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THERMAL RESPONSES OF LATE EMBRYOS AND HATCHLINGS OF THE SOOTY TERN[1]

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Abstract. Oxygen consumption ($\dot{V}O_2$) and body temperature (T_b) of young Sooty Terns (*Sterna fuscata*) were measured during late incubation and in the first 24 hr after hatching at ambient temperatures (T_a) between 28° and 38°C and between 15° and 43°C, respectively. Evaporative cooling by hatchlings at T_a of 36°–43°C was also measured. Significant increases in embryonic metabolic level occurred between external and internal pipping and between internal pipping and establishment of a pip hole. However, despite the improving access to oxygen produced by these events, $\dot{V}O_2$ and T_b both varied directly with T_a in an ectothermic pattern throughout the final stages of incubation. A capacity for sustained endothermy only became apparent in hatchlings. Their $\dot{V}O_2$ varied inversely with T_a between 27.5° and 36°C to an extent indicating a modest capacity for regulatory thermogenesis. This served to maintain T_b above 35°C in this range of T_a. The apparent abruptness of the appearance of this regulatory capacity after hatching suggests that emergence from the physical confinement of the egg could be an important proximate factor in the establishment of endothermy in this semi-precocial species. Hatchling Sooty Terns underwent a progressive fall in T_b with declining T_a below 27.5°C. On the other hand, they appeared quite proficient at evaporative cooling with increasing T_a between 36° and 43°C.

Key words: Cold-induced thermogenesis; development of homeothermy; evaporative cooling; hatchlings; late incubation embryos; metabolic level; pipping; semi-precocial; Sterna fuscata.

INTRODUCTION

The transition from poikilothermy to homeothermy differs in its position within the developmental schedules of altricial and precocial birds. The development of temperature regulation in the former occurs a number of days after hatching, whereas in the latter it involves an embryonic component that appears subject to a constraint imposed by the rate of diffusion of oxygen through the shell and the chorioallantoic membrane (Tazawa et al. 1988, Whittow and Tazawa 1991). Presumably, thermoregulation develops at intermediate stages of maturity in semi-altricial and semi-precocial (see Material and Methods) birds. The present report is the third in a series of studies of the acquisition of this capacity in semi-precocial tropical seabirds. The previous two investigations dealt with the surface-nesting Brown Noddy, *Anous stolidus* (Mathiu et al. 1991) and the burrow-nesting Wedge-tailed Shearwater, *Puffinus pacificus* (Mathiu et al. 1992), representatives of the orders Charadriiformes and Procellariiformes, respectively. These two species share the prolonged incubation characteristic of many tropical seabirds (Whittow 1980) and the sequence of events during pipping of their eggs is also similar. Pipping has a direct bearing on the development of thermoregulation in precocial birds, for the fracture of the shell and rupture of the internal shell membrane appear to be important events in moving the embryo beyond a state that Tazawa et al. (1988) referred to as "oxygen conductance limited," based on the work with the egg of the domestic fowl (*Gallus domesticus*). Removal of this constraint might allow a sufficiently advanced embryo to increase its oxygen uptake in response to

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cooling of the egg (Dawson 1984). However, no evidence of regulatory thermogenesis was found in either the Brown Noddy or the Wedge-tailed Shearwater while the embryos were *in situ* within pipped eggs. In fact, hatching appeared to be the climactic event in the development of endothermy, hatchlings of the two species acquiring the ability to maintain elevated rates of oxygen consumption in response to cooling either during or shortly after emergence from the egg (Mathiu et al. 1991, 1992).

Like the Brown Noddy, the Sooty Tern (*Sterna fuscata*), the subject of the present report, is a member of the family Laridae. The incubation biology of the two species is similar in certain respects: each lays a single egg directly on the surface of the ground and their embryos share a common pipping sequence. However, the incubation period of the Sooty Tern is 19% shorter and its relative embryonic growth rate (mean percentage of hatchling mass gained per day of incubation) 36% greater than in the larger Brown Noddy (Rahn et al. 1984). In view of these differences, we were interested to determine just when endothermy appears in the Sooty Tern. Comparison of the two species could facilitate understanding of the proximate events in the establishment of regulatory thermogenesis in semi-precocial birds.

