 retrieved loggers, 14 contained useful data. Eight loggers contained data exceeding 1 year and three loggers recorded for over 2 years (for details, see table 1 in Enstipp et al., 2017). Only two of the 14 birds for which we obtained recordings returned briefly to land after their initial departure from the colony (after ~1 month, for 3–4 days), while all others remained at sea until the following austral spring. Because our current analysis focuses on the second and third molt cycles of birds, i.e. after they spent their first and second year at sea, respectively, we consider all birds to be immatures, rather than juveniles. In November 2014, i.e. ~1 year after their first departure from the colony, eight immatures (~2 years old) returned briefly to shore before they departed for their pre-molt trip. Here, we consider a molt cycle to consist of a foraging trip

preceding molt (pre-molt trip), the molt period ashore, and a foraging trip succeeding molt (post-molt trip). Unfortunately, five of the eight loggers that recorded until the beginning of the second molt cycle (austral summer 2014–2015, after birds had spent their first year at sea) failed either while birds molted ashore or during the following post-molt trip at sea. Accordingly, we focused our analysis on the three birds for which the recording included two complete molt cycles (the second and third molt of their life; 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), resulting from vasomotor changes according to activity (i.e. peripheral vasoconstriction during dive bouts and vasodilation when resting at the surface after cessation of diving), affected the baseline of the pressure signal and caused an upward shift of up to 5 m, when birds were at the surface. Hence, a zero offset correction of depth data was required and 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. In addition, the temperature effect on the pressure sensor differed between the two logger coating types (different physical properties of wax and oil), potentially affecting the calculation of dive parameters differently between types. To avoid any potential bias, analysis for both logger coating types was kept separate. The loggers of the three birds included in the current analysis of peripheral temperature and dive/foraging effort and performance were all of the same coating type ('wax only'). After zero offset correction of depth data and the cutting of all recordings into appropriate sections, dive parameters and peripheral temperature data were extracted before further analysis (see Halsey et al., 2007).

### Dive/foraging effort and performance

We investigated a number of parameters concerning both dive/foraging effort and performance across both molt cycles (austral summer 2014–2015 and 2015–2016). Effort indicates 'how hard birds worked' during a trip, e.g. how much daily time they allocated to foraging. By contrast, performance, especially when investigated in relation to dive depth, should reflect physiological capacity. King

penguins feed mainly on patchily distributed, small mesopelagic fish that are located at great depth during the day (100–300 m; Bost et al., 2002). Optimal foraging models predict that within a dive cycle, penguins should maximize the time spent at feeding depth (i.e. bottom phase) and reduce the time spent in transit and at the surface between dives (Kramer, 1988; Ydenberg and Clark, 1989; Carbone and Houston, 1994; Thompson and Fedak, 2001; Mori et al., 2002). Such predictions are supported by Hanuise et al. (2013), who found that king penguins reduce transit time during periods of increased foraging activity. Accordingly, a potentially reduced dive/foraging performance of penguins during post-molt trips might indicate physiological constraints as a consequence of molt.

In our analysis we investigated parameters in two ways. First, we compared both dive/foraging effort and performance parameters between pre- and post-molt trips based on bird means calculated from daily means across entire trips ('daily means across trips'; Table 2). Second, to investigate the effect of maximum dive depth on performance parameters, we sorted all deep dives ( $\geq 50$  m) conducted by a bird during the pre- and post-molt periods into 10 m depth bins, according to the maximum depth reached during a dive. The 50 m depth threshold has been used in previous investigations (Ropert-Coudert et al., 2000; Charrassin et al., 2002; Halsey et al., 2007), because most foraging of adult king penguins seems to occur during dives exceeding this depth (Pütz et al., 1998). Hence, in the present study we distinguish between shallow ('traveling') dives ( $< 50$  m) and deep ('foraging') dives ( $\geq 50$  m). We then investigated how performance parameters changed with maximum depth reached during a dive and contrasted pre- and post-molt periods. Accordingly, dive depth in our study refers to maximum dive depth, i.e. mean dive depth refers to mean maximum dive depth, unless specified differently.

In the definition of dive/foraging effort and performance parameters, we followed the classification system for seabird diving behavior given in Halsey et al. (2007). The following five parameters were computed as an index of dive/foraging effort of birds during trips. (1) 'Time submerged' represents the cumulative time a bird spent underwater per day. We distinguished between the time submerged in dives  $> 1$  m (which include both shallow traveling and deep foraging dives) and in dives  $\geq 50$  m (which include only foraging dives). (2) 'Time foraging' represents the sum of the cumulative time per day that a bird spent underwater in dives  $\geq 50$  m and the recovery time spent at the surface between dives.

**Table 1. Summary of recorded molt cycles from immature king penguins**

Bird	Second molt cycle (2014–2015)					Third molt cycle (2015–2016)				
	Start pre-molt trip	Duration pre-molt trip (days)	Start molt	Duration molt (days)	Duration post-molt trip (days)	Start pre-molt trip	Duration pre-molt trip (days)	Start molt	Duration molt (days)	Duration post-molt trip (days)
LuL04	13/11/2014	50.0	02/01/2015							
LuL09	25/11/2014	40.4	04/01/2015	27.6						
LuL11	17/12/2014	28.3	14/01/2015	26.4						
LuL13	23/11/2014	33.4	26/12/2014	25.8	26.5	10/11/2015	30.5	10/12/2015	25.9	10.9
LuL14	15/11/2014	30.6	15/12/2014							
LuL15	27/11/2014	35.5	01/01/2015	29.5	22.5	08/11/2015	43.1	21/12/2015	28.9	18.4
LuL20	07/12/2014	16.5	23/12/2014	26.4	25.3	13/12/2015	13.6	26/12/2015	26.4	19.6
LuL29	09/11/2014	40.4	19/12/2014	28.6						
Mean	24/11/2014	34.4±3.5	29/12/2014	27.4±0.6	24.7±1.2	20/11/2015	29.1±8.6	19/12/2015	27.0±0.9	16.3±2.7
		±4.5 days		±3.4 days			±7.0 days		±2.9 days	

All dates are in the format dd/mm/yyyy. Mean indicates grand means  $\pm$  s.e.m. The second molt cycle is the second molt in the life of these immature penguins and occurred after birds spent  $\sim 1$  year at sea, when birds are  $\sim 2$  years old. Note that loggers in five of the eight birds failed during the second molt cycle and complete molt cycle information (for two cycles) is available for three birds only.

**Table 2. Summary of parameters indicating diving/foraging effort and performance during pre- and post-molt trips of immature king penguins**

Parameter	Pre-molt	Post-molt	F-value	P-value
<b>Diving/foraging effort</b>				
Time submerged day <sup>-1</sup> (h), [ $>1$ m]	10.71±0.48	9.42±0.51	7.4	<0.01*
Time submerged day <sup>-1</sup> (h)	6.97±0.8	5.28±0.81	18.55	<0.0001*
Time foraging day <sup>-1</sup> (h)	8.97±0.92	7.38±0.93	11.08	0.001*
No. of foraging dives day <sup>-1</sup>	99.33±7.26	79.80±7.44	11.26	<0.001*
Dive effort day <sup>-1</sup> (h×m), [ $>1$ m]	531.35±70.97	426.73±71.63	13.36	<0.001*
Available daylight day <sup>-1</sup> (h)	15.53±0.08	14.73±0.08	379.04	<0.0001*
<b>Diving/foraging performance</b>				
Dive duration (s)	259.41±7.67	235.63±7.75	34.85	<0.0001*
PDSI duration (s)	74.69±2.06	87.61±2.07	15.26	0.0001*
Dive depth (m)	106.80±5.93	111.96±5.98	3.26	0.07
Descent speed (m s <sup>-1</sup> )	1.07±0.03	1.15±0.03	45.02	<0.0001*
Ascent speed (m s <sup>-1</sup> )	1.01±0.02	1.11±0.02	52.10	<0.0001*
Bottom duration (s)	62.87±2.13	44.83±2.20	94.99	<0.0001*
Dive efficiency	0.20±0.01	0.15±0.01	65.95	<0.0001*
No. of wiggles day <sup>-1</sup>	172.22±19.68	106.46±20.09	25.49	<0.0001*
No. of wiggles h <sup>-1</sup> submerged	24.06±0.61	19.65±0.70	16.45	<0.0001*
No. of wiggles dive <sup>-1</sup>	1.74±0.05	1.29±0.06	45.27	<0.0001*

Values for pre- and post-molt are grand means±s.e.m., established from the daily means of individuals across trips ( $N=3$  birds). Values from both years were included in the LME model to test for significance and to compute grand means. For a definition of parameters, see Materials and Methods. For all parameters with the exception of 'time submerged/day [ $>1$  m]' and 'dive effort [ $>1$  m]', only dives  $\geq 50$  m were considered. \*Significant difference between pre- and post-molt trips ( $P<0.05$ ).

