

CALL FOR PAPERS | *Physiology and Pharmacology of Temperature Regulation*

Body temperature changes induced by huddling in breeding male emperor penguins

Caroline Gilbert,¹ Yvon Le Maho,¹ Martine Perret,² and André Ancel¹

¹Institut Pluridisciplinaire Hubert Curien, Département Ecologie, Physiologie et Ethologie and Département Interactions Physique, Chimie et Vivant, Unité Mixte de Recherche (UMR) 7178, Centre National de la Recherche Scientifique and Université Louis Pasteur, Strasbourg, France; and ²Département d'Ecologie et Gestion de la Biodiversité, UMR 5176, Centre National de la Recherche Scientifique and Muséum National d'Histoire Naturelle, Brunoy, France

Submitted 29 December 2005; accepted in final form 4 September 2006

Gilbert, Caroline, Yvon Le Maho, Martine Perret, and André Ancel. Body temperature changes induced by huddling in breeding male emperor penguins. *Am J Physiol Regul Integr Comp Physiol* 292: R176–R185, 2007. First published September 7, 2006; doi:10.1152/ajpregu.00912.2005.—Huddling is the key energy-saving mechanism for emperor penguins to endure their 4-mo incubation fast during the Antarctic winter, but the underlying physiological mechanisms of this energy saving have remained elusive. The question is whether their deep body (core) temperature may drop in association with energy sparing, taking into account that successful egg incubation requires a temperature of about 36°C and that ambient temperatures of up to 37.5°C may be reached within tight huddles. Using data loggers implanted into five unrestrained breeding males, we present here the first data on body temperature changes throughout the breeding cycle of emperor penguins, with particular emphasis on huddling bouts. During the pairing period, core temperature decreased progressively from $37.5 \pm 0.4^\circ\text{C}$ to $36.5 \pm 0.3^\circ\text{C}$, associated with a significant temperature drop of $0.5 \pm 0.3^\circ\text{C}$ during huddling. In case of egg loss, body temperature continued to decrease to $35.5 \pm 0.4^\circ\text{C}$, with a further 0.9°C decrease during huddling. By contrast, a constant core temperature of $36.9 \pm 0.2^\circ\text{C}$ was maintained during successful incubation, even during huddling, suggesting a trade-off between the demands for successful egg incubation and energy saving. However, such a limited drop in body temperature cannot explain the observed energy savings of breeding emperor penguins. Furthermore, we never observed any signs of hyperthermia in huddling birds that were exposed to ambient temperatures as high as above 35°C. We suggest that the energy savings of huddling birds is due to a metabolic depression, the extent of which depends on a reduction of body surface areas exposed to cold.

huddling; energy saving; hypothermia; emperor penguins; Antarctica

EMPEROR PENGUINS (*Aptenodytes forsteri*) breed in the middle of the Antarctic winter. Only the males take on the incubation duty and have to endure a fast of 4 mo (20, 33). With ambient temperatures below their thermoneutral zone (from -10°C to $+20^\circ\text{C}$; 24, 30), energetically costly heat production mechanisms have to be activated, so that a body temperature around 37.5°C to 38.5°C can be maintained (3, 24, 30). To save energy during their long fast, emperor penguins huddle together. It has

been shown that free-ranging males maintained a mass-specific field metabolic rate (sFMR) that was lower by 16% than that of males facing the same climatic conditions but being prevented from huddling effectively (1). Furthermore, sFMR in these free-ranging birds was also 25% lower than that of captive birds maintained within their thermoneutral zone (1, 24, 30).

Many avian and mammalian species use hibernation and torpor, during which deep body temperatures are reduced, to endure unfavorable periods (2, 10, 18, 25, 36). Could it be that a similar mechanism is, at least in part, responsible for the energy savings observed in freely breeding emperor penguins? Clearly, maintaining a lower core temperature during huddling periods could contribute to the reduction in metabolic rate observed in huddling penguins. Previous data (33) show that rectal temperatures of free-ranging huddling emperor penguins were on average 2.2°C lower than in birds isolated from the colony and therefore unable to huddle (35.7°C vs. 37.9°C , respectively). However, a sustained drop in body temperature would be in direct conflict with the requirements for successful egg incubation, as it has been demonstrated for other bird species (7, 40).

Hence, energy savings accrued from huddling might rely on mechanisms other than entering hypothermia. In particular, birds might save energy by reducing heat loss to the environment by minimizing body surface areas exposed to cold (5, 6, 41) and by being exposed to warmer temperatures within huddles (21, 23). In fact, in an earlier study, we found that during their breeding cycle males spent about 38% of their time exposed to temperatures above 0°C and huddled in discontinuous episodes that lasted 1.6 ± 1.7 h on average (11). However, males also spent about 13% of their time at ambient temperatures above 20°C , that is, above their upper critical temperature. Surface temperature of the back feathers rose rapidly when birds began to huddle, reaching 37.5°C during the pairing period (11). Hence, such elevated temperatures in huddles could potentially enhance heat stress.

Building on earlier studies (3, 14, 24, 30–34), our investigation reports the first recordings of deep body temperatures in free-ranging emperor penguins throughout their breeding cycle, using long-term implanted data loggers. First, we wanted to assess whether male emperor penguins lower their deep

Address for reprint requests and other correspondence: C. Gilbert, Institut Pluridisciplinaire Hubert Curien, Département Ecologie, Physiologie et Ethologie, UMR 7178, Centre National de la Recherche Scientifique and Université Louis Pasteur, 23, rue Becquerel, 67087 Strasbourg Cedex 02, France (e-mail: caroline.gilbert@c-strasbourg.fr).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

body temperature during breeding. Second, using external devices during pairing and visual observations of implanted males during incubation, we aimed to study deep body temperature variations during huddling. The purpose of our study was to gain new insights into the energy-saving mechanisms of huddling emperor penguins.

MATERIALS AND METHODS

The study was conducted between April and August 2001 at the emperor penguin colony of Pointe Géologie, near the French station of Dumont d'Urville, Adélie Land, Antarctica (66°40'S, 140°01'E). A meteorological station (Météo France), situated 500 m away from the colony, provided data for wind speed and temperature. Light durations for the colony site were downloaded from http://aa.usno.navy.mil/data/docs/RS_OneYear.html#formb. Between April and August, the day length varied from 2 h to 11.5 h.

Instruments and Deployment Protocol

In the middle of the pairing period, between April 22 and May 5, 2001, five pairs of emperor penguins were captured and instrumented as outlined below. We selected pairs in which the males had sufficient body fuel reserves that would ensure success in their incubating task, that is, a body mass ≥ 35 kg. After instrumentation, both mates were individually marked with colored strips (Tesa Tape, Charlotte, NC) and released together.

Data loggers (Mk7, Wildlife Computers, Redmond, WA), which enabled us to record internal temperatures with three probes (range: -40°C to $+60^{\circ}\text{C}$; resolution 0.05°C , accuracy 0.2°C), were surgically implanted in five males. Mk7 loggers were calibrated before and after deployment against a reference thermometer immersed in a thermostatic bath (temperature range: 20°C to 40°C in 5°C increments, accuracy of calibration: $0.1 \pm 0.1^{\circ}\text{C}$). Mk7 loggers weighed 36 g and measured $9 \times 2.4 \times 1.2$ cm (with 15- and 27-cm-long temperature probes, and an additional sensor embedded in the logger housing). To reduce any inflammatory reaction, the loggers were coated with wax and biomedical silicone (Silastic, Dow Corning, Midland, MI). Surgery was performed under general gaseous anesthesia with isoflurane (ForeneND; maintained between 1.5 and 2.5% in oxygen) and under strict conditions of sterility. Still, to avoid post-surgical infection or pain, antibiotics (oxytetracycline, Terramycine LAND) and anti-inflammatory (ketoprofen, KetofenND) were injected at the end of the surgery. The data loggers were implanted into the subcutaneous fat tissue in the lateral-abdominal region, so that the temperature probe within the logger housing recorded peripheral temperature. Another temperature probe was placed under the skin in the pectoral region to record subcutaneous temperature. The third temperature probe was inserted into the abdominal cavity, recording deep body temperature. Temperatures were recorded every 10 s, for about 2.5 mo.

