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Parental responses to unexpectedly cool eggs in Nazca boobies *Sula granti*

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Although incubation temperatures have been documented extensively in birds, few studies have followed fluctuations in temperatures throughout the length of the incubation period in natural nests. We recorded incubation temperatures of Nazca boobies *Sula granti* by replacing real booby eggs with a model egg containing an internal floating data logger for three-day intervals in 47 nests ("experimental group"). We also added the same logger eggs to 14 booby nests at the time of egg-laying, where they remained as the second egg in the clutches for the entire incubation period ("logger egg control group"). Finally, we measured surface temperatures of real eggs with an infrared sensor ("real egg control group"). In both control groups, the average temperature increased after laying, then stabilized for the remainder of the incubation period. The experimental group differed from the controls, because the cool logger egg could have been introduced at any point in the incubation cycle, not just at the beginning. Egg temperature in the experimental group had a parabolic relationship with day of incubation, because parents receiving a logger egg during the third quarter of incubation showed an exaggerated heating response during the subsequent two days. We infer from this that the parents are especially sensitive to egg temperature during this period, and it may thus represent a critical period of unknown nature for the embryo.

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Parent birds (except Megapodes) use contact incubation to transfer heat between their bodies and their eggs, using an abdominal brood patch (Lea and Klandorf 2002) or vascularized foot webs (Morgan et al. 2003). The embryo's temperature affects its development (Romanoff 1960, Lundy 1969, White and Kinney 1974, Drent 1975, Deeming and Ferguson 1991), and some argue that temperature is the most influential environmental effect on the embryo (Landauer 1967, Wilson 1991, Decuypere and Michels 1992). Heat provided by parents benefits embryos, but mounting evidence indicates that heat transfer during incubation is costly to parents (Monaghan and Nager 1997, Tinbergen and Williams 2002). Thus, egg temperature should be closely monitored and regulated by parents, to optimize the tradeoff between the embryo's developmental requirements and physiological costs to parents.

Few studies have evaluated variation in parental heat input or egg temperature across the incubation period, and even fewer have done so in natural environments. Using various methods, some studies showed no change in egg temperature with time (Webb 1987, Weathers and Sullivan 1989), while others detect an increase with time (Drent 1970, Norton 1972, Holcomb 1974, Caldwell and Cornwell 1975, Grant, et al. 1982, Webb 1987, Bergstrom 1989, Poussart et al. 2000), such as an initial increase in temperature after laying followed by a long period of constant temperature (Haftorn 1983, Morton and Pereya 1985, Webb 1987). Large initial increases in temperature were typically attributed to brood patch development while temperature increases at the end of

the cycle were thought to result from metabolic heat production by the growing embryo. As a body, these results indicate close regulation of egg temperature, and in particular, declining trends in egg temperature at any point in incubation are not typical.

Turner's (1991, 2002) models of egg heat flux suggest that the embryo's circulatory system increases heat loss from the egg during the latter part of the incubation period. While this heat loss may be partially offset by increased metabolic heat production as the embryo develops (Ackerman and Seagrave 1984, Ar and Sidis 2002), Turner's analyses with living and killed eggs indicate that the embryo's net contribution to the egg's heat content is negative. Parents may thus be required to make up the shortfall, if the embryo-driven heat loss would otherwise drop its own temperature below that which is optimal for development. Again, parental monitoring and regulation of egg temperature is expected to exist, due to the potentially complex thermal interaction of parent and embryo.

Most studies cited have recorded temperature by either inserting a thermistor or thermocouple into real or artificial eggs, but the trailing wire restricted the movement of the eggs in these cases. However, many have noted the importance of egg turning during incubation for heat flow and distribution of heat gradients within the egg (for review see Drent 1975). By limiting turning, wire-based measurement may have yielded unrealistic temperatures.