MATERIAL AND METHODS

EXPERIMENTAL SUBJECTS

The Sooty Tern (*Sterna fuscata*) is widely distributed over tropical and subtropical latitudes in the Atlantic, Pacific, and Indian Oceans and it nests on many islands in these waters. A single egg is laid in a nest that is little more than a shallow scrape in the ground. The incubation period is 28.6 ± 0.59 days in the Hawaiian breeding colony with which this study deals. Star fracturing of the eggshell (external pipping) and establishment of a pip hole occur 4.5 ± 0.36 and 1.3 ± 0.52 days before hatching, respectively (Brown 1977). Like other terns and gulls, Sooty Terns emerge from the egg fully clad in down. Quiescent at first, these semi-precocial young (in addition to being down-covered, semi-precocial young at hatching [1] have their eyes open, [2] possess some thermoregulatory capacity, [3] remain at the nest site, but have some locomotor capacity, and [4] are dependent on their parents for food and thermal protection) begin to call and to display limited locomotor capacities within a few hours after emergence from the egg. However, they do not move out of the exposed nest sites characterizing this species until three days after hatching (Murphy 1936). Our observations cover the approximately week-long period from just before the start of pipping through the first day after hatching and deal primarily with the ability of these birds to resist cooling. However, we also present observations on the capacities of hatchlings for evaporative cooling, in an effort to characterize their overall capacities for thermoregulation just after emergence from the egg.

Eggs for this study were collected from a colony of nesting Sooty Terns on Manana Island (21°20′N; 157°40′W—near Oahu in the Hawaiian Islands) and transferred to the laboratory at the University of Hawaii, Manoa, 24 km distant. The eggs were kept in a commercial incubator at an ambient temperature (T_a) of 36°C (incubation temperature for the Sooty Tern) and a relative humidity of 60% and turned twice daily. Measurements were made on embryos in intact eggs at the following stages (in order of increasing age): (1) *late-incubation eggs* 1–2 days before the start of pipping (identified by candling that indicated a large, well defined air space and general activity of the embryo, including movements that caused the bill periodically to distend the internal shell membrane into the air cell); (2) *externally pipped eggs* with star fractures in their shells; (3) *internally pipped eggs* in which the embryo's beak had penetrated through the internal shell membrane into the air cell; and (4) *pip-holed eggs* in which the opening in the shell exceeded ca. 1 cm^2 in area. Hatchlings that had emerged from previously unstudied eggs incubated in the laboratory were used in tests commencing in the first 3–24 hr after hatching, when their down had dried and become fluffy. These birds were held at 36°C before and after study.

PHYSIOLOGICAL MEASUREMENTS

Measurements of oxygen consumption and body temperature. Embryos and hatchlings were used only once in metabolic tests, all of which were conducted at constant T_a. The experimental subjects were placed in individual plexiglass chambers (volume, about 300 ml for the embryo-containing eggs and about 1 liter for hatchlings). In the case of most of the embryos and all of the hatchlings, up to three of these chambers were

positioned in a Hotpack Environmatic Chamber (model 1247 LA) at 15-min intervals and connected to separate open circuit metabolism systems. Dry air was pumped through each of these open circuit systems at ca. 200 ml min^{-1} and at 400–800 ml min^{-1} for embryos and hatchlings, respectively. The highest flow rates occurred in tests involving hatchlings at T_a of 40° and 43°C, and calculations (see Lasiewski et al. 1966) indicated that these rates maintained the absolute humidities in the chambers at or below 2 kPa. Rates of air flow through the open circuit metabolism systems and the fractional concentrations of oxygen in air entering and leaving the plexiglass chambers were measured with a Tylan FC-260 Mass Flow Controller (embryos) or a Brooks Sho-rate 150 rotameter (hatchlings) and an Ametek Oxygen Analyzer (model S-3A-II), respectively, used in conjunction with a Houston Instruments Omniscribe recording potentiometer. Equation 2 from Hill (1972) for upstream flow monitoring and analysis of dry, CO_2-free air was employed for calculation of rates of oxygen consumption ($\dot{V}O_2$) corrected to 0°C and 101.3 kPa. Drierite and Ascarite were used as desiccant and CO_2-absorbent, respectively.