To monitor their return to the colony, females were equipped with an Argos-VHF device (Sirtrack, Havelock North, New Zealand; 242 g, $13 \times 5 \times 3$ cm) glued to the back feathers. A Time Depth Recorder (TDR; Mk7, Wildlife Computers, Redmond, WA; 36 g, $9 \times 2.4 \times 1.2$ cm) was fixed above the Argos-VHF device, recording external temperature (from $+17^{\circ}\text{C}$ to $+42^{\circ}\text{C}$; resolution 0.05°C , accuracy 0.1°C), light (range 0 to 252 AU), and depth (resolution 0.5 m) every 10 s. The internal clocks of all loggers were synchronized at Greenwich Mean Time. Results from the TDR study are reported elsewhere (11).

Instrumented males and females were observed daily in the colony using binoculars, at a distance of at least 10 to 20 m. To minimize any potential stress effect (35), care was taken to walk slowly around the group of breeders. Females laid one egg between May 16 and 23, which corresponded well with the average egg-laying date (33). *Pair 4* split after instrumentation. All instruments were removed from

female 4 on May 15. *Male 4*, however, mated with another female, which laid an egg on June 9. All males began incubation after the females' departure. *Male 2* lost his egg accidentally around June 10, after a harsh, two-day blizzard but remained within the colony until July 10. Females foraged on average for 72 days, which corresponds to a conventional foraging trip length of about 75 days (33). They were recaptured just before their arrival in the colony to remove the logger, at the end of July. Females were released immediately after logger removal, and the exchange of the chick which took between 2 and 3 h, as usual (33), was observed. Males were recaptured just after the chick exchange, between July 10 and August 12. They were anesthetized to remove their internal data logger, and the correct positions of the temperature probes were visually confirmed. Males and females were weighed (Salter, Salter Brecknell Weighing, Fairmont, MN, Model 235 6M, 50 kg) before instrumentation and after logger removal. Breeding success of the entire colony during 2001 was low at 42%. Out of the five pairs studied, three were feeding their chicks until October 2001. Any impact of implanted loggers in our study must have been minor, similar to the conclusions of another study using long-term implanted data loggers (12). The experimental pairs in our study indeed resumed the expected breeding cycle, and their breeding success was similar to noninstrumented pairs.

All surgical procedures and experimentation were conducted by a trained veterinarian. Our study was approved by the Ethical Committee of the French Polar Institute (IPEV) and by the Scientific Committee of the IPEV, following the Scientific Committee for Antarctic Research code of conduct.

Data Analysis and Statistics

We use the term "breeding cycle" to describe the two following periods: the pairing period (when both males and females are in the colony) and the incubation period (when only males stay in the colony and females forage at sea).

To analyze core (i.e., deep body) temperature profiles during the breeding cycle (from the middle of the pairing period throughout the egg exchange to near the end of their incubation task), temperature data were averaged over hourly means. For the pairing period, linear regressions of hourly mean temperatures for each male were performed over the first 10 days of the data set, and then over the entire data set. Spearman rank correlations were performed between the estimated decrease in core temperature over the first 10 days of pairing, the males' initial body mass, and their core temperature on the first day of data. For the incubation period, linear regressions were performed for each male, except for *male 2*, taking into account 37 days. A Wilcoxon test was used to compare core temperatures of *male 2*, the unsuccessful breeder, before its egg loss and thereafter, over a period of 13 days for each condition ($n = 13$, considering daily means). Linear regressions were realized after normality and equality of variances had been tested. Analysis of body temperature was performed through Clock Lab software (Matlab 6.5, Mathworks, Natick, MA) on hourly means throughout the breeding cycle, and possible circadian periodicity was assessed through a Fourier analysis on a 10-day-period during both pairing and incubation periods.

Unfortunately, it was not possible to observe all egg exchanges, so we used a range of 2 days to define this period. All birds were observed in pairs before the beginning of this 2-day period, while only males incubating their egg alone were observed afterward. Within this 2-day period, we calculated an initial, maximum, and final mean core temperature using 6-h means. To test for differences between these three mean core temperatures, we used a Friedman ANOVA with repeated measures and Tukey's test for post hoc comparisons.

We analyzed changes in deep body temperature of males with respect to their huddling behavior.

1) During pairing, the beginning and the end of a huddle were identified from light recordings. A light record of zero indicated a huddling bout, when the bird's back was entirely covered by another

bird situated behind it. Night-time light records averaged 60 (AU), while day-time light records reached 120. Records of zero safely identified periods of huddling (11). Additional information about the density of huddles was provided by temperature sensors, as surface temperature increased when birds were packed closer to each other. Huddling patterns were classified into two categories: "tight huddles," in which surface temperature (T_s) rose exponentially to above 20°C, and "huddles," in which T_s never rose to 20°C. We chose a threshold of 20°C to define tight huddling bouts, because this is the upper critical temperature of emperor penguins (24, 30). External data loggers were attached to females only. However, because males typically initiate all movements within a pair (22, 33), and both mates huddle strictly side by side, we could also deduce information about the behavior of males from these recordings. Indeed, we observed four of the studied pairs entering a huddling group on 44 occasions, which represented about 9 h of continuous observations. In all cases, they were huddling side by side. Temperature data from the pairing period was divided into four categories: "tight huddling" (light = 0, $T_s \geq 20^\circ\text{C}$), "huddling" (light = 0, $T_s < 20^\circ\text{C}$), "nonhuddling at day" (light \neq 0, with sun of more than 6° above the horizon), and "nonhuddling at night" (light \neq 0). Friedman ANOVA with repeated measures and Tukey's test for post hoc comparisons were used to compare mean subcutaneous and core temperatures for these four categories.

2) After the females' departure, behavioral observations of the incubating males in the colony were used to define the following two conditions: when males stood isolated or in dispersed groups, they were considered to be "nonhuddling," whereas they were "huddling" when observed inside a huddle (when covered by at least 10 rows of animals). Behavioral observations of the five males during the incubation period amounted to 255 h in total. The observer located the studied males by identifying the colored mark on their backs using binoculars. Disturbance was minimized by maintaining a distance of at least 10 m away from the group of 2,500 breeders. Hence, when analyzing the body temperature data for the five males during incubation, we distinguished two periods, corresponding to the period when birds were observed "huddling" and "nonhuddling". To compare mean subcutaneous and core temperatures for these two categories, we used a Wilcoxon test for each individual.

All means are given \pm SD, and statistical significance was accepted at $P < 0.05$. All statistical tests were performed using SigmaStat, version 2.03 (SYSTAT Software, Point Richmond, CA) and StatView, version 5.0.1. (SAS Software, Cary, NC).

RESULTS

General Results

Males showed a conventional weight loss of 10.5 ± 0.6 kg during their fast, from 37.6 ± 2.4 kg at the beginning to 27.1 ± 2.4 kg at the end of their fast ($n = 5$). Subcutaneous, peripheral, and core temperatures were recorded for up to 75 days. In the case of *male 1*, the subcutaneous temperature records stopped just after its egg exchange, and these data were excluded from the analyses. External temperatures and light measurements recorded with instruments attached to females were analyzed from the day of instrumentation until their departure from the colony (period covered: April 22 to May 22). Behavioral observations of the five males during the incubation period amounted to 175 h for "nonhuddling" episodes and 80 h for "huddling" episodes.