We studied egg temperature of Nazca booby *Sula granti* (American Ornithologists' Union 2000) clutches using a new technique: artificial eggs with floating internal temperature loggers. The eggs could be turned by the parent, allowing the sensor to float near the top of the egg as a normal embryo would. All of the species studied previously transfer body heat to the eggs in the usual avian manner, through a ventrally located vascularized brood patch. Birds in the family Sulidae, comprising boobies and gannets, lack a brood patch and instead cover their eggs with the webbing of their feet (Bartholomew 1966, Whittow et al. 1989, Anderson 1993, Evans 1995; shown visually in Fig. 1 and 2 in Morgan et al. 2003). Unlike most other water birds that have webbing between only three toes (Hedges and Sibley 1994), these birds have a totipalmate foot with webbing between all four toes, that transfer heat to eggs (Morgan et al. 2003). No studies to date of totipalmate species have followed foot-mediated incubation temperatures throughout the incubation cycle, although several have measured incubation temperatures on a short-term basis (Howell and Bartholomew 1962, Whittow et al. 1989, Evans 1989, 1990, 1995).

In the course of collecting data on egg temperatures for another study (Morgan 2002), we discovered an unexpected pattern of parental regulation of egg temperature in Nazca boobies. Parents were given cool artificial eggs at various points to incubate for three days. Parents receiving eggs during the third quarter of the incubation period input more heat to the eggs than did other parents, yielding a parabolic relationship between time and egg temperature. We propose two explanations for this unusual relationship: (1) the normal time course of incubation temperature (influenced by parental, embryonic, and environmental heat fluxes) is in fact parabolic, or (2) the normal time course is not parabolic, but parents exhibit a hyper-sensitivity to a cool egg during the third quarter of incubation in comparison to other quarters. In this study, we bring data from real and artificial eggs to bear on these two hypotheses. Temperature of the top-most outer surface of real eggs under normal incubation ("real egg control group" provides the natural time course of incubation temperature. Temperature of the top-most inner surface provides similar data for continuously-incubated artificial eggs ("logger egg control group"). These two control groups can be compared to our "experimental" group, in which artificial eggs at ambient temperature are introduced to a clutch at a known point in the incubation time course. If all three time courses show the same parabolic relationship, then the normal time course of incubation is apparently parabolic. However, if the two controls show a similar non-parabolic time course, and the experimental eggs show a parabolic relationship, then the hypothesis of time-dependent sensitivity to egg temperature is supported. This study provides the first data for incubation temperatures recorded in the nest with a completely mobile sensor within an artificial egg, and the first data for incubation temperatures of Nazca boobies.

Methods

We studied Nazca boobies nesting on Española Island, Galápagos, Ecuador (1°20′S, 89°40′W; see Anderson and Ricklefs 1987), recording temperatures of real eggs or "logger" eggs made from plastic Easter eggs, size-matched to natural Nazca booby eggs. Each logger egg contained a small StowAway TidbiT temperature data logger (Onset Computer Corporation, Bourne, MA) with an effective range of $-20°C$ to $+50°C$. All loggers were calibrated against a National Institute of Standards and Technology (NIST)-certified platinum thermometer.

The logger's thermistor projects from the circular logger. Small styrofoam floats on the sides of the logger kept it buoyant in the water-filled egg, and brass pellets opposite the thermistor kept the thermistor oriented against the top-most inner surface of the egg. The thermistor's position thus mimicked that of the early embryo on top of the yolk in an actual egg. Logger eggs were sealed with silicone gel and coated with white gesso

paint to resemble a natural booby egg in color and texture.

Loggers recorded temperature every 2.5 or 3 min (Logger Egg Controls and Experimentals, respectively). After removal from the nest, eggs were opened and data from the loggers were downloaded to a computer using Boxcar Pro interface software (Onset Computer Corporation, Bourne, MA). Two eggs were opened before the end of the study to confirm that they were recording data. After data were downloaded, we discarded the first hour of data for each trial to exclude the initial warming of the egg to normal incubation temperatures and calculated the mean hourly temperature for each of the trials.