Dive cycles with a post-dive surface interval (PDSI) exceeding 15 min were excluded here, which concerned the last dives of a foraging bout and the occasional isolated deep dives. (3) 'Number of foraging dives' is the total number of dives  $\geq 50$  m. (4) 'Dive effort' was calculated as the product of the total time spent submerged per day (dives  $>1$  m) and mean depth during submergence (here, mean of all depth values during submergence, i.e. 'true' mean depth) and integrates both temporal and spatial aspects of diving/foraging. Lastly, (5) 'available daylight' was calculated as the number of hours between sunrise and sunset (nautical twilight) for the location of the Crozet Islands and the concerned periods (<http://www.timeanddate.com>). Although it was not possible to track birds in our study, they presumably foraged near the Polar Front, to the south of the Crozet Islands, during pre- and post-molt trips. Hence, any potential errors associated with the available daylight calculation should be similar for both trips.

We computed the following seven parameters as an index of dive/foraging performance. (1) 'Dive duration' is the period spent submerged during a dive cycle; the latter consists of a dive and the following post-dive interval, spent at the surface. (2) 'PDSI duration' is the total time spent at the surface between two foraging dives ( $\geq 50$  m) and excludes periods spent submerged during shallow dives ( $<50$  m), which occasionally occurred between foraging dives. In our analysis, we only included dives conducted within a foraging bout, excluding isolated deep dives (with a PDSI duration exceeding 15 min; Halsey et al., 2007). Typically, during a dive, steps and wiggles are detectable as specific changes in the dive profile and are used to define further parameters. A step is a slowing of the descent or ascent of a dive, while a wiggle is defined as the change from an increase in depth over time to a decrease in depth, followed by a further increase in depth, resulting in a concave depth profile (Halsey et al., 2007). (3) 'Bottom duration' was then defined as the time between the first and last wiggle or step of a dive occurring at a depth deeper than 75% of maximum dive depth. (4) 'Descent speed' is the vertical distance between the surface and the start of the bottom phase divided by the time required to cover that distance. Similarly, (5) 'ascent speed' is the vertical distance between the end of the bottom phase and the

surface divided by the time taken to swim that distance. (6) 'Dive efficiency' represents the fraction of a dive cycle spent in the bottom phase (bottom duration/dive cycle duration; Ydenberg and Clark, 1989; Charrassin et al., 2002). Lastly, we consider the (7) 'number of wiggles per dive' as a relative index of prey encounter/pursuit events (Wilson and Wilson, 1995; Simeone and Wilson, 2003; Bost et al., 2007; Hanuise et al., 2010).

Furthermore, we calculated 'vertical speeds' during descent/ascent in two ways: (1) as a single average value during the entire transit (see above) and (2) as sequential averages for 15 m steps (i.e. dividing vertical distance for every 15 m moved within the water column by duration taken). The 15 m steps were chosen based on the limitations associated with our sampling interval. In both cases, all dives were first categorized according to maximum depth reached during the dive and analysis was run according to depth bin (10 m bins in the first case and 50 m bins in the latter). A single average value during transit does not provide any indication as to how vertical speed might change over the course of a dive. Instead, it provides an average for vertical speed during dives to varying depth. By contrast, when calculated sequentially over a certain distance interval, it allows investigation of how vertical speed during dives to varying depth changes throughout transit. Such changes in vertical speed might be the consequence of changes in body angle, propulsion (flipper beat frequency and/or amplitude) and/or buoyancy (Hanuise et al., 2013) and will affect the heat balance of birds (i.e. heat production and heat loss).

#### Peripheral temperature during molt cycles

To investigate peripheral temperature patterns throughout molt cycles, we calculated daily means for temperature ( $T_{\text{mean}}$ ,  $T_{\text{max}}$  and  $T_{\text{min}}$ ) during the pre-molt trip, while molting ashore, and during the ensuing post-molt trip.  $T_{\text{mean}}$  is the average of all temperature values recorded per day, while  $T_{\text{max}}$  and  $T_{\text{min}}$  are the single highest and lowest temperatures recorded per day, respectively. When birds are foraging at sea,  $T_{\text{min}}$  is usually achieved during a dive/foraging bout, whereas  $T_{\text{max}}$  occurs during surface rest periods at night (Handrich et al., 1997; Enstipp et al., 2017).

### Peripheral temperature patterns at sea: compatible with feather synthesis?

In adult penguins, the synthesis of new feathers typically starts during the pre-molt trip at sea, and this has been interpreted as an adaptation to reduce the required molt duration on land (Brown, 1986; Groscolas and Cherel, 1992; Cherel et al., 1994). However, feather synthesis requires sufficient peripheral perfusion, which will increase heat loss and will be especially severe during deep diving. Hence, to investigate how birds deal with such conflicting demands, we firstly calculated the daily amount of time when peripheral temperatures of birds were at normothermia (defined as  $\geq 36^{\circ}\text{C}$ ; Lewden et al., 2017a), indicating peripheral perfusion. When at sea, birds typically dive/forage throughout most of the available daylight hours. During these dive bouts, peripheral perfusion is curtailed, as indicated by substantially reduced peripheral temperatures (Enstipp et al., 2017), reducing overall heat loss. However, at night, when birds rest at the surface, peripheral temperatures return to normothermia, indicating peripheral perfusion (Lewden et al., 2017b; Enstipp et al., 2017). Hence, sufficient peripheral perfusion to fuel feather synthesis might be restricted to nightly rest periods. Accordingly, we secondly calculated the percentage of time that peripheral temperatures of birds during a selected 4 h rest period at night were at normothermia.

Because we did not record the geographical position of birds and juvenile king penguins have been shown to roam over large oceanic areas during their first year at sea (e.g. up to  $\sim 4000$  km to the west of the Crozet Islands; Orger et al., 2016), the exact start and end of night periods, as experienced by the birds, were unknown. However, deep dives ( $\geq 50$  m) during the night are rare in adult king penguins (Pütz et al., 1998) and birds typically discontinue deep diving activity when light levels become insufficient in the evening, and resume the following morning. Hence, it was possible to estimate the approximate night periods based on changes in bird diving behavior, which typically shows a systematic increase in dive depth near dawn and a systematic decrease near dusk. Night periods between nautical twilight in the evening and morning at the time of the molt cycles in our birds were  $\sim 5\text{--}7$  h near the Crozet Islands. Accordingly, we selected a 4 h night rest period for each day during the pre- and post-molt trips, starting 1 h after the end of the last deep dive of the day. Occasionally, birds discontinued foraging before the evening (e.g. at midday). These days could be detected by the resulting longer-than-typical rest period and were excluded from the analysis. Similarly, during some of the night rest periods, birds conducted shallow dives, most likely traveling. Because our focus was on resting periods at the surface, we excluded periods when birds spent  $>0.5$  h of the 4 h night rest period submerged.

### Statistical analysis

All statistical analyses were conducted in JMP (version Pro 11.2.0, SAS Institute Inc., Cary, NC, USA). Linear mixed-effects (LME) model analysis with restricted maximum likelihood (REML) estimation was used to test for differences in parameters (trip duration, peripheral temperatures, dive/foraging effort and performance parameters) with molt status (pre-molt versus post-molt). Where appropriate, interaction terms were included in the respective model and removed if not significant. Molt status, year (second and third molt cycle) and, where appropriate, dive depth were included as fixed effects, while bird ID was included as a random effect to account for repeated measures. Analysis was run separately for the second and third molt cycle, if results differed between years. Significance for all statistical tests was accepted at  $P < 0.05$ . All values presented are means  $\pm$  s.e.m., unless specified differently.