Mean core temperature recorded for the five males throughout their breeding cycle was $36.7 \pm 0.3^\circ\text{C}$ ($n = 5$). Subcutaneous and peripheral temperatures were $35.4 \pm 0.4^\circ\text{C}$ ($n = 4$) and $36.0 \pm 0.6^\circ\text{C}$ ($n = 5$), respectively. Supported also by our visual observations during data logger removal, these three

different temperatures suggest that the probes indeed recorded thermal core, peripheral, and subcutaneous temperatures. We chose to focus only on the analysis of the recordings from core and subcutaneous probes, which represent the core and outer shell temperatures, respectively.

Core Temperature Profiles During the Breeding Cycle

Profiles of the core temperature for the five males, reflecting the effective heat production and temperature maintenance of the body, are presented in Fig. 1, A–E. These profiles can be split into three periods, which correspond to the different key episodes of their breeding cycle: the pairing period, the egg exchange, and the incubation period.

Pairing period. During the pairing period, each male showed a decrease in its core temperature (Fig. 1, A–E). The mean core temperature on the first day of instrumentation (i.e., midpairing) for the 5 males was of $37.5 \pm 0.4^\circ\text{C}$, while it was $36.5 \pm 0.3^\circ\text{C}$ on the last day of their pairing period (Table 1). Hence, core temperature decreased on average by 1°C during this period. The mean estimated decrease during the first 10 days of pairing (since instrumentation) was $0.08 \pm 0.05^\circ\text{C}/\text{day}$, while temperature decreased by $0.06 \pm 0.03^\circ\text{C}/\text{day}$ when considering the entire studied pairing period (Table 1). Hence, core temperature stabilized toward the end of the pairing period in all males. The measured core temperature at this point was $0.7 \pm 0.5^\circ\text{C}$ higher than the estimated core temperature calculated with the mean decrease during the first 10 days ($0.08^\circ\text{C}/\text{day}$; $35.8 \pm 0.7^\circ\text{C}$) (Table 1). The scope of the temperature decline varied between individuals, so that the estimated decrease over the first 10 days ranged from 0.03 to $0.14^\circ\text{C}/\text{day}$ (Table 1). Body mass was not correlated with this estimated temperature decrease ($r < 0.0001$, $P = 1$). However, core temperature on the first day of pairing was positively correlated with the estimated temperature decrease in most individuals ($r = 0.9$, $P = 0.08$). The average date of arrival for male and female emperor penguins at the Pointe Géologie colony in 2001 peaked around April 5. The estimated temperature decrease over the entire period studied ranged from 0.03 to $0.09^\circ\text{C}/\text{day}$. Consequently, the estimated temperature decrease over the course of a 45-day period would be $2.7 \pm 1.3^\circ\text{C}$ on average, ranging from 1.3 to 3.9°C (Table 1). Assuming a linear temperature decrease, temperature at the point of arrival in the colony should have been $\sim 39.2^\circ\text{C}$. However, actual temperature at arrival was likely lower than this estimate. Core temperature recorded on the first day (pairing period) was often positively correlated with the following temperature decline. Hence, individuals may adjust their temperature decrease depending on their initial core temperature. At the end of the pairing period, mean core temperature stabilized at around 36.5°C .

Egg exchange. During the period of egg exchange, the mean initial, maximum, and final core temperatures for the 5 birds were $36.5 \pm 0.6^\circ\text{C}$, $37.7 \pm 0.2^\circ\text{C}$, and $36.6 \pm 0.3^\circ\text{C}$, respectively. Maximum core temperature was significantly higher than initial core temperature (by $1.2 \pm 0.7^\circ\text{C}$; $F_{2,14} = 7.6$, $P = 0.02$; Tukey's post hoc test, $P < 0.05$). Clearly, egg exchange is a very active phase for a pair, in which the pair keeps displaying and singing for hours at a time (20, 22, 33).

Incubation period. We will now consider two cases separately: the four males that were successful breeders (Fig. 1, A,

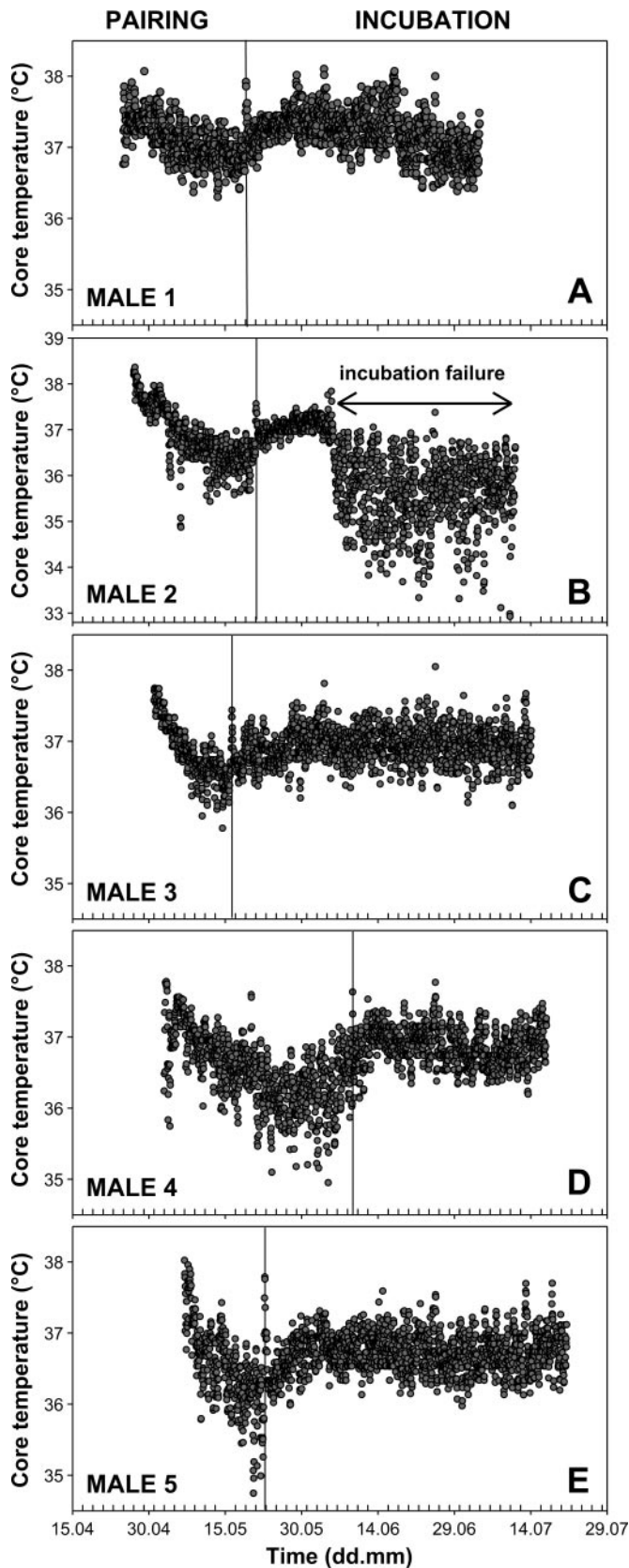


Fig. 1. Core temperature profiles during the breeding cycle of the five implanted males. (Vertical black line denotes egg exchange.)

C–E), and male 2, which lost its egg after 20 days of incubation (Fig. 1B). The core temperature of the four successful breeders remained roughly constant throughout incubation, averaging $36.9 \pm 0.2^\circ\text{C}$ (Fig. 2). The slopes of the linear regressions of core temperature against time for these four males illustrate that core temperature decline during this period was small (mean: $0.004 \pm 0.001^\circ\text{C}/\text{day}$) and significantly different from the greater temperature decrease during pairing ($F_{1,1} = 600.0$, $P < 0.001$, $n = 4,760$). Hence, average temperature decline during the entire 65 days of incubation was 0.2°C .