Experimental group

Nazca boobies incubate each egg of their 1–2 egg clutches for 38–49 days total, with hatching normally taking place between days 42–44 (Anderson 1993). For the experimental group, studied during the 1999–2000 breeding season, this incubation period was divided into four incubation classes: Class A clutches, 0–10 days old; Class B clutches, 11–20 days old; Class C clutches, 21–30 days old, and Class D clutches, 31 days or older. For each "trial" using four logger eggs, we selected a one-egg clutch from each incubation category and replaced the real egg with a cool (ambient temperature) logger egg. Each trial lasted 72 hours (Fig. 1). Real eggs were temporarily cross-fostered into other nests during the trial. Nests with logger eggs were checked daily to ensure that they were being incubated. After 72 hours, logger eggs were removed, real eggs were returned to their original nests, and four other clutches were selected for the subsequent trial. This procedure was repeated for 13 trials and a total of 47 nests. Five trials did not have nests in every incubation class due to a shortage of clutches in some age classes or due to abandonment of clutches during a trial.

Differences between trials, incubation classes, and the presence of autocorrelation within the recorded egg temperatures were analyzed using the following model in the general linear model procedure in SAS (SAS Institute, Inc., Cary, NC):

$$Y_{tch} = \mu + \alpha_t + \beta_c + \gamma_h + \beta \times Y_{tch^{-1}} + \varepsilon \quad (1)$$

Where: Y = temperature
t = incubation class (A–D)
C = cohort
H = hour
$Y_{tch^{-1}}$ = temperature recorded in previous hour

We used hourly mean temperatures for the 48 hrs following the first midnight of the trial (Fig. 1). The 1999–2000 field season was unusual in that few boobies in the study colony laid eggs and even fewer incubated their eggs for more than a few weeks. As a result, some clutches were used in more than one trial and in more than one incubation class (we did not use any clutch more than once in a single incubation class). Because we used clutches more than once, we performed the general linear model analysis twice. We first analyzed data including repeated nests and then analyzed data not including repeated nests (the second data set contains fewer individuals in each class and fewer trials).

In both analyses, autocorrelation in egg temperature was detected between one hour and the next ($P < 0.001$ in both cases, Tables 1 and 2). Consequently, and because of the imbalanced nature of the data (unequal

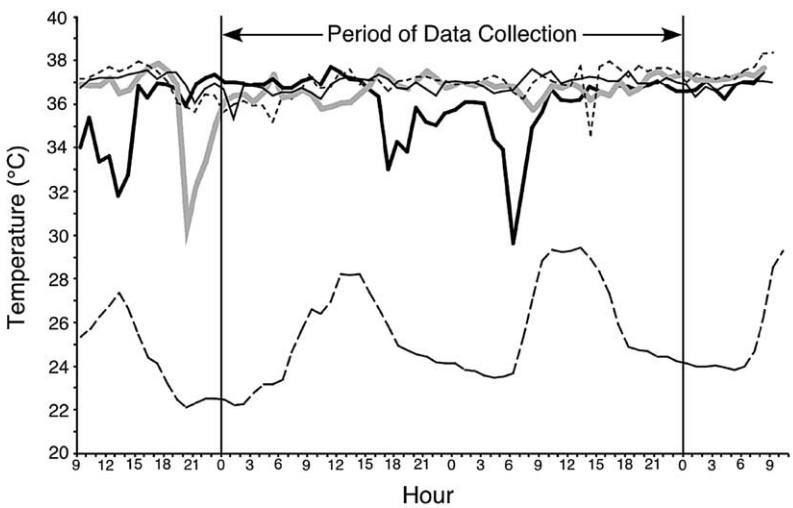

Fig. 1. Incubation and ambient temperatures through a 72-hour incubation cycle for one trial of the experimental group. Ambient temperature (represented by the thin black dashed curve at bottom of figure) cycles throughout the 72 hours, while logger egg temperatures in each of the four incubation classes (representative traces for: Class A, thick black solid curve; Class B, thick gray solid curve; Class C, gray dashed curve at top, and Class D, thin black solid curve) remained centered around 36.7°C. Peaks and drops in temperatures recorded in the artificial eggs are probably due to movement by the incubating parent (Morgan et al. 2003).