## RESULTS

### Molt cycles

In November–December 2014, eight immatures ( $\sim 2$  years old) returned to land after having spent an average of  $334.5 \pm 6.2$  days at sea. After  $6.9 \pm 0.7$  days ashore, birds initiated their pre-molt foraging trip of the second molt cycle of their life. Similarly, in November–December 2015, three immatures (now  $\sim 3$  years old) returned to shore after they had spent an average of  $259.5 \pm 7.5$  days at sea. They remained ashore for an average of  $6.3 \pm 1.7$  days, before they initiated the pre-molt foraging trip of their third molt cycle. On average, when both molt cycles are considered, duration of the pre-molt trip was longer than that of the post-molt trip ( $32.7 \pm 3.0$  versus  $22.2 \pm 4.4$  days;  $F = 5.0$ ,  $P = 0.049$ ). Table 1 shows the trip durations for both molt cycles. Molt duration ashore, i.e. the time between arrival from the pre-molt trip and departure for the post-molt trip, did not differ between years ( $27.3 \pm 0.6$  days on average;  $F = 0.89$ ,  $P = 0.44$ ; Table 1). When immatures returned from their post-molt foraging trip, they remained ashore only for a few days (range: 1.5–5.5 days) before returning to sea. After their second molt cycle, immatures ( $N = 3$ ) remained at sea until the following austral spring, apart from occasional short periods ashore ( $\sim 2\text{--}3$  days). After their third molt cycle, the three immatures returned to shore frequently for short periods during the austral summer of 2015–2016 and were eventually recaptured in their natal colony.

### Dive/foraging effort during pre- and post-molt trips

All parameters computed to investigate the dive/foraging effort during trips showed significantly greater values for the pre-molt trip when compared with the post-molt trip (Table 2). For example, birds spent a significantly greater amount of time submerged per day in foraging dives during the pre-molt trip when compared with the post-molt trip ( $6.97 \pm 0.92$  versus  $5.28 \pm 0.81$  h; Table 2). Although the total time available for foraging per day (daylight hours) was  $\sim 50$  min longer during pre-molt, birds also allocated a greater relative fraction of this period to foraging (58% versus 50% during pre- and post-molt, respectively; Table 1). Dive effort was the only parameter that differed significantly between years and was greater for the pre-molt trip during year 2 (third molt cycle) when compared with year 1 (second molt cycle;  $F = 10.37$ ,  $P < 0.01$ ).

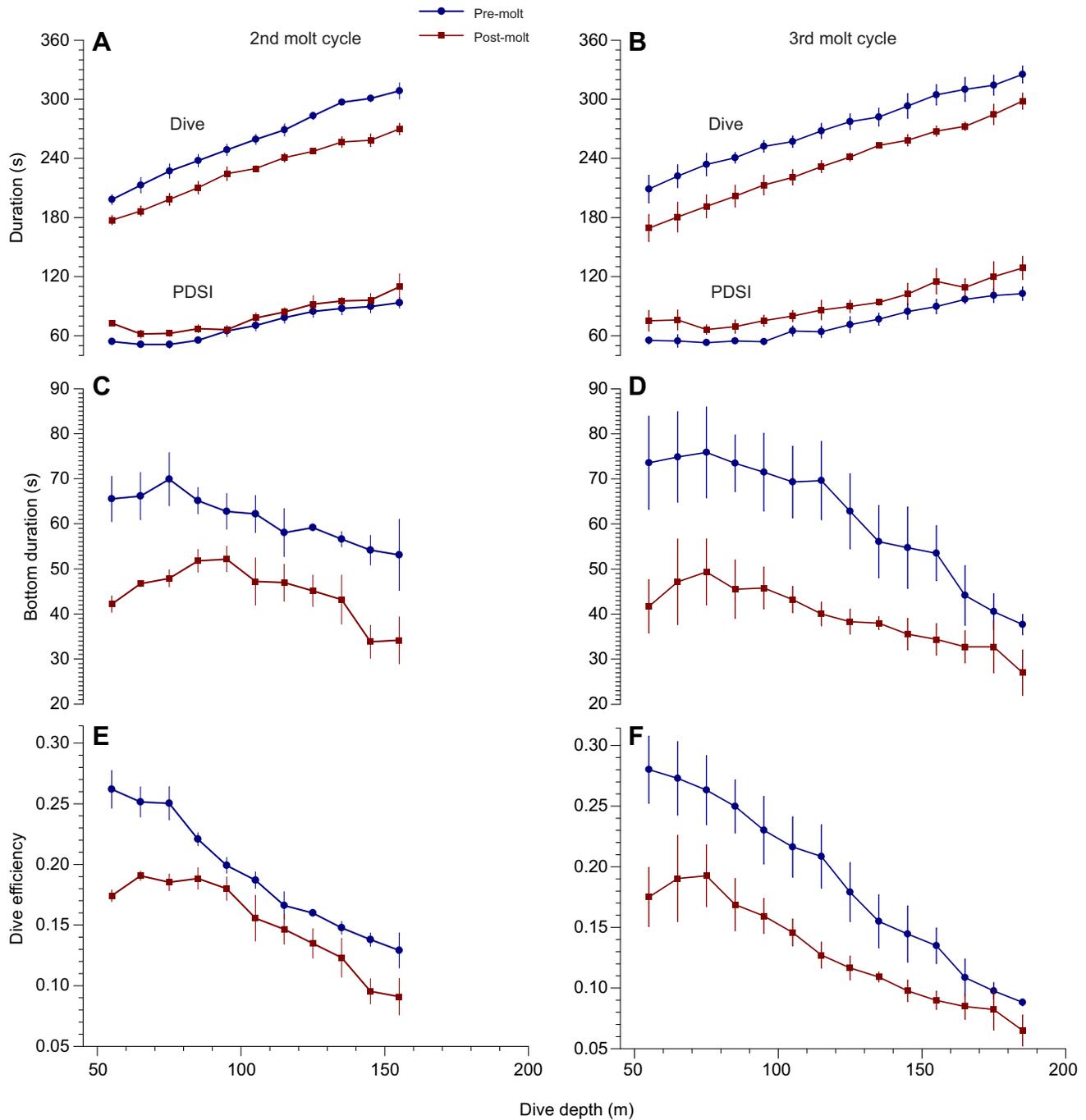
### Dive/foraging performance during pre- and post-molt trips

#### Daily means across trips

Birds dived to similar depths during both pre-molt ( $106.80 \pm 5.93$  m) and post-molt trips ( $111.96 \pm 5.98$  m). By contrast, all other parameters related to dive/foraging performance (all dives  $\geq 50$  m) differed significantly between pre- and post-molt trips (Table 2). Although most parameter values were greater during the pre-molt trip (e.g. dive duration, bottom duration, dive efficiency, number of wiggles), PDSI duration and transit speeds (during descent/ascent) were significantly greater during the post-molt trip (Table 2).