Before male 2 lost its egg, its core temperature profile was the same as in the other four males, stabilizing at a core temperature of $37.0 \pm 0.2^\circ\text{C}$ (Fig. 1B). After egg loss, its core temperature dropped significantly to $35.5 \pm 0.4^\circ\text{C}$ [Wilcoxon (W) = 91, $P < 0.001$; range: 32.8°C to 37.4°C]. This is in contrast to the successful breeders that maintained a core temperature of about 37°C throughout incubation. Hence, the maintenance of a stable and high core temperature ($\sim 37^\circ\text{C}$) might be a prerequisite for a successful incubation.

No circadian periodicity could be detected for body temperature either during the pairing period or during the incubation period. Some ultradian periodicities of about 6 to 7 h were found, but they were not related to day length variation or to the huddling position of the bird. As illustrated in Fig. 3, core temperature throughout the breeding cycle declined during pairing. This was followed by a temperature stabilization during successful incubation. No clear circadian periodicity was detectable throughout the period studied.

Changes of Subcutaneous and Core Temperature During Huddling

Pairing period. For all birds, subcutaneous temperature was significantly higher during tight huddling than during episodes of nonhuddling during the daytime (by $0.7 \pm 0.2^\circ\text{C}$; post hoc test, $P < 0.05$; Table 2 and Fig. 4, top).

In contrast, core temperature during “tight huddling” was significantly lower than during nonhuddling episodes in the daytime (by $0.5 \pm 0.3^\circ\text{C}$; post hoc test, $P < 0.05$; Table 2). This pattern of core temperature decrease when birds are engaged in tight huddles was similar in all birds, except for male 3, in which core temperature remained constant (Fig. 4, bottom).

Hence, huddling during the pairing period, especially tight huddling, was associated with a cooling of the thermal core, while, at the same time, the temperature of the thermal envelope increased.

Incubation period. Subcutaneous temperature during huddling ($35.5 \pm 0.6^\circ\text{C}$) was elevated when compared with nonhuddling episodes ($34.9 \pm 0.3^\circ\text{C}$). However, despite the fact that this pattern of subcutaneous temperature increase while huddling was similar for all four birds (Fig. 5, top), the $0.6 \pm 0.3^\circ\text{C}$ temperature difference was not significant (Table 2). In contrast, core temperature during huddling ($36.6 \pm 0.7^\circ\text{C}$) was decreased, albeit not significantly, by $0.3 \pm 0.4^\circ\text{C}$, when compared with nonhuddling periods ($36.9 \pm 0.4^\circ\text{C}$; Table 2). If we consider the individual responses, it appears that core temperature of the four successful breeders did not change between huddling ($36.9 \pm 0.3^\circ\text{C}$) and nonhuddling periods ($37.0 \pm 0.2^\circ\text{C}$; Table 2 and Fig. 5, bottom). In contrast, core temperature of male 2, which had lost its egg during incuba-

Table 1. *Body mass (day of instrumentation) and estimated decrease in body temperatures (T_{core}) for five breeding male emperor penguins during their pairing period*

	Initial Body Mass, kg	Estimated Decrease ($^{\circ}\text{C}/\text{day}$) in T_{core} ($n = 240$: First 10 Days of Pairing)	Estimated T_{core} ($^{\circ}\text{C}$) at the End of the PP	Measured T_{core} ($^{\circ}\text{C}$) on the First Day of the PP ($n = 24$)	Measured T_{core} ($^{\circ}\text{C}$) on the Last Day of the PP ($n = 24$)	Estimated Decrease ($^{\circ}\text{C}/\text{day}$) in T_{core} During the Entire PP
Male 1	39	0.03	36.7 (23 days)	37.3 (0.3)	36.8 (0.2)	0.03 ($n = 552$)
Male 2	36	0.14	34.9 (23 days)	38.1 (0.2)	36.3 (0.4)	0.08 ($n = 552$)
Male 3	41	0.12	35.9 (15 days)	37.6 (0.1)	36.4 (0.1)	0.08 ($n = 360$)
Male 4	35	0.04	35.7 (36 days)	37.1 (0.6)	36.7 (0.2)	0.03 ($n = 864$)
Male 5	37	0.10	35.8 (15 days)	37.5 (0.3)	36.1 (0.4)	0.09 ($n = 360$)
Grand mean ($n = 5$)		0.08 ± 0.05	35.8 ± 0.7 (22.4 \pm 8.6 days)	$37.5 (0.4)$	$36.5 (0.3)$	$0.06 (0.03)$

Estimated T_{core} ($^{\circ}\text{C}$) at the end of the pairing period (PP) was calculated using the estimated decrease ($^{\circ}\text{C}/\text{day}$) in T_{core} during the first 10 days of pairing, taking into account the duration of each pairing period (in days) for each breeding male. Values are presented as means \pm SD (in parentheses). The relationships between T_{core} , ($^{\circ}\text{C}$) and time (t , h) during the first 10 days of data were best described by Male 1: $T_{core} = 37.4 - 0.0012 \cdot t$, ($F_{1,239} = 30.0$, $P < 0.001$); Male 2: $T_{core} = 38.1 - 0.0058 \cdot t$, ($F_{1,239} = 297.4$, $P < 0.001$); Male 3: $T_{core} = 37.6 - 0.0048 \cdot t$, ($F_{1,239} = 1080.8$, $P < 0.001$); Male 4: $T_{core} = 37.2 - 0.0017 \cdot t$, ($F_{1,239} = 31.9$, $P < 0.001$); and Male 5: $T_{core} = 37.3 - 0.0042 \cdot t$, ($F_{1,239} = 137.3$, $P < 0.001$). Slopes differ between individuals ($F_{1,4} = 77.1$, $P < 0.001$, $n = 1200$). The relationships between T_{core} ($^{\circ}\text{C}$) and time (t , h) during the entire data set for pairing were best described by Male 1: $T_{core} = 37.4 - 0.0012 \cdot t$, ($F_{1,551} = 363.0$, $P < 0.001$); Male 2: $T_{core} = 37.8 - 0.0033 \cdot t$, ($F_{1,551} = 1170.8$, $P < 0.001$); Male 3: $T_{core} = 37.5 - 0.0033 \cdot t$, ($F_{1,359} = 1046.4$, $P < 0.001$); Male 4: $T_{core} = 37.1 - 0.0012 \cdot t$, ($F_{1,863} = 532.5$, $P < 0.001$); and Male 5: $T_{core} = 37.2 - 0.0036 \cdot t$, ($F_{1,359} = 336.7$, $P < 0.001$).

tion, decreased by 0.9°C during huddling ($35.4 \pm 0.8^{\circ}\text{C}$) when compared with nonhuddling episodes ($36.3 \pm 0.6^{\circ}\text{C}$; Table 2 and Fig. 5, *bottom*).

Hence, huddling during incubation was associated with the maintenance of the thermal core, unless incubation failed, and a slight temperature increase of the thermal envelope.

DISCUSSION

General Results

The breeding success of the five pairs equipped with devices was similar to that of other free-ranging pairs in the colony. This suggests that disturbance linked to instrumentation was minor. Furthermore, the breeding cycle of the equipped birds

was very similar to the average breeding cycle observed in this colony. Lastly, none of the first temperature records obtained from the birds showed any rise in body temperature after surgery, indicating that handling and surgery were well tolerated. The daily body mass loss for the five equipped males was 119 ± 13 g/day, which is similar to previous reports [124 g/day (33), 133 g/day (15), and 137 g/day (1)].

Core Temperature Profiles

We followed core temperature profiles of five males during the breeding cycle, namely, the pairing period, the egg exchange, and the incubation period, which we will consider separately in the following section.