Table 1. Results from general linear model, Type III sums of squares analysis, using repeated nests from the experimental group.

Variable	df	SS	MS	F-value	P
Class	3	23.27	7.76	23.10	<0.0001
Trial	12	6.92	0.58	1.72	0.0571
Time	46	25.14	0.55	1.63	0.0051
Previous temperature	1	789.64	789.64	2351.22	<0.0001

numbers of nests in each trial or each incubation class), we used Type III sums of squares values for our analyses.

We also calculated the mean for each day of incubation using all of the data from all trials. We fitted both exponential and second-order polynomial regressions to the data using Sigmaplot software (SPSS Inc., Chicago, USA).

Logger egg control group

We located newly laid eggs in the 2000–01 breeding season during daily searches of the breeding colony. Logger eggs were placed as second eggs in 14 nests with newly laid eggs and remained in the nest until the first egg hatched or until the first egg reached 45–49 days old, much past the typical age of hatching. If a second egg was laid after the logger egg was introduced, that egg was fostered into another nest for the remainder of its incubation.

We calculated the mean temperature for each day of the incubation cycle. We fitted both exponential and second-order polynomial regressions to the data by using Sigmaplot.

The key difference between the experimental and the logger egg control groups is the time at which birds received a cool logger egg. The logger egg control group received a logger egg cooler than their normal incubation temperature at the beginning of their incubation cycle; the experimental group could have received a cool logger egg at any point in the incubation cycle.

Real egg control group

During the 2002–03 breeding season, we measured the external surface temperature of one known-age egg in each of 212 clutches with an infrared emission sensor (Raynger ST20 Pro standard, Raytek Corp., Santa Cruz, CA) on 6 November between 06.25–08.30 h. We gently displaced each incubating bird from its clutch and measured the temperature of a 1 cm diameter circle of the top-most egg surface within 10 s. We then waited 10 s and took a second measurement in the same manner. We then allowed the parent to return to its clutch. We used a paired t test to determine whether the serial measurements differed, and calculated the Spearman r correlation coefficient of the two measurements to assess repeatability. We used an ANCOVA to determine whether the first temperature measurement was related to time of day (covariate) or several categorical variables (incubator sex, clutch size, and incubation class).

During all three data collection periods, a bare logger was suspended in the shade in the middle of the study colony to record ambient temperature every three minutes; mean temperatures were calculated by hour.

Results

Experimental group

In all nest classes and for all trials of both logger egg control and experimental groups, logger egg temperature always exceeded ambient temperature; mean internal logger egg temperature was 36.7°C, and remained relatively steady (S.E.M. = 0.08), while the ambient temperature cycled between 22°C and 35°C over each 24-hour period (Fig. 1). Examination of the mean values for experimental logger egg temperature revealed differences among nest classes. The mean temperature of all class C clutches (21–30 days old) was consistently higher over the 24-hour cycle than those of the other incubation classes (Fig. 2) and class D clutch (31 days or older) temperatures were higher than class A (1–10 days old) and class B (11–20 days old) temperatures most of the time. The results of the general linear model analysis using repeated clutches (Table 1) indicated a significant effect of class on incubation temperature ($P < 0.001$), supporting our conclusion that experimental class C

Table 2. Results from general linear model, Type III sums of squares analysis, using only non-repeated nests from the experimental group.