#### The effect of maximum dive depth

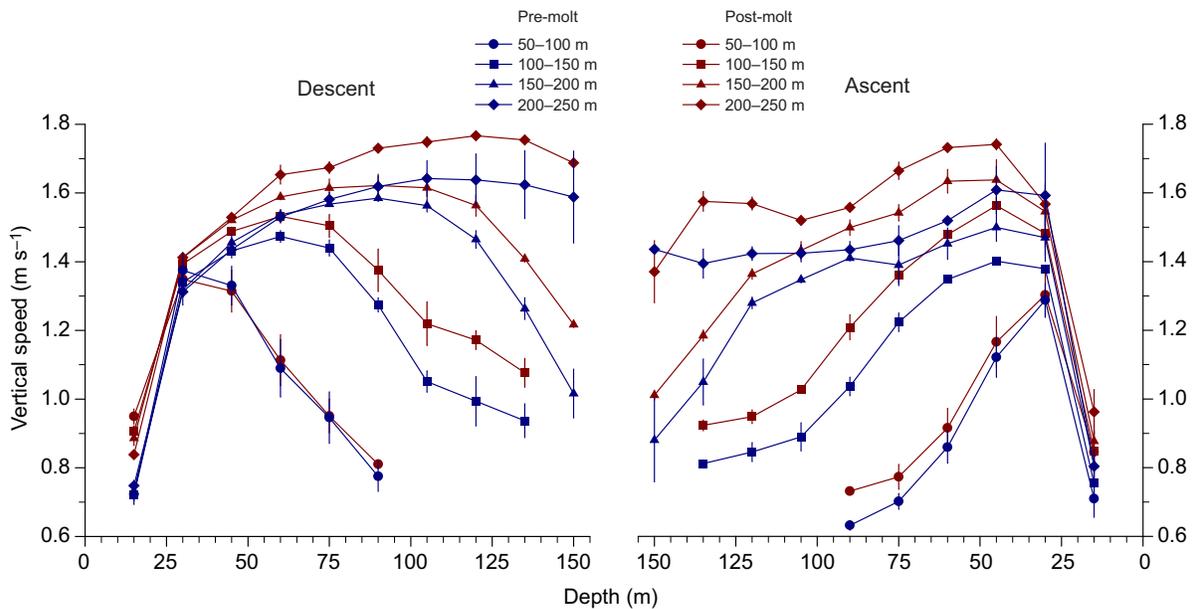
LME model analysis showed that all performance parameters changed significantly with depth ( $P < 0.0001$  for all parameters and for both years; Fig. 1). While dive duration, PDSI duration and descent/ascent speed increased significantly with depth, bottom duration and dive efficiency declined significantly with depth. Importantly, all parameters differed significantly between the pre- and post-molt periods ( $P < 0.0001$  for all parameters and for both years; Fig. 1). With the exception of the PDSI duration and transit speeds (descent/ascent), values for all performance parameters were significantly greater during the pre-molt period when compared



**Fig. 1. Parameters indicative of the dive/foraging performance of three immature king penguins in relation to maximum dive depth, contrasting pre- and post-molt trips.** Values are grand means  $\pm$  s.e.m. established from bird means (10 m depth bins; all dives  $\geq 50$  m). (A,C,E) Results for the second molt cycle ( $n=6680$  dives during pre-molt, blue symbols;  $n=4923$  dives during post-molt, red symbols); (B,D,F) results for the third molt cycle ( $n=7554$  dives during pre-molt;  $n=4003$  dives during post-molt). All performance parameters changed significantly with maximum dive depth. Most importantly, all parameters differed significantly between the pre-molt and post-molt period during both years.

with the post-molt period. By contrast, PDSI duration and transit speeds were significantly greater during the post-molt period. On average for a given maximum depth, dive duration was 12% shorter (year 2: 14%) and PDSI duration was 13% longer (year 2: 30%) during the post-molt trip when compared with the pre-molt trip. Bottom duration, which gives an indication of the time available for foraging, was greatly reduced during the post-molt period when compared with the pre-molt period (on average by 27% and 38% for year 1 and year 2, respectively). Consequently, dive efficiency was

considerably lower during the post-molt period (reduced on average by 21% and 33% during year 1 and year 2, respectively). Transit speeds (during both descent and ascent) differed less across the molt cycle and were on average 9% (year 2: 5%) faster when descending and 10% (both years) faster when ascending during post-molt when compared with pre-molt. Similarly, when calculated over 15 m steps, vertical speeds during both descent to and ascent from different final depth (50 m depth bins) were significantly faster during post-molt when compared with pre-molt ( $P < 0.01$  and



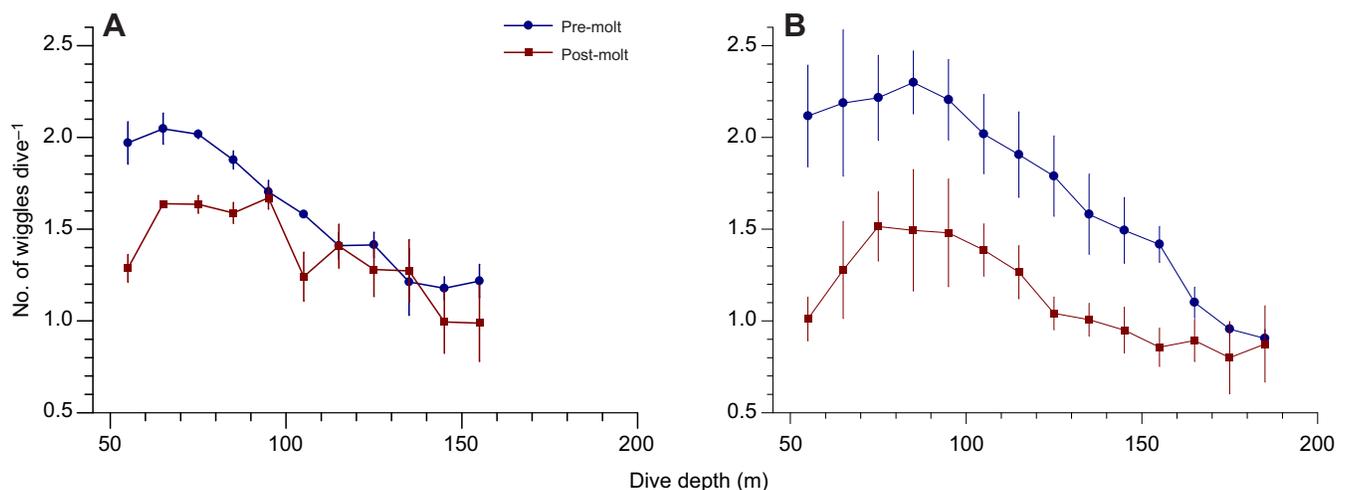
**Fig. 2. Vertical transit speeds of three immature king penguins during dives to differing final depth, contrasting pre- and post-molt trips (second molt cycle, 2014–2015).** Values during descent and ascent were calculated over 15 m steps for four 50 m depth bins (all dives  $\geq 50$  m) and represent grand means  $\pm$  s.e.m., established from bird means. Pre- and post-molt trips are indicated by blue and red symbols, respectively ( $n=6680$  dives during pre-molt and  $n=4923$  dives during post-molt). Vertical speeds during both descent to and ascent from different final depth were significantly faster during post-molt when compared with pre-molt. Across the different depth bins, vertical speed reached at a particular depth during descent and ascent increased with final dive depth.

$P < 0.0001$  for descent and ascent speeds, respectively; Fig. 2). Overall, for all depth bins, vertical speed during descent first increased before it leveled off and decreased, once birds neared their final depth (Fig. 2). Across the different depth bins, vertical speed reached at a particular depth during descent increased with final dive depth (Fig. 2). Similarly, the vertical speed reached at a given depth during ascent was higher when ascending from greater depth. During ascent, vertical speed for all depth bins also first increased before it leveled off and strongly decreased once birds reached the upper 30 m (Fig. 2). Lastly, the number of detected wiggles per dive declined significantly with depth and was lower during post-molt

(on average 15% and 37% lower during year 1 and year 2, respectively; Fig. 3) when compared with pre-molt. However, when taking into account bottom duration (i.e. number of wiggles per second bottom duration), the difference between pre- and post-molt disappeared (year 2;  $F=0.02$ ,  $P=0.9$ ) or even reverted (year 1;  $F=41.62$ ,  $P < 0.0001$ ).

#### Peripheral temperature during molt cycles

During the molting period ashore, peripheral temperatures were stable ( $T_{\text{mean}}: 38.5 \pm 0.1^\circ\text{C}$ ) and did not differ between years ( $F=3.84$ ,  $P=0.05$ ), reflecting continuous perfusion of the



**Fig. 3. Number of wiggles per dive in relation to maximum dive depth for three immature king penguins during pre- and post-molt trips across two molt cycles.** (A) Second molt cycle; (B) third molt cycle. Values are grand means  $\pm$  s.e.m., established from bird means [ $n=6680$  dives and 7554 dives during the pre-molt trip of cycles 2 and 3, respectively (blue symbols);  $n=4923$  dives and 4003 dives during the post-molt trip of cycles 2 and 3, respectively (red symbols)]. Overall, the number of wiggles per dive declined with dive depth and for a given maximum depth was lower during post-molt trips when compared with pre-molt trips.

integument (Fig. 4). This contrasts with peripheral temperatures recorded during pre- and post-molt trips, which showed a strong difference between periods of inactivity ( $T_{\max}$ ) and diving/foraging periods ( $T_{\min}$ ; Fig. 4). Furthermore, peripheral temperatures ( $T_{\text{mean}}$ ,  $T_{\max}$ ,  $T_{\min}$ ) recorded during pre-molt trips were significantly lower than during post-molt trips ( $P < 0.0001$  for all; Fig. 4). When considering both molt cycles, on average,  $T_{\text{mean}}$ ,  $T_{\max}$  and  $T_{\min}$  were  $22.3 \pm 0.3^\circ\text{C}$ ,  $38.1 \pm 0.2^\circ\text{C}$  and  $15.7 \pm 0.2^\circ\text{C}$ , respectively, during pre-molt trips and  $33.4 \pm 0.4^\circ\text{C}$ ,  $38.8 \pm 0.2^\circ\text{C}$  and  $26.2 \pm 0.2^\circ\text{C}$  during post-molt trips.