The pairing period was characterized by a core temperature decrease of about 1°C , from 37.5°C (midpairing) to 36.5°C . The slope of this decrease appeared to be positively correlated with the initial temperature of the studied birds, recorded at midpairing. Core temperature stabilized at about 36.5°C (36.1 to 36.8°C) for all individuals at the end of their pairing period. In earlier studies (16), it was found that rectal temperatures changed during the breeding cycle and were minimal in April–May ($34.6 \pm 1.9^{\circ}\text{C}$), but these findings were not discussed by the authors. For gentoo and emperor penguins, a core temperature decrease of 0.5°C was reported in birds that were isolated from the colony but continued with their fast (8, 28). However, these studies also showed that the temperature dropped further by 1.3 to 1.5°C once birds had reached their critical body mass (37). It was suggested (8) that this temperature drop at the point when critical body mass was reached is due to the adjustment of the thermal set point to a lower temperature. Following the suggestion of Dewasmes and colleagues (8), the continuous decrease in core temperature that we recorded in breeding emperor penguins throughout their pairing period could be interpreted as a change in their defended set point. Indeed, the decrease in core temperature along the pairing period seemed to be adjusted relative to the initial core temperature, so that core temperature stabilized at the end of pairing at about 36.5°C .

Egg-laying defines the end of the pairing period. During egg exchange, core temperature of the five males increased by

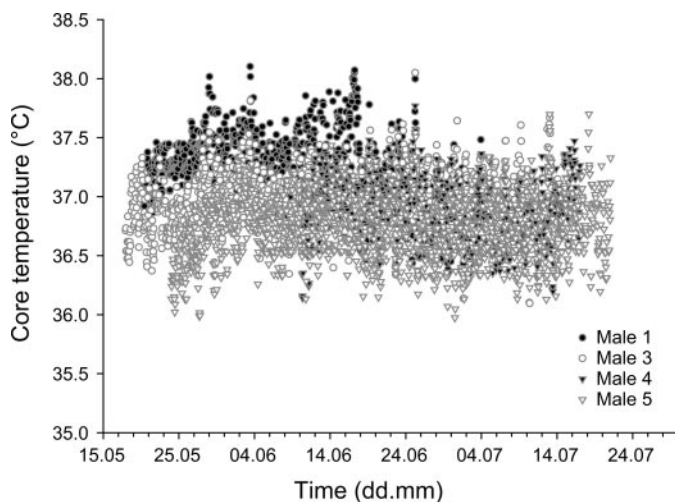


Fig. 2. Core temperature profiles for the successful breeders during incubation. The relationships between core temperature (T_{core}) and date were best described by Male 1: $T_{core} = 37.4 - 0.0002 \cdot t$ ($F_{1,889} = 33.2$, $P < 0.001$), $0.005^{\circ}\text{C}/\text{day}$; Male 3: $T_{core} = 36.9 - 0.00017 \cdot t$ ($F_{1,889} = 31.3$, $P < 0.001$), $0.004^{\circ}\text{C}/\text{day}$; Male 4: $T_{core} = 36.9 - 0.00014 \cdot t$ ($F_{1,889} = 19.8$, $P < 0.001$), $0.003^{\circ}\text{C}/\text{day}$; and Male 5: $T_{core} = 36.7 - 0.00011 \cdot t$ ($F_{1,889} = 9.5$, $P = 0.002$), $0.003^{\circ}\text{C}/\text{day}$, where T_{core} is core temperature in $^{\circ}\text{C}$ and t is time in hours. Slopes differ significantly between individuals ($F_{1,3} = 291.4$, $P < 0.001$, $n = 3,560$).

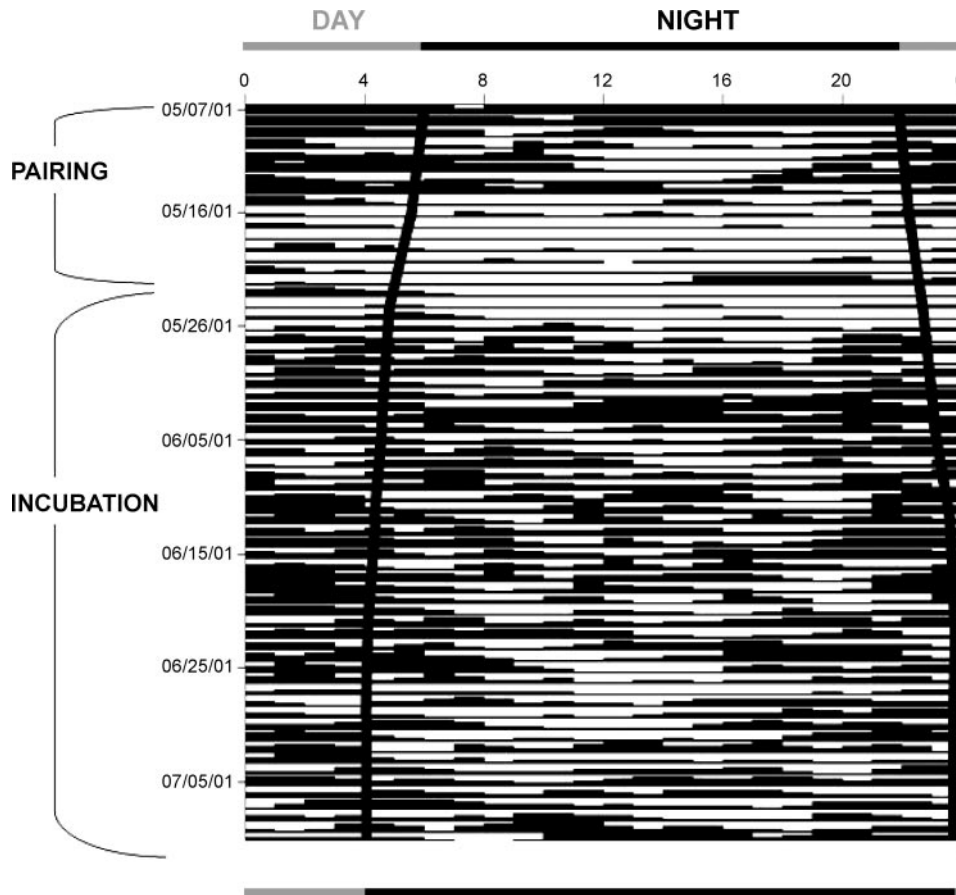


Fig. 3. Daily variations of core temperature during the breeding cycle of male 5 (schematic representation given by the Clock Lab analysis). One line corresponds to one day along the breeding period (pairing and incubation) from May 7th to July 10th. Oscillations of core temperature around the overall mean value (36.7°C) are represented in black when temperature was above the mean and in white when it was below the mean. Day length variation during the studied period is indicated by the vertical lines. A clear decrease in core temperature is visible during pairing, and high temperature values were recorded during the incubation period. No circadian rhythmicity is detectable.

1.2°C when compared with the end of the pairing period. Singing and displaying are of major importance for the pair to strengthen their bond, and they will recognize each other through their vocal signature (22). Hence, the 1.2°C increase in core temperature during egg exchange is most likely associated with the high activity level of the birds at that time.

