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Magellanic Penguin (*Spheniscus magellanicus)* Embryos Tolerate High Temperature Variations and Low Temperatures during Incubation

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Abstract.—Maintaining appropriate developmental temperatures during avian incubation is costly to the parents, so embryos may experience pronounced variations in temperature that can lead to embryo mortality and extended incubation periods, or that could affect the offspring phenotype in several bird species. The egg temperatures (N = 28 eggs) of free-living Magellanic Penguins (*Spheniscus magellanicus*) were recorded in a breeding colony in Puerto Deseado, Santa Cruz, Argentina. Three nests had atypical incubation patterns. Two nests experienced high temperature drops (average = 11.7°C, minimum = 6.5°C, duration = 9 h) and another nest had a broad daily temperature range (max-min), i.e. 13.9 ± 0.9°C for the first egg and 14.1 ± 0.8°C for the second egg (range = 8-22°C during egg laying and 18-37°C during advanced incubation). Thermal anomalies during incubation did not affect the embryonic viability, hatchling mass or fledging success. The survival of embryos despite these atypical incubation patterns may be an adaptive mechanism during the harsh weather conditions normally experienced by eggs throughout incubation. *Received 1 April 2012, accepted 16 June 2012.*

Key words.—cold tolerance, egg temperatures, embryo viability, incubation, Magellanic Penguin, reproduction, *Spheniscus magellanicus.*

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Parents have a major influence on the development of avian embryos throughout the incubation period. However, maintaining appropriate developmental temperatures throughout incubation is costly to the parents (Williams 1996). Therefore, fluctuations in parental investments during incubation may lead to pronounced variations in the incubation temperature, which may be directly harmful to the developing young (Olson *et al.* 2006). High and stable incubation temperatures are prerequisites for normal embryonic growth and maturation (Webb 1987; Deeming and Ferguson 1991; Nilsson 2006). Many studies report development defects after the heating or cooling of eggs (Webb 1987). Embryos of the Western Gull (*Larus occidentalis*) are killed after exposure to temperatures of 5-10°C for 10 h (Bennett *et al.* 1981). Moreover, <1°C differences in the incubation temperature affected the offspring phenotype of Wood Ducks (*Aix sponsa*) (DuRant 2011) while a decrease of 2°C can cause embryo mortality in fowl (*Gallus gallus)* (Romanoff *et al.* 1938). However, nest abandonment during incubation is common in some Procellariiformes (Boersma 1982), and this leads to variations in the incubation length, but does not affect embryonic viability (Boersma and Wheelwright 1979).

The aim of this study was to record individual egg temperatures of Magellanic Penguins (*Spheniscus magellanicus*) throughout the entire incubation period. We detected atypical incubation patterns that could be lethal to embryos in other avian orders. The report describes these anomalies and analyzes their impact on the embryonic viability or hatchling mass.

METHODS

Study Area

Isla Quiroga is situated 80 m offshore in Puerto Deseado, Santa Cruz Province, Argentina (47°45'S, 65°53'W), hosting a colony of approximately 1,000 breeding pairs of Magellanic Penguins.

Study Species

Magellanic Penguins lay two eggs four days apart in October (Boersma *et al.* 1990). The eggs hatch after 39-41 days of incubation (Rebstock and Boersma 2011). Males and females share the incubation duties by alternating two incubation shifts of 15 days each, which are followed by shorter shifts (2-5 days) until hatching (Boersma *et al.* 1990). The two eggs hatch two days apart in mid-November (Boersma *et al.* 1990; Frere *et al.* 1998).

Incubation Temperature

The study checked 14 nests daily during October, 2010 to determine the laying order (E1 = first egg laid, E2 = second egg laid). On the laying day, we fixed a thermochron temperature data logger (iButton DS1921G#F50; ± 0.5°C) with a small strip of medical adhesive tape to the shell surface between the poles of each egg (28 eggs). We programmed iButtons to log the temperature every 15 min, until the egg was approximately 31 days old. The hatchability of eggs was not affected by iButtons (data not shown).

We defined the temperature range as the difference between the daily maximum and minimum temperatures.

We assumed that 26°C was the physiological zero temperature for Magellanic Penguins, i.e. the temperature at which no development occurs (Weinrich and Baker 1978).

Methodological Validation

During October, 2011, we performed a trial to establish whether the recordings were affected by the position of the iButtons. As penguins rotated the eggs and we fixed the iButtons on one side of the egg, the iButtons recorded the temperature when in contact with the skin of the brood patch, as well as the temperature when the iButtons made contact with the ground. However, the embryo floats on the surface of the yolk, so the temperature at the center of the egg may be a better estimate of the temperature the embryo experiences (Beaulieu *et al.* 2010). Therefore, we selected two eggs from different nests and we attached three iButtons to each egg, with two located in opposite positions between both poles and another at the blunt end (as shown in Rebstock and Boersma 2011). Temperatures were recorded simultaneously every 5 min for three days (N = 2 eggs), which facilitated an analysis of the temperatures of eggs in contact with the ground and the brood patch at the same time.