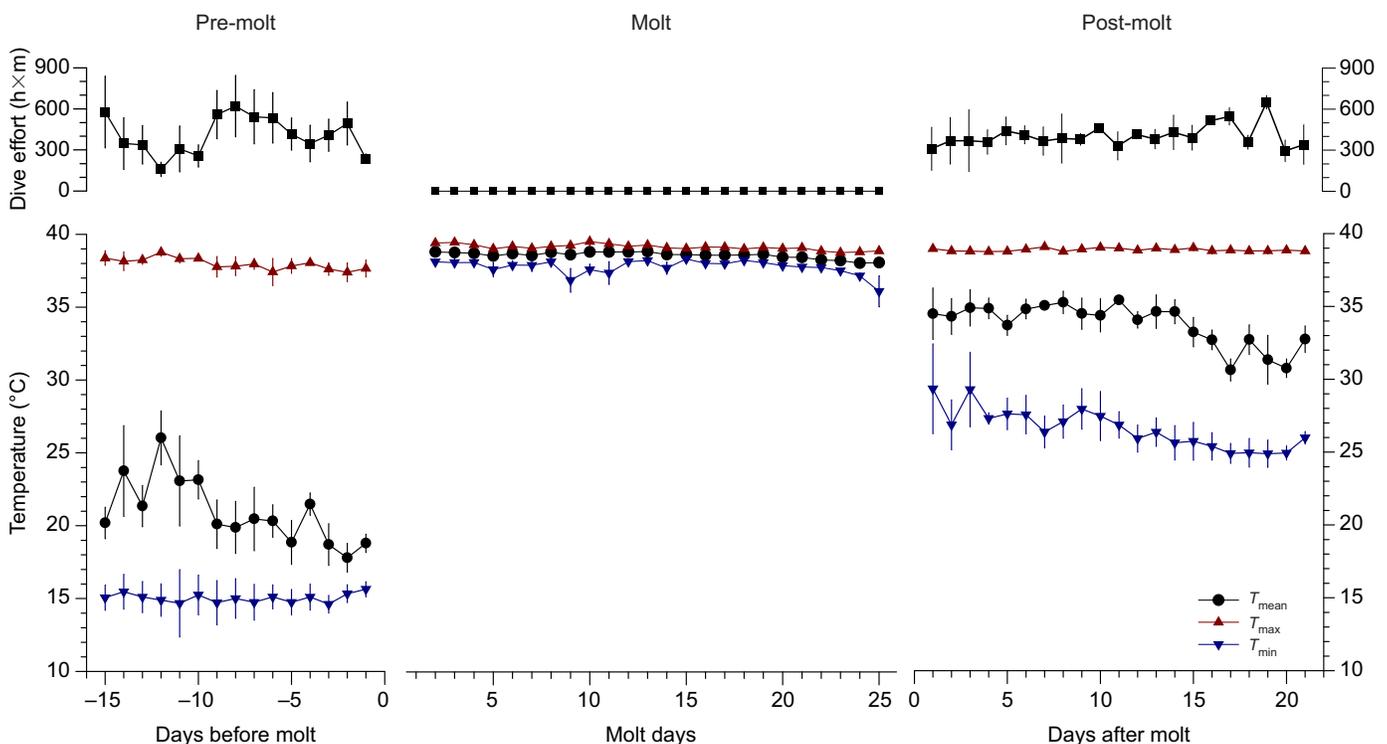
#### Daily peripheral temperature patterns at sea: compatible with feather synthesis?

During pre-molt trips, the peripheral temperature of birds was on average for  $4.74 \pm 0.33$  h per 24 h period at normothermic levels (i.e. for 19.8%) significantly less than during post-molt trips ( $12.17 \pm 0.39$  h; for 50.7%;  $F = 177.75$ ,  $P < 0.0001$ ) and this did not differ between years ( $F = 2.88$ ,  $P = 0.09$ ). If only the last week during pre-molt and the first week during post-molt are considered, i.e. periods when molt processes are likely to be of importance, the situation remained unchanged during post-molt ( $12.58 \pm 0.46$  h; 52.4%), but during pre-molt, the normothermic period was even further reduced ( $2.71 \pm 0.46$  h, 11.3%). When considering the 4 h night rest periods, the peripheral temperature of birds was on average for  $40.75 \pm 3.3\%$  of these periods at normothermia during pre-molt trips, while during post-molt trips peripheral perfusion, as inferred from temperature, was almost continuous ( $97.0 \pm 3.5\%$  at normothermia;  $F = 262.35$ ,  $P < 0.0001$ ). For post-molt trips these values did not differ between years, but values for pre-molt trips did ( $28.75 \pm 5.75\%$  versus  $59.25 \pm 4.5\%$  during the second and third molt cycle, respectively). Fig. 5

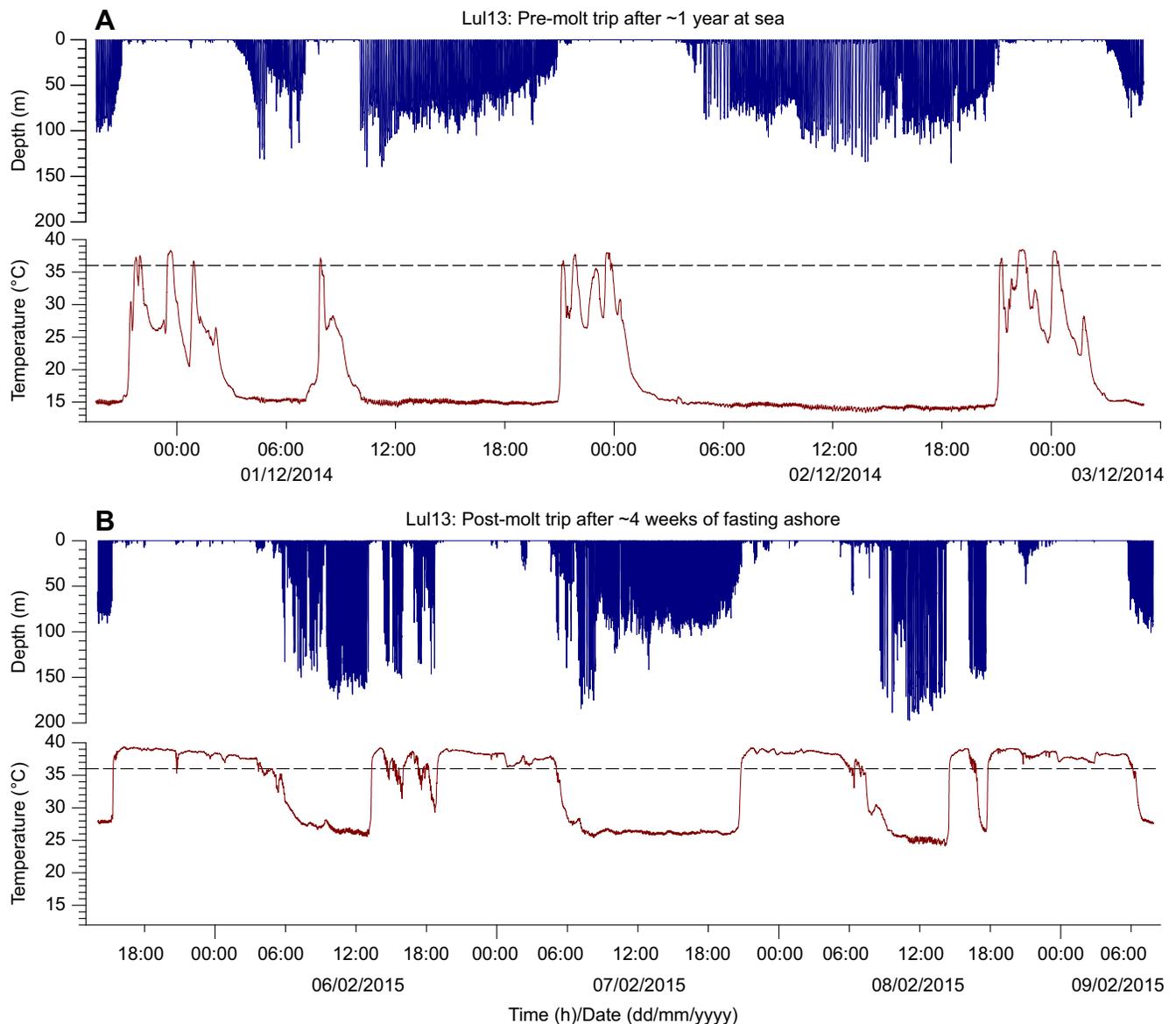
shows the typical temperature pattern observed for one immature bird during a section of the pre- and post-molt trips of the second molt cycle. The following is worth noting. (1) Peripheral temperature drops, associated with extended foraging bouts, fell to substantially lower levels during pre-molt when compared with post-molt, indicating considerable differences in body condition/peripheral insulation during both periods (Enstipp et al., 2017). (2) During non-foraging periods (i.e. no deep dives), particularly at night, peripheral temperatures were maintained at normothermia during post-molt, indicating perfusion. (3) By contrast, during pre-molt, birds showed periodic peripheral temperature drops below normothermic levels throughout these extended resting periods at the surface, indicating at least a considerable reduction in peripheral perfusion and intermittent reperfusion.