During the subsequent incubation period, successful males maintained their core temperature at a level of 37°C. This seems consistent with the need of breeders to incubate their eggs, when temperature of the brood pouch is maintained at about 36°C (34). However, their subcutaneous temperature,

measured in the pectoral region, was only about 35°C. This is not surprising, as regional heterothermy has been demonstrated in king and emperor penguins, during both resting phases and when diving at sea (31, 32). A strikingly different picture emerged from *male 2*, which failed during incubation. While *male 2* was incubating its egg, its core temperature was maintained at 37°C. Concomitantly with the egg loss, its core temperature dropped to an average value of 35.7°C. This suggests that incubation might prevent the breeders from lowering their body temperature further, despite the need to save energy. This is consistent with results from studies on various

Table 2. Subcutaneous and core temperatures for five male emperor penguins during the pairing period and during incubation

	Nonhuddling		Huddling		
	at day	at night	<20°C	Tight huddling	
Pairing					
Subcutaneous temperature (n = 3)	35.6 (0.3)	35.7 (0.3)	35.9 (0.0)	36.4 (0.1)	$F_{3,11} = 8.46, P = 0.017$
Core temperature (n = 4)	37.0 (0.2)	36.8 (0.4)	36.7 (0.3)	36.5 (0.4)	$F_{3,15} = 10.23, P = 0.003$
Incubation					
Subcutaneous temperature (all breeders, n = 4)	34.9 (0.3)		35.5 (0.6)		$W = 10, P = 0.013$
Core temperature (all breeders, n = 5)	36.9 (0.4)		36.6 (0.7)		$W = 11, P = 0.19$
Core temperature (successful breeders, n = 4)	37.0 (0.2)		36.9 (0.3)		NA
Core temperature (unsuccessful breeder, n = 1)	36.3 (0.6)		35.4 (0.8)		NA

Values are expressed as grand means ± SD (in parentheses), which were based on individual bird means (unsuccessful breeder; nonhuddling: n = 11,726 and huddling: n = 5,754 events). Subcutaneous temperatures from *male 1* were excluded, and so were core and subcutaneous temperatures from *male 4* during the pairing period (*pair 4* split after instrumentation). W, Wilcoxon test.

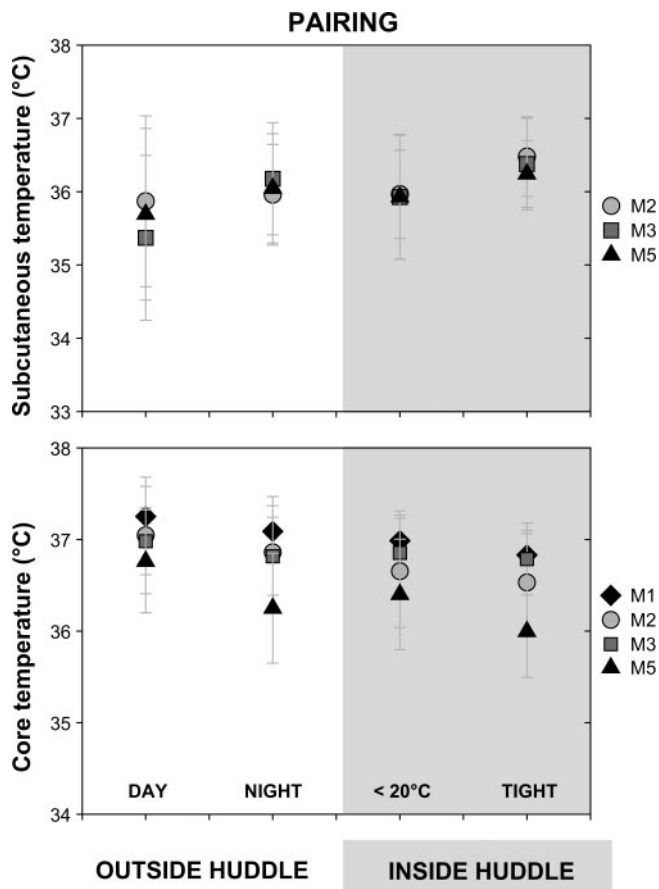


Fig. 4. Subcutaneous and core temperatures of male emperor penguins associated with nonhuddling (day and night), huddling, and tight huddling episodes during the pairing period.

bird species, which showed that incubating birds resist entering torpor or nocturnal hypothermia, unless incubation is interrupted (7, 40). A sustained drop in core temperature in an incubating adult emperor penguin would presumably delay egg development or even result in the death, or malformation, of the embryo. Hence, there is a clear trade-off between the demands associated with incubation and the need for saving energy.

Core Temperatures During the Breeding Cycle and Implications for Metabolic Rate

Our study is the first to provide long-term deep body temperature records of unrestrained breeding emperor penguins at their colony. Deep body temperatures of the five free-ranging penguins throughout their breeding cycle were on average $36.7 \pm 0.3^\circ\text{C}$. The average core temperature that we measured was lower than the stomach temperatures previously reported for captive emperor penguins during rest within their thermoneutral zone. Mean stomach temperatures in these studies ranged from 37.8°C to 38.9°C , that is, 1.2 to 2.2°C higher than our measurements (3, 24, 30). However, our temperature measurements fall well within the temperature range reported by previous authors (14, 34). Mean rectal temperatures of 36.7°C for breeding birds and 37.1°C for incubating and huddling birds were measured in these studies. Other authors (24, 28), comparing body temperature records of emperor and

gentoo penguins, argued that stomach temperature should be about 0.7°C higher than cloacal temperature. Another study (31) recorded temperatures in diving emperor penguins. Temperatures within the abdominal cavity and the inferior vena cava during diving were 37.2°C and 37.1°C , respectively. During rest at night, when birds were exposed to cold ambient temperatures below their thermoneutral zone, core temperatures ranged from 36.0 to 38.7°C for the inferior vena cava and from 34.2 to 38.4°C for the abdominal cavity, depending on the individual (31). Considering these temperature recordings, the normal core temperature for emperor penguins at rest and within their thermoneutral zone should be about 37.5°C to 38°C . This temperature range corresponds to the core temperature we recorded at midpairing (from 37.1 to 38.1°C). A core temperature reduction during the pairing period to 36.5°C and a mean core temperature of 36.7°C (when averaged over the entire breeding cycle), may therefore amount to significant energy savings during the breeding cycle of emperor penguins. Indeed, torpor and hypothermia (i.e., a decline in deep body temperature) are efficient strategies to decrease energy expenditure in birds (4, 25). Dewasmes and colleagues (8) discussed the decrease in sMR linked to a decreased body temperature for emperor penguins (four birds in their study had mean stomach temperatures ranging from 38.5°C to 40°C). They suggested a potential reduction in sMR as high as 40% for a core temperature decrease of 1°C . However, this might be an overestima-

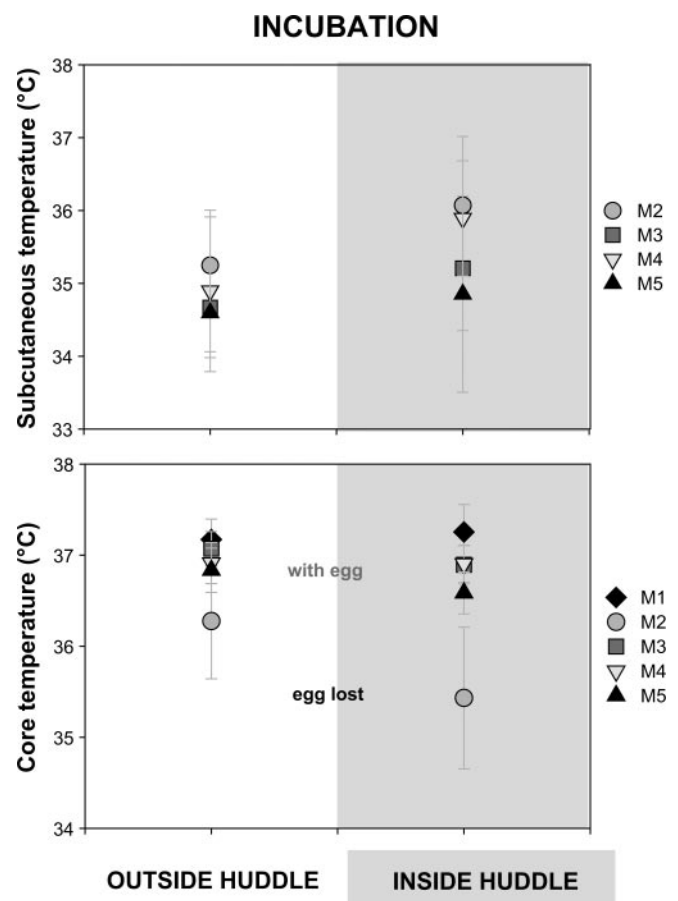


Fig. 5. Subcutaneous and core temperatures of male emperor penguins associated with episodes of nonhuddling and huddling during incubation.

tion. Let's consider a core temperature decline of 1°C (from 37.7°C to 36.7°C) and assume that the apparent Q_{10} is between 2 and 3. If average ambient temperature and conductance remain equal, then the metabolic rate of emperor penguins under these circumstances might be reduced by about 7 to 10% (4, 17).