Chamber temperatures in the metabolism systems were monitored with thermistor probes (Yellow Springs Instruments no. 401). Body temperatures (T_b) of the tern embryos were measured with a needle thermistor probe (YSI no. 524) inserted 2.5 cm into the egg near the midpoint of its long axis. In eggs with pip holes, a blunt YSI thermistor probe (no. 402) was inserted through the hole and the tip positioned between the embryo's folded limbs and its body. A probe of this type was also used to obtain the cloacal temperatures of hatchlings. All thermistors were used with a YSI Telethermometer (no. 46 TUC), following calibration against a Brooklyn P-M Thermo Co. thermometer (no. 4 N 389) accurate to 0.05°C.

Observations on respiratory frequency and pulmocutaneous evaporation. Measurements on respiratory frequency (f) were made just before hatchlings were removed from the chambers after 11 of the metabolic tests performed at T_a of 36°–43°C. We substituted a rubber stopper containing a connection for a Grass PT-5 pressure transducer for the stopper containing the connections for the incurrent and excurrent air lines of the metabolism circuit in the lid of the plexiglass chamber. Nylon tubing served as the link between this new connection and the transducer, which was used in conjunction with a Grass Polygraph (model 5D) and a Grass low level DC preamplifier (model 5P1H). Pressure changes associated with respiration were recorded over 1–2 min, periods that were brief enough to avoid a rise in f due to a buildup of CO_2.

Measurements of pulmocutaneous evaporation by hatchlings (hereafter total evaporative water loss, or TEWL) accompanied 18 of the determinations of $\dot{V}O_2$ at T_a of 36°–43°C. A shallow container of mineral oil was added to the bottom of each chamber to cover any excrement voided during experiments and prevent it from adding water vapor to the system. The hatchling rested on a wire mesh platform above this container during a test. The TEWL was determined over a precise time interval by measuring the increase in mass of an absorbent train containing Drierite. All weighings were made with an analytical balance (Mettler H6). The absorbent train was connected to the outlet port of the metabolism chamber by a 1-m length of 6.3-mm copper tubing to prevent any errors from diffusion of water vapor into the system that might have occurred through plastic tubing. This tubing was maintained at a temperature warm enough to prevent condensation of water vapor, even at the highest rates of water loss by the hatchlings.

Duration of tests and timing of physiological measurements. Metabolic tests lasted 90 min and 2 hr for eggs and hatchlings, respectively. The values we report for $\dot{V}O_2$ and TEWL were measured during the last 15 min and last 20 min of tests, respectively. At these times the hatchlings were a minimum of nearly 5 hr old, for, as noted above, no tests were initiated until at least 3 hr after hatching to allow the birds' down to dry and become fluffy. Placement of the drying tubes for capture of water vapor in the metabolism circuits produced a transient change in the rate of air flow. Starting the measurement of $\dot{V}O_2$ 5 min after the connection of these tubes served to eliminate the effect of this change. As noted above, respiratory frequencies were measured immediately after the conclusion of some of these tests. Body temperature was recorded as the eggs or hatchlings were removed from the plexiglass chambers.

OBSERVATIONS ON BODY MASS, MUSCLE MASS, AND DOWN

Information on body mass of embryos and hatchlings was necessary for conversion of data on $\dot{V}O_2$ and TEWL to mass-specific terms and

TABLE 1. Masses of eggs and yolk-free embryos (mean ± SD in g) of the Sooty Tern during the final stages of incubation.