Variable	df	SS	MS	F-value	P
Class	3	1.65	0.55	2.08	0.10
Trial	10	3.20	0.32	1.21	0.28
Time	46	18.06	0.39	1.48	0.02
Previous temperature	1	336.27	336.27	1268.03	<0.0001

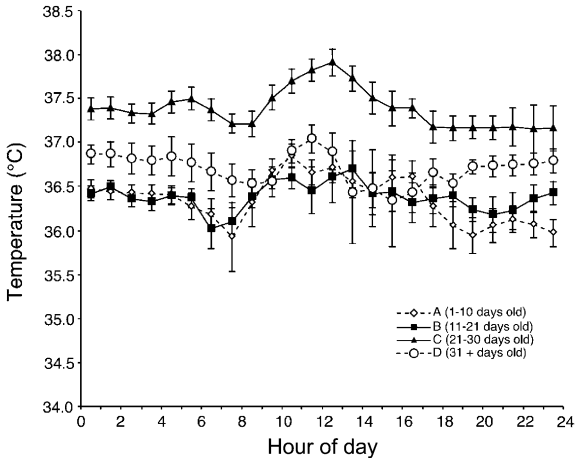

Fig. 2. Mean internal egg temperature for all clutches over a 24-hour period for each incubation class in the experimental group. Significant variation in incubation temperature exists between class C and the remaining three classes of egg clutches. Class D clutches are also, at times, significantly warmer than those of class A or B, but never of class C. Error bars represent one standard error of the mean.

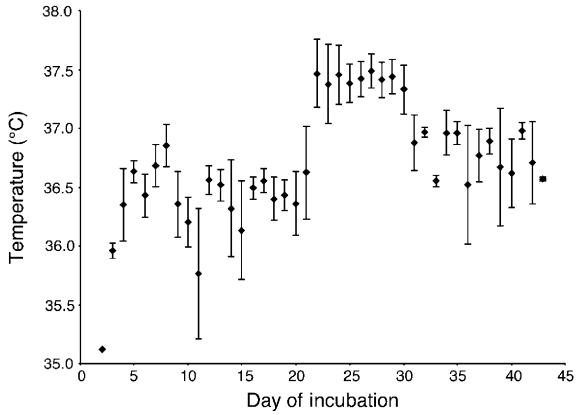

Fig. 3. Variation in mean temperature of all clutches over the entire 44 days of incubation for the experimental group. Significant variation can be seen in the mean temperature of all clutches over the entire incubation period, with significantly higher values between days 22 and 31, corresponding to class C clutches. Error bars represent one standard error of the mean. Each point represents means from 2–8 clutches.

nests were maintained at higher temperatures than other incubation classes. The same analysis of data from the much smaller number of unrepeated nests showed the same trend approaching statistical significance ($P = 0.10$; Table 2). In our remaining analyses of the experimental group, we used the entire dataset (including repeated nests) to maximize the sample size.

The general linear model also indicated a significant effect of time of day on egg temperature ($P = 0.005$), apparently due to the slight increase in egg temperatures across all classes during the middle, hottest part of the day (Fig. 2); however, no post-hoc test exists for this model to test this hypothesis.

To depict variation in incubation temperatures across the entire incubation cycle, we plotted mean temperature versus day of incubation cycle (Fig. 3). Because the data are apparently non-linear, we chose to fit them with two models: a second order polynomial function (of the form $\text{Temperature} = a + b \times \text{Day} + c \times \text{Day}^2$; where a, b, and c are constants) and a simpler exponential function (of the form $\text{Temperature} = a + b(1 - e^{-c \times \text{Day}})$, and again, a, b, and c are constants). A polynomial would be expected to best fit data that are bell-shaped or smoothly curved, whereas the exponential model would best describe data where the measured variable approaches some asymptotic value. Fig. 4A shows that a polynomial model provided a better fit to the data from the experimental group than the exponential model (compare R^2 values), indicating a maximum temperature mid-cycle, followed by a decline. The first derivative of the equation for the polynomial curve indicated a maximum at 28.6 days, within our class C incubation period.

Because many of the eggs laid during the 1999–2000 season did not hatch, we separated the nests in which eggs hatched from those in which eggs failed, either because they were abandoned or because the embryos were not viable. We repeated the curve fits with data from only those nests in which an egg eventually hatched (Fig. 4B). Again, the polynomial curve was a slightly better fit to the data than the exponential model. The maximum, at 28.5 days, was similar to that of the regression of all nests.