Pioneering studies (27, 33) showed that birds isolated in small groups within the colony had rectal temperatures about 1°C higher than free-ranging birds that were able to huddle ($36.9 \pm 1.1^\circ\text{C}$ vs. $35.7 \pm 1.1^\circ\text{C}$ and $36.3 \pm 0.8^\circ\text{C}$ vs. $35.2 \pm 0.8^\circ\text{C}$, respectively; unfortunately, no indication of the incubating status of the birds was given). Furthermore, isolated individuals had a mean rectal temperature that was a further 1°C higher than in birds captive in small groups (27, 33). Besides a higher body temperature, birds isolated in small groups also had a daily body mass loss that was on average 50 g/day higher than birds that could huddle (27, 33, 34). This would imply that the energy savings accrued during huddling are at least, in part, due to a lowering of body temperature, which appears not to be possible in case of ineffective huddling. In support of this, it has been shown that huddling emperor penguins were able to lower their metabolic rate by 16% (1). From isotope-derived body composition changes, sFMR was estimated to be 1.5 W/kg and 1.8 W/kg for free-ranging males and isolated males in small groups, respectively. The question arises, why huddling birds decrease their body temperature, while birds unable to huddle maintain a higher core temperature?

Changes in Body Temperatures During Huddling

Frequent engagement in tight huddles, where ambient temperatures increase above the upper critical temperature (20°C) seems to play an important role in the energy-saving strategies of huddling emperor penguins. By contrast, these warm ambient temperatures were shown to induce hyperthermia and heat stress in emperor penguins (13, 30). When exposed to 15°C in the laboratory, their body temperature increased from 38 to 40°C within 2 h (13). At 25°C, their evaporative water loss may be nearly five times the basal rate at cold temperatures (30). By contrast, however, despite ambient temperatures up to 37.5°C, our data show that those penguins huddling tightly did not enter hyperthermia. Could then the loss of metabolic heat for a tight huddling bird take place through its cold-exposed body surfaces (i.e., the feet, the upper part of the back, the neck, and head), therefore preventing hyperthermia? This seems unlikely, for the following reasons. First, the conductive heat loss from the feet to the ground is presumably minor. The surface in contact with the ice is reduced to only several square centimeters, as penguins stand on their heels, claws, and tail. Furthermore, the cutaneous temperature of feet of incubating penguins averages only 3.3°C, because of a vascular counter-current system in their extremities (34). Second, the heat loss through head, neck, and upper back are unlikely to be efficient thermal windows for penguins to evacuate heat. Indeed, penguins huddling tightly during blizzards often accumulate snow that does not melt on their head and upper back, suggesting that heat flux through these surfaces are reduced (Fig. 6). Third, no birds were observed either panting or hyperventilating when tight huddling, which would have suggested an urgent need to dissipate heat (C. Gilbert, personal observation). Furthermore,



Fig. 6. Incubating birds huddling tightly during a blizzard. Please note the snow on their heads, necks, and upper backs.

birds inside such groups are densely packed, with up to 8–10 birds/m² (33). With a resulting high pressure inside tight huddles, birds would surely not be able to pant.

How then can this paradox be solved? We found that during huddling, the shell temperature of penguins tends to increase by 0.6 to 0.7°C, while their core temperature is either maintained during incubation or slightly decreased during pairing (by 0.5°C). Such a pattern of increased shell temperature and slightly decreased core temperature, which was also shown in other species (26, 29), could be explained by an increase in peripheral blood flow during huddling. The resulting change in thermal conductance would transfer heat from the core to the shell. In that case, the decrease in core temperature could lead to a reduction in metabolic rate. However, cold-exposed body surfaces and, therefore, heat loss are greatly reduced during tight huddling. Hence, even if core temperature could be reduced at the beginning of huddling, such a passive decrease in metabolic rate would be insufficient to prevent an increase in core temperature after hours of tight huddling. Cold-exposed body surfaces under these circumstances might be unable to dissipate enough heat.

Hence, a possible explanation for the constancy (or slight decrease) of core temperature inside dense huddles, in contrast to the expected temperature rise, is that metabolic rate of birds is depressed accordingly. Such a reduction in metabolic rate could possibly be achieved by entering sleep. Indeed, during tight huddles, we usually observed that birds had their eyes closed. It has been shown that the proportion of sleep increases during the fast of emperor penguins (9), and this is typically associated with a drop in metabolic rate of about 8% (39). Moreover, we showed that a large proportion of huddles in emperor penguins occur at night (94% of the time spent in huddles) (11). However, surprisingly, no nycthemeral rhythm in body temperature was visible in our recordings.

Following our hypothesis, a reduction in metabolic rate would induce a core temperature decrease, while the increase in shell temperature would be passive, because birds are packed together densely. If we consider the case of an isolated animal, any significant metabolic depression would lead to general body cooling because of the immense heat loss to the environment. Such a strategy would be associated with two disadvantages. First, it would not allow a successful incubation. Second, birds would have to expend large amounts of energy to rewarm. Indeed, 3.34 kJ are needed to increase the body temperature of a 1-kg mammal by 1°C (38). For an

emperor penguin with a mass of 30 kg, about 100 kJ would be needed to increase its temperature by 1°C, which represents about 3% of its total energy expenditure per day. By contrast, in a huddling bird, the surface area exposed to the cold is drastically reduced. Consequently, no dorsal, ventral, or lateral heat flow can occur in the huddling bird because neighbors are at the same temperature. There is, therefore, no risk of general body cooling for a huddling bird that lowers its metabolic rate. There is also no risk of overheating, provided that metabolic heat production is reduced to the same degree as exposure of body surfaces to cold, following Newton's law of cooling. The warm ambient temperatures that huddling generates would likely become a problem when—and only when—the birds switch from reduced to normal metabolic rate. During these transitions, changes in metabolic rate and in the exposed surface areas must occur fairly simultaneously. Because this depends on the proximity and behavior of neighboring birds, a remarkable synchrony is required between congeners. This suggests that a control of the metabolic transition should use very efficient mechanical or thermal temperature sensors, such as the spinal temperature sensors, which play a major role in bird thermoregulation (19).

In conclusion, we suggest that the reduction of cold-exposed body surfaces while huddling, and especially tight huddling, allows a downregulation of body temperature by 1°C in free-ranging birds when compared with captive animals. The maintenance of a constant core temperature of about 37°C by those birds incubating, indicates that they have a slightly higher metabolic rate than birds that failed their incubation. However, in both incubating and nonincubating birds, most of the energy sparing can be explained by a metabolic depression associated with the reduction of cold-exposed body surfaces; this depression explains why the birds neither suffer of hyperthermia despite the very high ambient temperatures generated by the tight huddles nor become hypothermic.

ACKNOWLEDGMENTS

We thank the 51st expedition at Dumont d'Urville station for technical assistance, Dr A. Malan for very useful suggestions, and Drs. S. Blanc, M. Enstipp, and G. Robertson for manuscript revision.