Egg stage	*n*	(a) Egg mass	(b) Yolk-free embryo mass	(b/a) %
Late incubation, unpipped	26	32.55 ± 2.86	15.92 ± 2.66	49
Externally pipped	25	32.35 ± 1.56	17.99 ± 1.38	56
Internally pipped	28	31.51 ± 2.03	21.36 ± 2.03	68
Pip-holed	26	28.84 ± 2.12	20.80 ± 1.63	72

for analyses involving allometric relationships. Masses of eggs and hatchlings were measured with an Ohaus triple beam balance immediately before and just after metabolic tests. Shortly thereafter, each egg was sacrificed and opened to remove and weigh the embryo. Then the yolk sac was removed and weighed to allow determination of yolk-free embryonic mass. Both measurements involving embryos were carried out using a Sartorius top loading balance (model 1265 MP) accurate to 0.001 g.

Plumage measurements were made on hatchlings to provide information on the extent of integumentary insulation. The respective thicknesses of the down covering the dorsal surface of the lumbar region, the sternal area, and the crown of the head were determined for hatchling Sooty Terns after their down had dried. A small sample was plucked from each of these sites and fastened to a piece of paper using Scotch Magic Tape. The length of the feathers in the sample was measured to the nearest mm. Additionally, six hatchlings were sacrificed by cervical dislocation and their down plucked and weighed on the Mettler H6 analytical balance.

The respective masses of the leg and breast muscles (the principal leg muscles are the Mm. gastrocnemius; iliotibialis lateralis; and femorotibialis externus, femorotibialis medius, and femorotibialis internus; the breast muscles are the Mm. pectoralis and supracoricoideus) of hatchlings were determined for estimation of the amount of tissue available at these sites for shivering thermogenesis. Nine hatchlings were weighed, sacrificed by decapitation, and the skin quickly removed. The breast muscles were dissected using scissors. A good deal of the leg musculature was also removed in this manner. However, completion of the process required scraping the leg bones with a scalpel. The tissues were covered and weighed on the Mettler H6 analytical balance. The dissections and weighings were completed within 2–4 min to minimize mass loss due to evaporation.

STATISTICS

Means are reported ± one standard deviation (SD). Regression equations and correlation coefficients were obtained from the Sigmaplot Program (Jandel Scientific). Group means were compared with the Tukey HSD Test. Regressions of embryonic $\dot{V}O_2$ or T_b on T_a were compared by analysis of covariance (ANCOVA). For each comparison, an F^* test was used to establish whether a simple regression of the particular variable on T_a described the data as well as did a full model incorporating dependence on temperature, developmental stage, and interaction between temperature and developmental stage. If the full model did not offer a better description of the data, the two developmental stages would not differ in $\dot{V}O_2$ or T_b over the range of T_a examined. All probabilities are single-tailed values. Application of the various tests was facilitated by use of Systat software (Wilkinson 1987).

RESULTS

EMBRYOS

Masses of late-incubation eggs and embryos. Mean mass of Sooty Tern eggs decreased over the final stages of incubation (Table 1). On the other hand, yolk-free embryonic mass tended to increase through the internal pipping stage, with the differences between the means for embryos in unpipped and externally pipped eggs and between externally and internally pipped eggs being statistically significant ($P < 0.01$, Tukey HSD test). The mean mass of embryos in pip-holed eggs was slightly lower than that of ones at the preceding stage, but the difference is not statistically significant ($P > 0.05$, Tukey HSD test). Over the approximately 4-day period between the initiation of pipping and the establishment