Logger egg control group

Since logger eggs remained in these nests for the duration of the incubation cycle, we analyzed them using only the curve fitting techniques outlined previously. Fig. 4C demonstrates that, in contrast to the experimental group, logger egg control temperatures were better fit by the exponential model than by the polynomial. Temperature rose rapidly over the first nine days of incubation, and thereafter remained relatively constant. A second difference between the experimentals and logger egg controls was that the temperatures recorded were lower in the controls (Fig. 4C). Logger egg controls were also maintained at temperatures consistently higher than ambient (maximum 35.0°C; compare Fig. 4C).

Real egg control group

All egg surface temperatures (range 31.8–37.4°C) exceeded all ambient temperatures (20.0–23.4°C) during the sampling period. The first temperature, taken 1–10 s

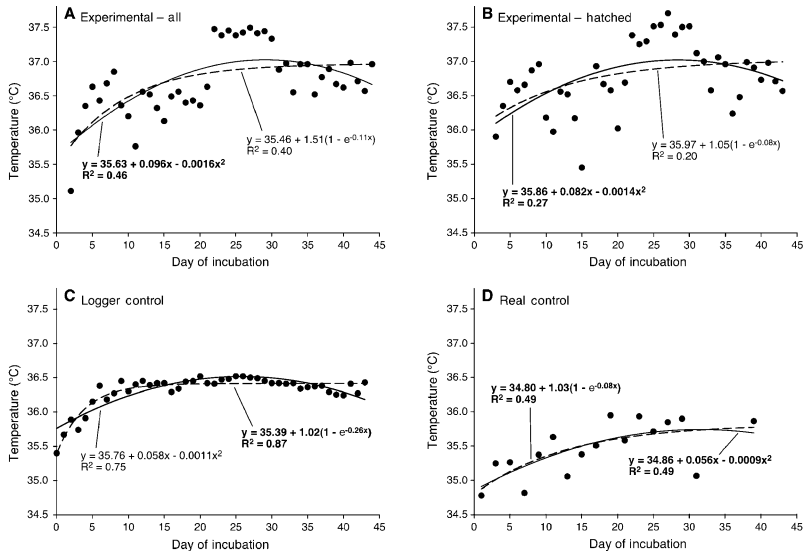

Fig. 4. Regression analyses of temperature and day of incubation for Nazca boobies. In all panels, the solid curve is a second-order polynomial fitted to the data; the dashed curve is an exponential fit. The equation that provided a better fit to the data (judged by higher R^2 value) is indicated by bold type. **A**: Regression analysis of mean temperature of all clutches and day of incubation for the experimental group. The second-order polynomial curve provides the better fit to the data; first derivative of the equation yields a maximum of 28.6 days, which falls approximately 66% of the way through the incubation cycle (within class C). **B**: Regression analysis of mean temperature of clutches with eggs that hatched and day of incubation for the experimental group. Again, the polynomial curve was the better fit to the data; the first derivative of the equation gives a maximum of 28.5 days, which is almost the same as the maximum for all clutches. **C**: Regression analysis of mean temperature of all clutches and day of incubation for the logger control group. The exponential model provided a superior fit to the data. **D**: Regression analysis of mean surface temperature of Nazca booby eggs and day of incubation. The solid curve is a second-order polynomial fitted to the data; the dashed curve is an exponential fit, and the dash-dot line is a linear fit. Both curves provided a better fit to the data (judged by higher R^2 value) than a linear regression did ($R^2 = 0.42$). The second-order polynomial and exponential curves fit the data equally well.

after displacing the parent, differed from the second, taken 10 s after the first (35.4 vs 35.3°C; paired t test, $t = 3.84$, $df = 211$, $P < 0.001$), providing an estimate of egg cooling rate. Thus, first temperatures may have underestimated true surface temperature before the parent was displaced by approximately 0.1°C. The repeatability of surface temperature was high, as judged by the correlation between the first and second temperatures (Spearman $r = 0.96$, $n = 212$, $P < 0.001$). ANCOVA of surface temperature revealed a significant effect of incubation class ($F_{3,175} = 6.48$, $P < 0.001$), but not of time of day ($F_{1,187} = 1.20$, $P = 0.27$), incubator sex ($F_{3,175} = 1.06$, $P = 0.30$), clutch size ($F_{3,175} = 0.03$, $P = 0.86$), nor any interaction (all $P > 0.10$). An exponential curve fit the egg age x temperature relationship as well as a more complex second order polynomial did (Fig. 4D, compare R^2 values).