GRANTS

Field work was financially and logistically supported by the Institut Polaire Français Paul-Emile Victor and the Terres Australes et Antarctiques Françaises.

REFERENCES

1. Ancel A, Visser H, Handrich Y, Masman D, and Le Maho Y. Energy saving in huddling penguins. *Nature* 385: 304–305, 1997.
2. Barclay RMR, Lausen CL, and Hollis L. What's hot and what's not: defining torpor in free-ranging birds and mammals. *Can J Zool* 79: 1885–1890, 2001.
3. Boyd JC and Sladen WJL. Telemetry studies of the internal body temperatures of Adélie and emperor penguins at cape Crozier, Ross Island, Antarctica. *The Auk* 88: 366–380, 1971.
4. Butler PJ and Woakes AJ. Seasonal hypothermia in a large migrating bird: saving energy for fat deposition? *J Exp Biol* 204: 1361–1367, 2001.
5. Canals M, Rosenmann M, and Bozinovic F. Geometrical aspects of the energetic effectiveness of huddling in small mammals. *Acta Theriol* 42: 321–328, 1997.
6. Contreras LC. Bioenergetics of huddling—Test of a psycho-social hypothesis. *J Mammal* 65: 256–262, 1984.
7. Csada RD and Brigham RM. Reproduction constrains the use of daily torpor by free-ranging common poorwills (*Phalaenoptilus nuttallii*) (Aves: Caprimulgidae). *J Zool, Lond* 234: 209–216, 1994.
8. Dewasmes G, Le Maho Y, Cornet A, and Groscolas R. Resting metabolic rate and cost of locomotion in long-term fasting emperor penguins. *J Appl Physiol* 49: 888–896, 1980.
9. Dewasmes G, Buchet C, Geloën A, and Le Maho Y. Sleep changes in emperor penguins during fasting. *Am J Physiol* 25: R476–R480, 1989.
10. Geiser F. Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu Rev Physiol* 66: 239–274, 2004.
11. Gilbert C, Robertson G, Le Maho Y, Naito Y, and Ancel A. Huddling behavior in emperor penguins: Dynamics of huddling. *Physiol Behav* 88: 479–488, 2006.
12. Green A, Tanton JL, Woakes AJ, Boyd IL, and Butler P. Effects of long-term implanted data loggers on macaroni penguins *Eudyptes chrysolophus*. *J Avian Biol* 35: 370–376, 2004.
13. Groscolas R. Effet d'une exposition à la chaleur sur la température corporelle et quelques paramètres hématologiques du manchot empereur, *Aptenodytes forsteri*. *Comp Biochem Physiol* 50A: 533–543, 1975.
14. Groscolas R. Changes in body mass, body temperature and plasma fuel levels during the natural breeding fast in male and female emperor penguins *Aptenodytes forsteri*. *J Comp Physiol B* 156: 521–527, 1986.
15. Groscolas R. The use of body mass loss to estimate metabolic rate in fasting sea birds: a critical examination based on emperor penguins (*Aptenodytes forsteri*). *Comp Biochem Physiol* 90A: 361–366, 1988.
16. Guillard R and Prévost J. Observations écologiques à la colonie de manchots empereurs de Pointe Géologie (Terre Adélie) en 1963. *L'Oiseau et la RFO* 34: 33–51, 1964.
17. Heldmaier G and Ruf TP. Body temperature and metabolic rate during natural hypothermia in endotherms. *J Comp Physiol B* 162: 696–706, 1992.
18. Heldmaier G, Ortman S, and Elvert R. Natural hypometabolism during hibernation and daily torpor in mammals. *Respir Physiol Neurobiol* 141: 317–329, 2004.
19. Inomoto T and Simon E. Extracerebral deep-body cold sensitivity in the Pekin duck. *Am J Physiol* 241: R136–R145, 1981.
20. Isenmann P. Contribution à l'éthologie et à l'écologie du manchot empereur (*Aptenodytes forsteri* Gray) à la colonie de Pointe Géologie (Terre Adélie). *L'Oiseau et la RFO* 40: 136–159, 1971.
21. Jarman M. Experiments on the emperor penguin in various thermal environments. *Bull Brit Antarctic Survey* 33–34: 57–63, 1973.
22. Jouventin P. Comportement et structure sociale chez le manchot empereur. *La Terre et la Vie* 25: 510–586, 1971.
23. Kirkwood R and Robertson G. The occurrence and purpose of huddling by Emperor penguins during foraging trips. *Emu* 99: 40–45, 1999.
24. Le Maho Y, Delclitte P, and Chatonnet J. Thermoregulation in fasting emperor penguins under natural conditions. *Am J Physiol* 231: 913–922, 1976.
25. McKechnie AE and Lovegrove BG. Avian facultative hypothermic responses: a review. *Condor* 104: 705–724, 2002.
26. Mercer JB and Hammel HT. Total calorimetry and temperature regulation in the nine-banded armadillo. *Acta Physiol Scand* 135: 579–589, 1989.
27. Mougin JL. Observations écologiques à la colonie de manchots empereurs de Pointe Géologie (Terre Adélie) en 1964. *L'Oiseau et la RFO* 36: 167–226, 1966.
28. Mougin JL. Enregistrements continus de températures internes chez quelques *Spheniscidae*. I Le manchot papou *Pygoscelis papua* de l'île de la Possession (Archipel Crozet) *L'Oiseau et la RFO* 42: 84–110, 1972.
29. Ostnes JE and Bech C. Ontogeny of deep-body cold sensitivity in Pekin ducklings *Anas platyrhynchos*. *J Comp Physiol B* 167: 241–248, 1997.
30. Pinshow B, Fedak MA, Battles DR, and Schmidt-Nielsen K. Energy expenditure for thermoregulation and locomotion in emperor penguins. *Am J Physiol* 231: 903–912, 1976.
31. Ponganis PJ, Van Dam RP, Knower T, and Levenson DH. Temperature regulation in emperor penguins foraging under sea ice. *Comp Biochem Physiol* 129A: 811–820, 2001.
32. Ponganis PJ, Van Dam RP, Levenson DH, Knower T, Ponganis KV, and Marshall G. Regional heterothermy and conservation of core temperature in emperor penguins diving under sea ice. *Comp Biochem Physiol* 135A: 477–487, 2003.
33. Prévost J. *Ecologie du manchot empereur*. Expéditions polaires françaises, Hermann Press: Paris, 1961, p. 1–204.
34. Prévost J, and Sapin-Jaloustre J. A propos des premières mesures de topographie thermique chez les Sphéniscidés de la Terre Adélie. *L'Oiseau et la RFO* 34: 52–90, 1964.

35. **Regel J and Pütz K.** Effect of human disturbance on body temperature and energy expenditure in penguins. *Polar Biol* 18: 246–253, 1997.
36. **Reinertsen RE.** Physiological and ecological aspects of hypothermia. In: *Avian energetics and nutritional ecology*, edited by Carey C: Chapman and Hall, New York, 1996, p. 125–157.
37. **Robin JP, Frain M, Sardet C, Groscolas R, and Le Maho Y.** Protein and lipid utilization during long-term fasting in emperor penguins. *Am J Physiol* 254: R61–R68, 1988.
38. **Schmidt-Nielsen K.** *Animal Physiology. Adaptation and Environment* (5th ed.), New York: Cambridge University Press, 1997, p. 241–297.
39. **Stahel CD, Megirian D, and Nicol SC.** Sleep and metabolic rate in the little penguin, *Eudyptula minor*. *J Comp Physiol B* 154: 487–494, 1984.
40. **Vehrencamp SL.** Body temperature of incubating versus non-incubating roadrunners. *Condor* 84: 203–207, 1982.
41. **Vickery WL and Millar JS.** The energetics of huddling by endotherms. *Oikos* 43: 88–93, 1984.