Chick Stage

We checked the nests daily for hatching after the eggs were 35 days old. We painted the newborn chicks with waterproof markers to recognize them on subsequent visits (first chicks to hatch: right foot and wing; second chicks: left foot and wing) and we also weighed them. We checked chicks periodically until 60 days old.

We presented all of the results as the mean ± standard error and we performed statistical analyses using Statistica 7.0 (Statsoft Inc. 2004) with a significance level of P < 0.05.

RESULTS

Incubation Temperature in Typical Nests

The mean daily egg temperature increased progressively with egg age and stabilized when eggs were 17 days old at 34.2 ± 0.1°C (Fig. 1). The maximum daily egg temperatures were constant throughout the entire incubation period ($\overline{x}$ = 37.9 ± 0.1°C), although the daily minimum temperatures ($\overline{x}$ = 24.7 ± 0.2°C) were low during the first four days (average four days = 18.1 ± 0.5°C) before increasing with egg age (Pearson's correlation coefficient: r = 0.74, P < 0.01).

In the first three days after being laid, the temperature of E1 was below physiological zero (26°C), reaching 26.3°C at four days, whereas E2 was always incubated at >26°C. E1 reached 32°C at 13 days and E2 at eleven days, although both achieved stable temperatures (34.2°C) together (Fig. 1).

Deviations in the Typical Incubation Pattern

One nest had a wider daily temperature range than other typical nests (Fig. 2; Mann-Whitney U-test; typical nests *vs.* atypical nest: Z= -3.67, P < 0.001, N = 34 days). The mean daily temperature range was 13.9°C ± 0.9°C for E1 and 14.1 ± 0.8°C for E2, although the range never dropped below 8°C. The maximum temperature range was 28°C at four days for E1, whereas E2 had not been laid at that point (Fig. 2). There was no significant difference in the daily temperature ranges of E1 and E2 (Wilcoxon matched pairs test: Z = 0.62, P = 0.53, N = 30 days).

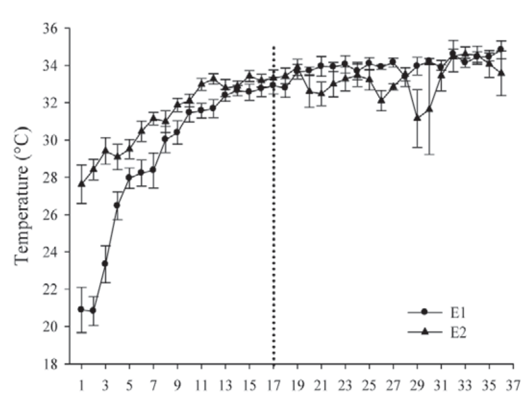

Figure 1. Daily mean temperatures of Magellanic Penguins throughout the incubation period for first (circles) and second (triangles) laid eggs. The dotted line shows the plateau for both eggs at 17 days.

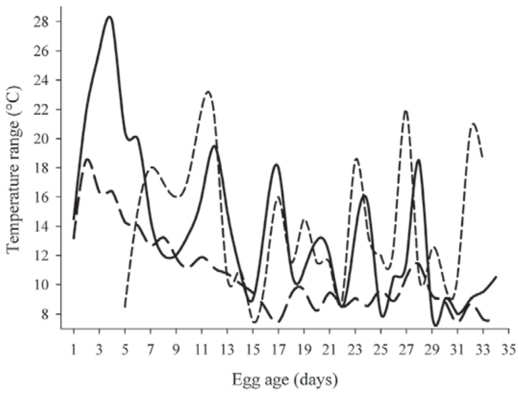

Figure 2. Temperature ranges of Magellanic Penguins throughout the incubation of first (solid line) and second (short dashed line) laid eggs in the atypical nest, and the daily average range for both eggs in all typical nests throughout the incubation period (long dashed line).

In other two nests, the incubation temperature dropped below the physiological zero for a few hours when the eggs were 25-29 days old (Table 1). The first eggs achieved higher mean temperatures than second eggs during the temperature drops (Wilcoxon matched pairs test; nest A: $Z = 2.44$, $P < 0.01$, $N = 41$ days; nest B: $Z = 4.46$, $P < 0.01$, $N = 26$ days).

Only one egg failed to hatch, which disappeared before hatching (probably due to predation), whereas all other chicks fledged successfully. The chick weights upon hatching in atypical nests ($N = 3$) were similar to those in typical nests ($N = 8$), (Mann-Whitney U-test: $Z = -1.33$, $P = 0.19$).