Discussion

Our data clearly show that Nazca boobies, incubating at least in part by means of their feet, maintain the temperature of their own eggs and logger eggs at relatively constant temperatures greater than ambient (Fig. 1). Further, temperatures are not held constant throughout the incubation period, but vary in a predictable way: in both real and artificial eggs under continuous incubation, lower temperatures at the beginning of the incubation cycle are followed by higher temperatures that are sustained until the end of the period (Fig. 4C–D). In these two groups, egg temperature did not decline near the end of incubation.

The experimental group differed from the two controls in the pattern of parental heat input: experimental temperatures exhibited a discontinuity, such that they were distinctly higher during the class C period than in the adjacent days (Fig. 4A–B). In contrast, temperatures of the two control groups rose rapidly after the time of laying, and then remained approximately constant after approaching an asymptote. Additionally, temperatures recorded from experimentals were overall higher than those from logger controls, and this difference is most marked during the class C period of the incubation cycle. These results are consistent with a hypothesis of hypersensitivity of incubating Nazca

boobies to artificially low egg temperatures during the third quarter of incubation. They are not consistent with the hypothesis that the normal time course of incubation results in a parabolic relationship of temperature and time.

Our data seem to indicate that experimental parents reacted to the sudden introduction of a cool egg by producing such heat that the logger egg "overshot" the normal incubation temperature. Indeed, we showed elsewhere that when challenged to warm a cool (ambient temperature), large albatross egg, incubating Nazca boobies often shiver, indicating higher than normal heat production (Morgan et al. 2003). We suggest that the warming response of the incubating parent is highly exaggerated specifically during the C period, and we speculate that the C period may represent a critical period in the development of Nazca booby embryos, such that low temperatures cannot be tolerated during that time.

The idea of periods of particular importance, or "critical periods", during incubation is not new. Critical periods of mortality have been found in both the chicken and the turkey. Payne (1919) found an early peak of mortality around 20–30% through the incubation cycle in chickens and a second peak later at about 85–95% through the cycle. Martin and Insko (1935) found the same in their studies of both natural and artificial incubation of bronze turkey eggs. Under natural incubation, turkey eggs showed an increase in temperature from the ninth to the tenth day of incubation (approximately 35% of the way through incubation), and a corresponding reduction in growth under constant-temperature artificial incubation. The same pattern was observed between days 19 and 20 (approximately 70% of the cycle). Martin and Insko (1935) maintain that these two breaks in temperature and growth correspond to points where the embryo is switching from mostly carbohydrate metabolism to protein metabolism and from protein to fat metabolism, respectively. Romanoff (1929) found the same reduction in growth on days 9 (approximately 45% of the cycle) and 16 (approximately 76% of the cycle) in chicken embryos under artificial incubation.

Some neuromuscular and functional critical periods have also been demonstrated. Avrutina et al. (1985) found that exposing eggs to cooler temperatures between 13–19 days (61%–90% of cycle) altered the stress response of the chicks that hatched from those eggs. Oppenheim and Levin (1975) found that chick embryos temporarily exposed to temperatures 2–3°C above normal during day 15 (71% of cycle) had increased neuromuscular activity while day 15 embryos exposed to temperatures 2–3°C below normal had decreased activity. They also found that day 20 embryos (95% of cycle) had decreased activity with exposure to both the same lower and higher temperatures. Several authors have reported that slightly lowering temperature late in the incubation cycle increased hatchability. Romanoff et al. (1938) found that lower temperatures late in incubation decreased mortality in chick embryos. In fact, decreasing chick temperature during artificial incubation after 16 days by about 1.5°C produces maximum hatchability (Romanoff 1936), perhaps indicating the end of a critical period (of unknown nature) somewhere around 76% of the incubation cycle.