#### DISCUSSION

Our study found an important decline in the dive and foraging performance of immature king penguins following their annual molt fast ashore. Most importantly, dive and especially bottom duration for a given dive depth were significantly shorter after molt, while PDSI duration was significantly greater, leading to an overall reduced dive efficiency during post-molt trips. Although possible differences in prey availability between pre- and post-molt trips might have contributed to some of the observed changes in performance (but see below), the greater PDSI duration for dives of a shorter duration during post-molt strongly suggests physiological constraints following the annual molt. We also found peripheral temperature patterns that differed greatly before and after the molt. The lower peripheral temperatures recorded before their molt indicate that immatures were well insulated at this moment (less heat



**Fig. 4. Dive effort and peripheral temperature across the second molt cycle of three immature king penguins.** Values are grand means  $\pm$  s.e.m., established from the daily means of individuals. Because trip duration varied between birds, only the periods when data for all birds were available are shown. Dive effort, though variable between days, was on average greater during the pre-molt trip when compared with the post-molt trip. Peripheral temperatures recorded during pre-molt trips, especially  $T_{\min}$  (blue triangles), were significantly lower than during post-molt trips, reflecting differences in body condition/insulation of birds. During the molt period ashore, peripheral temperatures were high and stable, indicating maintained peripheral perfusion.



**Fig. 5. Dive and peripheral temperature patterns observed during sections of the pre- and post-molt trips of an immature king penguin.** During both trips (A: pre-molt; B: post-molt), the bird dived/foraged extensively throughout most of the daylight hours, while during the night it mostly rested at the surface. Peripheral temperature drops, associated with extended foraging bouts, fell to substantially lower levels during pre-molt when compared with post-molt. During post-molt, peripheral temperatures were maintained at normothermia ( $\geq 36^{\circ}\text{C}$ ; dashed line) throughout non-foraging periods, indicating perfusion. However, during pre-molt, the bird showed periodic peripheral temperature drops below normothermic levels throughout these extended resting periods, indicating a considerable reduction in peripheral perfusion. Note that time refers to the local time at the Crozet Islands and, therefore, only approximately reflects the local time experienced by the bird.

arriving at the logger, insulated by subcutaneous fat; see fig. 1 in Enstipp et al., 2017). During molt, birds lost most of this insulation (especially subcutaneous fat), so that the recorded peripheral temperatures during post-molt were considerably higher, indicating increased heat loss. Peripheral perfusion during pre-molt, as inferred from peripheral temperature recordings, was restricted to short periods during nocturnal rest, suggesting that feather synthesis did not start until birds reached the shore. By contrast, during post-molt, peripheral perfusion was continuous throughout the nightly rest periods, extended into daylight hours, and was at times maintained during (shallow) diving. Such extensive peripheral perfusion during post-molt might have been required to finish molt processes but also to rapidly deposit a new

subcutaneous fat layer to improve insulation. Finally, and most importantly, all the changes we observed between pre- and post-molt trips of immature king penguins persisted across two molt cycles. Accordingly, these changes occur annually and are likely also present in adult birds.

#### Molt cycles

In both years, immatures began their molt in December/January, within a 2- to 4-week range between birds (Table 1). For comparison, adult king penguins start their annual molt between August and January, depending on whether they successfully raised a chick in the preceding breeding season (Weimerskirch et al., 1992; Gauthier-Clerc et al., 2002). Molt duration of immature birds was

stable with an average of 27 days during both years, with little variation (maximum difference: ~4 days), regardless of when the molt started (Table 1). This is unlike in adult king penguins, where molt duration declines over the course of the season, which has been attributed to a time constraint for reproduction (Weimerskirch et al., 1992; Gauthier-Clerc et al., 2002). Immature king penguins are clearly not affected by such a constraint and this might explain their stable molt duration. The duration of immature foraging trips preceding the molt period ashore was on average longer than that of post-molt trips (Table 1). By contrast, in adult king penguins, trip duration is typically shorter during pre-molt (~17 days; Adams and Brown, 1990) when compared with post-molt (24 days; Weimerskirch et al., 1992; but see Gauthier-Clerc et al., 2002, who reported a range of 11–29 days). A longer pre-molt trip in immatures might be explained by the need to lay down sufficient nutrient/energy reserves for the upcoming molt, whereas after the post-molt trip they returned to sea within a few days. Hence, unlike the situation in adult birds at this point, immatures did not have to lay down energy reserves for the upcoming breeding fast. The shorter post-molt trip duration of immatures might have also been motivated by courtship attempts, especially after the third molt cycle.

#### **Dive/foraging effort during pre- and post-molt trips**

Little is known about the foraging behavior of penguins at sea when they prepare for their annual molt (pre-molt trip) and breeding season (post-molt trip; but see Charrassin and Bost, 2001; Charrassin et al., 2002). Immature king penguins in our study worked hard in preparation for molt. During the pre-molt trip, birds spent a longer period per day underwater, they foraged for a longer period (and also allocated a relatively greater fraction of available daylight hours to foraging), and the overall dive effort was greater than during the post-molt trip (Table 2). This might not be surprising, as immature penguins, unlike adults, were under no pressure to build up nutrient/energy reserves during the post-molt trip in preparation for a breeding fast. Despite this, immature penguins also worked hard during the post-molt trip and likely recovered their body condition in substantial parts. Adult king penguins increase their body mass by 45–47% during both trips, when preparing for molt and breeding (Cherel, 1995). During both trips, adults store large amounts of lipid (mainly subcutaneously), whereas during pre-molt, birds also preferentially store protein and associated water (mainly in the pectoral muscles). Cherel (1995) also found that the daily body mass gain of adults and the estimated daily food consumption were greater during pre-molt than during post-molt, which might indicate that these birds, like our immatures, worked harder during pre-molt.