Methodological Validation

In both eggs, there were no significant differences between the temperature recordings made on opposites sides of the eggs at the same time (paired t-test, EGG 1: $t = 1.85$, $P = 0.065$, $N = 579$; EGG 2: $t = -0.69$, $P = 0.48$, $N = 579$). The temperature differences between both sides (|side A at time Xi - side B at time Xi|) were 3.74 ± 0.11°C for EGG 1 and 5.51 ± 0.17°C for EGG 2. For one egg, there was no significant difference between the recordings made on both sides and the blunt end (ANOVA one factor: $F_{2,1706} = 0.77$, $P = 0.46$), whereas in the other egg sides A and B differed with the blunt end being colder (paired t-test, side A: $t = -7.59$, $P < 0.001$, $N = 579$; side B: $t = -10.96$, $P < 0.001$, $N = 579$).

DISCUSSION

High and stable egg temperatures were achieved in the later stages of the incubation period. The delayed onset of full incubation is not common, although in most birds, the onset of incubation occurs before the clutch is complete (Clark and Wilson 1981). However, it has been shown in other penguins that vascularization and the area of the brood patch increases throughout the incubation period, which delays the achievement of a stable incubation temperature (Farner 1958; St. Clair 1992). The incubation pattern could also be attributable to these physiological constraints in Magellanic Penguins.

The incubation temperature trajectories of E1 and E2 were similar to those reported by Rebstock and Boersma (2011), whereas the temperatures during laying were not. If 26°C is considered to be the physiological zero, the first eggs began their development on the fourth day after being laid. In this study, it was also found that the hatching asynchrony was 1.8 days (data not shown), thus incubation onset may not be the only factor that determined hatching asynchrony.

The egg temperature ranges were mainly due to variations in the minimum temperatures because the maximum temperatures were highly constant. Hyperthermia appears

Table 1. Egg temperatures (mean and minimum) recorded during drops and their duration in two atypical nests of Magellanic Penguins.

Nest - laying order	Mean temperature (°C)	Minimum temperature (°C)	Drop duration (h)
A - E1	16.4	10.5	5.8
A - E2	11.7	6.5	9.0
B - E1	20.4	17.0	1.5
B - E2	12.8	9.0	2.0

to be more injurious to the embryo than hypothermia (Webb 1987), which suggests a reason for a maximum constant temperature. However, the maximum temperature may have been stable because this could have been the maximum temperature that the brood patch can achieve.

Variations in the typical incubation patterns may not be unusual because atypical nests represented 14% of all the nests analyzed. Magellanic Penguins have 100% attentiveness to nests from the moment they lay their eggs (Deeming 2002). Thus, these drops may have been due to inattentiveness to the eggs by adults, although they were probably at or very close to the nest, which was confirmed by the temperatures recorded in one nest where the second egg reached 6.5°C and the first egg reached 25°C.

In many species, broad temperature variations during incubation are detrimental to embryonic development. Thermal extremes can cause the immediate death of embryos (e.g. Baldwin and Kendeigh 1932), sublethal teratogenic effects (Thompson *et al.* 1976), or result in a slowdown in development (reviewed by Webb 1987). Magellanic Penguin eggs can tolerate drastic fluctuations in temperature and very low temperatures, while still managing to hatch successfully. The short duration of the drop and/or the developmental stage when the drops occurred could be important for success. Advanced embryos are less sensitive to temperature variations because they have some level of internal heat production (Calder and Booser 1973; Ar and Sidis 2002, but also see Tazawa and Rahn 1986). Cold tolerance could be an adaptive egg mechanism for coping with the harsh weather conditions that normally occur in Magellanic Penguin breeding areas during incubation.

There was also no effect of these anomalies on the hatchling mass or fledging success. There may have been an effect on the offspring phenotype that was not evaluated, because most adults maintained nest temperatures within a high, narrow range, despite the cost of incubation. Many studies show that embryos incubated at low temperatures have slower growth and lower body condition, as well as reduced immune responses (DuRant *et al.* 2010), thermoregulatory performance (DuRant *et al.* 2012), and locomotory performance (Hopkins *et al.* 2011).

The methodology used in this study was validated because the temperature difference between the two opposite sides of an egg was not significant.

The results also agreed with Rebstock and Boersma's (2011) study of typical nests, which showed that the incubation temperature stabilized later during the incubation period and lower than that of other bird species. Most importantly, this study showed that short-lived temperature drops during the advanced incubation period and broad temperature variations throughout the entire incubation period did not affect the embryonic viability, hatchling mass or fledging success. Further studies should be carried out to determine the causes of this broad thermal environment during incubation and its possible consequences for offspring phenotypes.

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