The initial increase in temperature shown in our data could be reflective of gradual, increased function in the feet acting as brood patches and/or increased attentiveness by the parents. Drent (1975), reviewing his work on herring gulls, showed that egg temperatures continue to increase for the first week of incubation (approximately 20% of the way into the incubation cycle) which he attributed to both parental attentiveness and possibly increased heating from the developing brood patch. On the other hand, Farner (1958) found that the brood patch of yellow-eyed penguins *Megadyptes antipodes* did not reach full incubation temperature and vascularization until 36% of the way through the incubation period, day 15 of 42. Afton (1979) attributed the increase in temperature in the first four days of incubation in the northern shoveler (approximately 20% of the cycle) to increased brood patch development, although brood patch temperatures were not significantly related to embryonic age. Finally, increased attentiveness of yellow-eyed juncos was thought to cause an increase in minimum egg temperatures as the incubation period progressed (Weathers and Sullivan 1989). The time course of vasculature development in foot webs of incubating Nazca boobies is currently unknown; attentiveness during incubation is essentially 100% (D. J. Anderson unpubl. data).

Incubation is known to be costly to parents, requiring energetic input of heat or even energetic dissipation of heat and requiring valuable time that could be used for foraging or reproduction (for review see Drent 1972). For Nazca boobies, the target incubation temperature might be closer to those recorded at our maxima, but parents may vary in their ability to maintain a high temperature for the entire incubation period. Given the tradeoff between parental incubation costs and embryonic developmental requirements, some parents may settle for increasing their input during only the most critical phase.

Our data suggest a heightened sensitivity of parents to the unexpected appearance of a cool egg during the third quarter of incubation, but we are puzzled by the fact that parents bring the egg temperature to an elevated level for a period of days. At present we have no explanation for this exaggerated response. However, if our suggestion is correct that the third quarter of incubation is in fact a critical period and departure from the target incubation temperature is heavily penalized, then the variance of egg

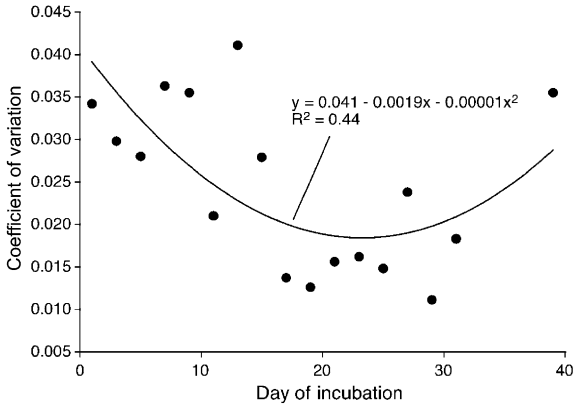

Fig. 5. Coefficient of variation of the mean recorded surface temperature of Nazca booby eggs versus day of incubation. The fitted curve is a second-order polynomial; variability is minimized during the third quarter of the incubation cycle.

temperature should be minimized during this quarter. A second-order polynomial regression of mean daily coefficient of variation from the logger control group on day of incubation cycle approached significance ($y = 0.021 - 0.00057x + 0.00001x^2$, $F_{2,38} = 2.53$, $P = 0.092$, $R^2 = 0.12$). The minimum of the curve fell in the middle of the C period (28.5 days), suggesting that our hypothesized critical period did have less variation in temperature than the rest of the incubation cycle. Temperatures from the real egg controls provided a similar result (Fig. 5; $F_{2,14} = 5.61$, $P = 0.016$). Future work on this topic should include a focus on the functional consequences for the embryo, if any, of lower mean egg temperature and higher variance in temperature during the third quarter of the incubation period.

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