#### **Dive/foraging performance during pre- and post-molt trips**

The dive/foraging performance of immature king penguins was strongly affected by their molt fast ashore, as indicated by the daily mean values for various performance parameters across trips (Table 2). However, the dive/foraging performance of birds strongly depends on the final depth reached during a dive. Hence, a meaningful comparison between performance parameters across the molt cycle requires to take into account dive depth, so that parameters can be compared for a given maximum depth. Such a comparison revealed that, with the exception of vertical speeds during descent and ascent (Fig. 2), all performance parameters were decreased during post-molt when compared with pre-molt throughout the observed depth range (Figs 1–3). Most importantly, dive duration for a given depth was significantly

shorter during post-molt and so was bottom duration (Fig. 1A–D). The latter represents the phase of a dive when most feeding occurs (Charrassin et al., 2002; Halsey et al., 2007). Accordingly, a shorter bottom duration for a given depth during post-molt means that birds had less time available for feeding when compared with pre-molt. Furthermore, although dive duration for a given depth was shorter during post-molt, PDSI duration was longer (Fig. 1A,B). The latter is the time spent at the surface, during which birds recover from the previous dive and prepare for the next (i.e. gas exchange). In general, longer dives require a longer recovery time at the surface (Kooyman, 1989; Halsey and Butler, 2006; Halsey et al., 2006; see also Fig. 1A,B). However, the longer PDSI duration for a shorter dive duration during post-molt indicates a considerably reduced dive performance and is most likely a consequence of the severe physiological changes that occurred during molt. Because immatures lost about half of their pectoral muscle mass during molt ashore, their oxygen stores (Mb–O<sub>2</sub>) must have been considerably lower during post-molt, reducing aerobic dive capacity. Hence, it is possible that birds used up a greater fraction of their reduced oxygen stores when diving during post-molt, so that they needed a longer recovery time at the surface to reload their oxygen stores and eliminate CO<sub>2</sub>. A longer recovery period at the surface might also have been the consequence of an increased contribution of anaerobic metabolism (especially phosphocreatine, PCr) during diving. While reliance on anaerobic metabolism might be limited to extended dives (Baldwin et al., 1984; Baldwin, 1988; Ponganis et al., 1997d), PCr recovery might be similarly fast as Mb–O<sub>2</sub> recovery (Williams et al., 2012). For emperor penguins, which possess a large anaerobic energy reserve in form of glycogen and PCr in their pectoral muscles, it was suggested that Mb–O<sub>2</sub> and PCr recovery should occur within most observed post-dive surface intervals (Williams et al., 2012). A stronger depletion of the reduced oxygen stores during post-molt (and/or a potential increase in anaerobic metabolism) could be the consequence of an increased work load to generate sufficient heat so that the presumably high heat loss can be balanced and core temperature is maintained (see discussion on vertical speed changes below). In our calculation of PDSI duration, we only included the time spent at the surface between deep dives and excluded the occasional periods spent submerged in shallow dives between deep dives, because gas exchange occurs only at the surface. Furthermore, although these periods might serve a physiological function in dive recovery (Fahlman et al., 2007), their inclusion in the analysis did not change the results. The differences in dive duration, PDSI duration and bottom duration between pre- and post-molt trips had consequences for the calculated dive efficiency, which was lower during post-molt trips when compared with pre-molt trips (Fig. 1E,F). Furthermore, the number of wiggles per dive was, on average and for a given depth, greater during pre-molt, likely indicating a greater foraging success during that period (Fig. 3). Together, these findings suggest that dive capacity of immature king penguins following molt was reduced, with consequences for their ability to forage effectively.

Charrassin et al. (2002) studied how dive/foraging parameters changed in adult king penguins throughout their annual cycle. They also found a significant reduction in performance during the post-molt period (October) when compared with the preceding winter period (June–September). Dive duration and especially bottom duration were significantly reduced, while PDSI duration was increased, resulting in the lowest dive efficiency of the annual cycle. Performance in adult birds was exceptionally good during winter when compared with summer and autumn, so that the contrast with the post-molt period was particularly strong (Charrassin et al.,

2002). Although in that study the effect of dive depth on performance was not specifically addressed and the winter period preceding molt was considerably longer than the pre-molt trips in our study, it nevertheless supports our finding of a decreased dive/foraging performance in immatures following molt.

However, although we were able to detect a clear difference in dive/foraging performance of birds between pre- and post-molt trips, we do not know where immatures foraged during both periods, nor do we have any understanding of the prey abundance and availability to birds. One could argue that the changes in dive duration for a given depth we observed between pre- and post-molt trips might be related to differences in prey availability (Charrassin et al., 2002). For example, it is conceivable that at a higher prey availability, birds find prey faster and, therefore, terminate their dives sooner than at a lower prey availability. The shorter dive duration for a given depth during post-molt might then reflect a greater prey availability. Shorter dive and bottom durations when foraging in high quality patches have been reported for southern elephant seals (*Mirounga leonina*), but might be explained by seals foraging on single, large prey items (Thums et al., 2013). However, king penguins forage predominately on relatively small, schooling fish, found at great depth. Hence, these birds should try to capture multiple prey items during the bottom phase of their dives and reduce overall transit time during foraging bouts (Hanuise et al., 2013). Accordingly, birds will likely decide to stay submerged for longer and take advantage of a high prey availability, thereby extending dive duration. In this case, the longer dive duration for a given depth during pre-molt might indicate a greater prey availability.

Changes in dive profile (i.e. wiggles) have been used as indicators of feeding success in diving animals, including king penguins (Bost et al., 2007; Hanuise et al., 2010), and might also convey information concerning prey availability. However, because noticeable differences in size/mass of prey species taken by king penguins exist (Cherel and Ridoux, 1992; Hindell, 1988), the number of prey items caught per day might vary considerably, especially across seasons, so that the number of wiggles can only be a relative index of food intake. The low sampling rate in our study (0.2 Hz) also means that the number of detected wiggles will not reflect prey encounter/capture rates accurately (Simeone and Wilson, 2003), but nevertheless allows a relative comparison across foraging trips. To explore potential differences in prey availability between periods, we investigated how the daily number of wiggles varied with the amount of time immatures spent submerged per day (dives  $\geq 50$  m). Not surprisingly, this showed that the daily number of wiggles increased with the time spent submerged. However, the overall relationship did not differ between the pre- and post-molt trips during the third molt cycle ( $F=2.74$ ,  $P=0.10$ ) and only reached marginal significance during the second molt cycle ( $F=4.16$ ,  $P=0.044$ ). Hence, if we accept such a relationship to convey information about prey availability, the situation should have been similar between foraging trips. This is also supported by our finding that for a given bottom duration, the number of wiggles per dive was similar during pre- and post-molt. Accordingly, the lower number of wiggles per dive during post-molt (Fig. 3) is likely explained by the reduced bottom duration (absolute and relative) during this period (Fig. 1C,D), reducing the time available for feeding. Altogether, this supports our suggestion that differences in dive performance during pre- and post-molt trips are likely a consequence of physiological constraint following molt.

By contrast, vertical speed during descent and ascent was consistently faster during post-molt trips than during pre-molt trips

(Fig. 2), and this requires explanation. Changes in vertical speed can be accomplished through changes in body angle and/or changes in swimming speed. In king penguins, vertical speed changes seem to be mostly driven by changes in body angle (Hanuise et al., 2013). After the early descent phase, in which buoyancy is of great importance, drag becomes the main force determining the mechanical costs of underwater swimming (Lovvorn et al., 1991, 2001; Lovvorn, 2001). Drag is strongly affected by speed, body size and shape, while the roughness and vibration of feathers influence the flow around the body, further affecting drag (Lovvorn et al., 2001). Penguins are highly streamlined, and drag coefficients, calculated from experimental drag measurements, are extremely low (Bannasch et al., 1994; Bannasch, 1995). After molt ashore, body mass and body diameter of penguins are substantially reduced (loss of most subcutaneous fat). A smaller body diameter during post-molt and a renewed plumage should reduce the hydrodynamic drag further and, for a given speed, decrease propulsive requirements (less work per stroke) and energetic costs. Buoyancy should also be considerably lower during post-molt (loss of most subcutaneous fat during molt). Hence, it is possible that the overall small increase in transit speed during post-molt (on average  $\sim 5$ – $10\%$ ) was a result of birds maintaining a constant work rate during diving, rather than reducing power output to maintain speed. Given the presumably lower dive capacity of our immature king penguins during post-molt, one might expect birds to reduce locomotion costs as much as possible, to increase aerobic dive duration, especially bottom duration. Regulating the required work against drag within a relatively narrow range (i.e. maintaining muscle contraction speed and work per contraction) might maximize muscle efficiency during diving (Lovvorn, 2001), and has been observed in other wing-propelled divers (Brünnich's guillemot, *Uria lomvia*; Lovvorn et al., 2004). Alternatively, during post-molt, birds might increase work load and, hence, energy expenditure during diving (either by increasing actual swim speed or by transiting at a steeper dive angle) to increase metabolic heat production and so maintain core temperature and offset heat loss to some degree (see Ciancio et al., 2016). Although this might seem counterintuitive, because it will increase oxygen consumption underwater and/or lead to an increase in anaerobic metabolism, it agrees with the increased surface intervals observed during post-molt (Fig. 1A,B). However, Ciancio et al. (2016) found no evidence for such a behavioral response in Magellanic penguins (*Spheniscus magellanicus*) during deep foraging dives in cold water. These birds did not increase swim speed to offset heat loss via increased heat production, as the authors had predicted. Nevertheless, this aspect deserves further investigation.