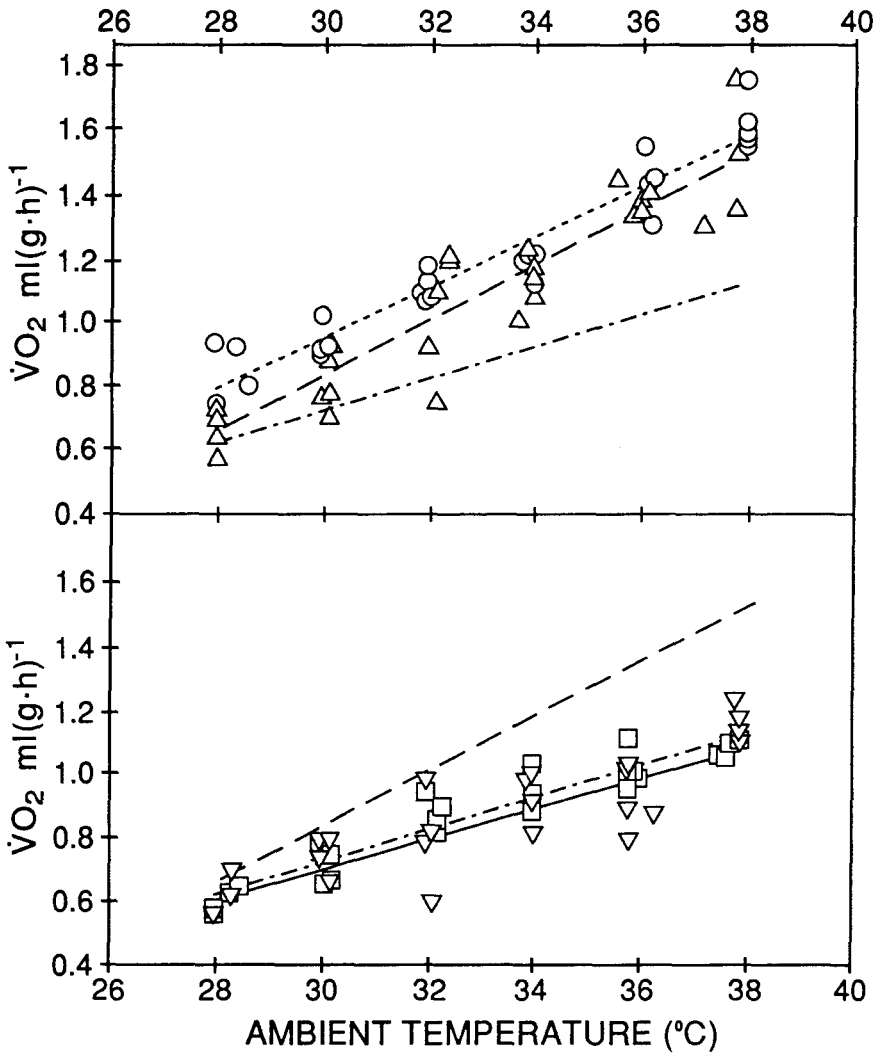

FIGURE 1. Oxygen consumption ($\dot{V}O_2$) of embryos in unpipped (▽, ——) and externally pipped (□, −·−·−) eggs of the Sooty Tern at various ambient temperatures (lower panel) and of embryos in internally pipped (△, — — —), and pip-holed (○, ·····) eggs (upper panel). For comparison, the regression lines for embryos in internally pipped eggs (— — —) and in externally pipped eggs (−·−·−) are included in the lower and upper panels respectively. The equations for the linear regression lines for the various stages are given in Table 2 (equations 1, 3, 5, and 7).

of a pip hole, yolk-free embryonic mass increased from 49 to 72% of concurrent egg mass.