### Peripheral temperature during molt cycles and thermoregulation

When immatures departed for their first pre-molt trip during the austral spring, they possessed a substantial layer of subcutaneous fat and were well insulated, as indicated by the lower peripheral temperatures recorded during this period, especially  $T_{\min}$  (Fig. 4; less core heat arriving at the well-insulated logger). During foraging, when birds conduct deep dives in quick succession, peripheral perfusion is curtailed (vasoconstriction), leading to a strong temperature gradient between the warm core and the cool shell, reducing heat loss and, therefore, thermoregulatory costs. Hence, the daily minimum peripheral temperature ( $T_{\min}$ ), which is typically recorded during foraging bouts, provides a relative index of body insulation (Enstipp et al., 2017). For a given peripheral perfusion level (strong vasoconstriction during diving), a lower  $T_{\min}$  indicates

a greater insulation and lower heat loss in comparison with a higher  $T_{\min}$ . During the first 6 months of their first year at sea, the daily  $T_{\min}$  of juvenile king penguins declines continuously, before reaching a stable level, presumably indicating the maximum amount of body insulation (Enstipp et al., 2017), which is maintained (apart from small oscillations) until molt. During the molt period ashore (i.e. in air), peripheral temperatures of immatures were elevated and stable, reflecting continuous perfusion of the integument to facilitate feather synthesis (Fig. 4). Initially, heat loss, associated with peripheral perfusion, will be kept at bay by a sufficient air layer within the plumage (Jarman, 1973; Le Maho et al., 1976; Stahel and Nicol, 1982), while new feathers develop beneath the old feathers. When new feathers are ~60% grown, the old feathers will be gradually replaced (Groscolas and Cherel, 1992). In adult king penguins, daily body mass loss at this point (after ~10–15 days ashore) increases sharply, as continued feather synthesis and increased heat loss (owing to a strong decline in thermal insulation) elevate energy expenditure approximately twofold over resting (Cherel et al., 1988; Adams and Brown, 1990; Groscolas and Cherel, 1992). During the molt fast ashore, body insulation of king penguins is severely altered, owing to the loss of most subcutaneous and abdominal fat. This is indicated by the higher peripheral temperatures recorded during the post-molt trip, especially  $T_{\min}$  (more core heat arriving at the now little-insulated logger), when compared with the pre-molt trip (Fig. 4). In principle, the higher  $T_{\min}$  values could also be the result of altered peripheral perfusion patterns as a consequence of continued molt requirements. For example, perfusion of the skin and/or underlying muscles after molt could be greater than during pre-molt, so that more heat reaches the logger. However, because  $T_{\min}$  is typically recorded during diving, when the periphery will be vasoconstricted as part of the dive response to preserve finite oxygen stores (Butler and Jones, 1997; Butler, 2004), this is unlikely. In any case, the higher temperatures recorded during post-molt ( $T_{\text{mean}}$ ,  $T_{\text{max}}$ ,  $T_{\min}$ ) mean that heat loss will be greatly increased over the pre-molt situation (Enstipp et al., 2017).

#### Daily peripheral temperature patterns at sea: compatible with feather synthesis?

During both pre-molt and post-molt trips, immature king penguins spent about half of every day (~12 h) in diving activity (traveling and foraging; sum of submergence and PDSI durations), while they rested for the remainder. Because the periphery will be vasoconstricted during diving, we would expect the observed peripheral temperature pattern to reflect the behavioral pattern (i.e. ~12 h hypothermia during diving and ~12 h normothermia during resting). Although this was the case for post-molt trips (on average ~12.2 h per day normothermic), the situation during pre-molt clearly differed, as birds spent on average only ~4.7 h per day at normothermia. This means that during pre-molt trips, peripheral vasoconstriction, as inferred from temperature recordings, was not restricted to diving activity but also occurred during extended resting phases at the surface (Fig. 5A). This is confirmed by the peripheral temperature patterns observed during selected 4 h rest periods at night. Although peripheral perfusion during these nightly rest periods was almost continuous during post-molt (~97% of rest period), it was restricted to only ~41% of the time during pre-molt. Furthermore, during post-molt trips, normothermia and, hence, peripheral perfusion was at times maintained even during (shallow) diving (Fig. 5B). Hence, the observed peripheral temperature patterns during pre-molt raise the question of whether they are compatible with molt processes, especially feather synthesis.

The relatively short periods per day spent at normothermia throughout pre-molt trips, which were even further reduced during the last week of a pre-molt trip (on average ~2.7 h per day), suggest that molt processes in immature king penguins had not started yet. This is supported by the observation of Groscolas (1978), who found that some juvenile emperor penguins returned for molt before feather growth had started. However, in adult penguins, feather synthesis certainly starts during their intense pre-molt foraging trip (Brown, 1986; Groscolas, 1978; Groscolas and Cherel, 1992; Cherel et al., 1994), but how birds accomplish this is unclear. Presumably, peripheral perfusion will also be curtailed during their intense and long foraging bouts, restricting nutrient supply for feather synthesis to the relatively short resting periods at the surface during the night. Alternatively, birds might relax peripheral vasoconstriction during diving/foraging and perfuse the integument, at least intermittently. However, this will presumably come at a high energetic cost, as it will increase heat loss, especially during deep foraging dives. Clearly, it would be interesting to study these temperature patterns across molt cycles in adult king penguins, especially when linked with an investigation concerning locomotion patterns (i.e. flipper stroke frequency and amplitude).

Lastly, what might explain the observed differences in peripheral temperature patterns between pre- and post-molt trips? During pre-molt, birds were well insulated (subcutaneous fat layer) and surplus energy was presumably stored preferentially in abdominal fat (and the pectoral muscles), so that peripheral perfusion could be kept to a minimum. By contrast, during post-molt, birds were lean, as they lost most of their abdominal and subcutaneous fat during the molt fast ashore. Hence, to keep heat loss at bay, a rapid build-up of the subcutaneous fat layer is required, which will force birds to maintain peripheral perfusion to a much greater extent, so that free fatty acids can be deposited (Lewden et al., 2017a,b). In addition, completion of molt processes might also force birds to maintain peripheral perfusion, which might be especially relevant during the first days of the post-molt trip.

In conclusion, we found that the dive and foraging performance of immature king penguins was significantly decreased during post-molt foraging trips, which is most likely a consequence of the severe physiological changes that birds undergo during their annual molt ashore. Despite this, immatures successfully mastered this challenging situation and recovered their body condition, as indicated by a progressive decline in  $T_{\min}$  (Enstipp et al., 2017). Some of the shortcomings associated with the atrophy of pectoral muscle during molt and the reduction in its oxidative capacity to produce sufficient ATP for force production might be alleviated by changes in mitochondrial efficiency. For example, fasting in king penguin chicks has been shown to increase the efficiency of mitochondrial ATP synthesis in pectoralis muscle (ATP/O coupling; Montemier et al., 2014; Bourguignon et al., 2017). Accordingly, for a given activity level, pectoral muscle functions at a lower cost in these birds. Hence, variations in mitochondrial efficiency might have a strong effect on animal performance. This has been demonstrated in humans, where experimental treatments that elevated mitochondrial coupling increased exercise efficiency (muscle power output per  $O_2$ ) and the capacity for sustained exercise (Conley, 2016). Further studies are required to test whether such changes in mitochondrial efficiency are a potential mechanism for king penguins to ensure sufficient force production with a greatly downsized pectoral muscle machinery after their annual molt, to guarantee a quick recovery of body condition during their post-molt trip.

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

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