Thermal responses of embryos during late incubation. After the 75-min point in tests of late-incubation embryos in unpipped, externally pipped, internally pipped, and pip-holed eggs, direct linear relations exist between $\dot{V}O_2$ and T_a and between T_b and T_a (Figs. 1, 2; equations 1–8 in Table 2). For metabolic rate between 28° and 38°C through these stages, Q_{10} values are near 2 (Table 2), emphasizing further the essentially ectothermic pattern of response by embryos of this age. Analysis of covariance indicates that the $\dot{V}O_2$-T_a regressions for late incubation embryos in unpipped and externally pipped eggs (Table 2) do not differ significantly (F^* = 1.34, dfn = 1, dfd = 48, P [corrected for multiple comparisons] = 0.25). On the other hand, those for embryos in externally pipped and internally pipped eggs (Table 2) do (F^* = 98.24, dfn = 1,

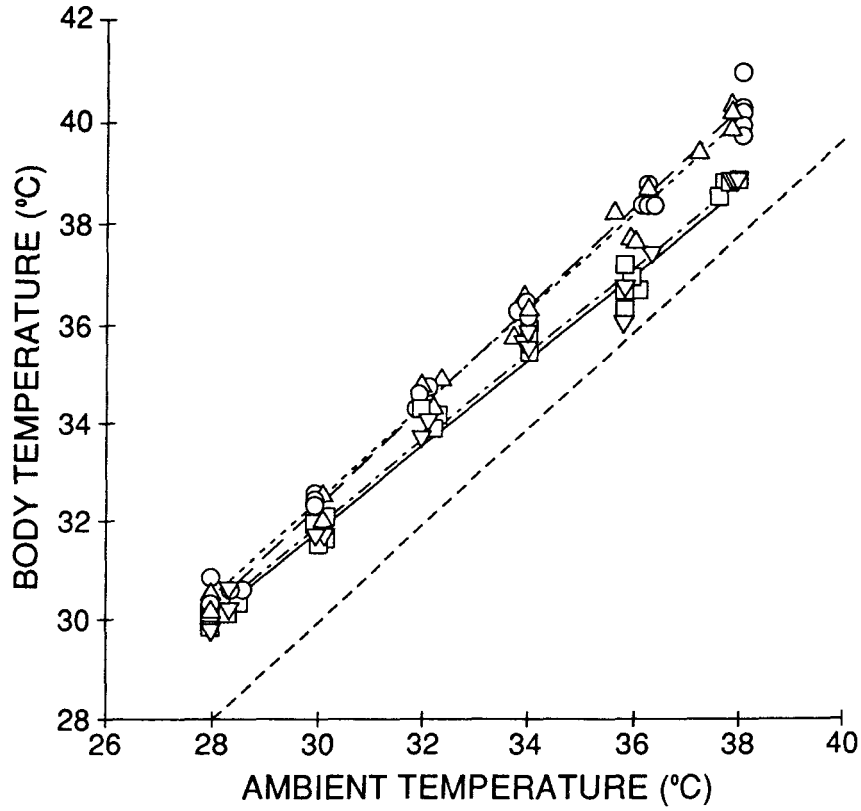

FIGURE 2. Body temperature of embryos in unpipped (▽, ——), externally pipped (□, –·–·–), internally pipped (△, — — —), and pip-holed eggs (○, ·····) of the Sooty Tern at various ambient temperatures. The equations for the respective linear regression lines for the various stages are given in Table 2 (equations 2, 4, 6, 8). The lower diagonal line (– – –) links equivalent values of body and ambient temperature.

dfd = 50, P [corrected for multiple comparisons] < 0.0001). This is also the case for embryos in internally pipped and pip-holed eggs (Table 2; F^* = 15.43, dfn = 1, dfd = 51, P [corrected for multiple comparisons] = 0.0008). An F-test based on an ANCOVA incorporating data for embryos at all four stages yielded a highly significant interaction term between developmental stage and T_a as predictors of $\dot{V}O_2$ (F = 13.16, dfn = 3, dfd = 97, P [corrected for multiple comparisons] < 0.001).

The T_b on T_a regressions for the final stages of incubation are relatively homogeneous (Fig. 2; Table 2). Those for embryos in unpipped and externally pipped eggs do not differ significantly (F^* = 0.39, dfn = 1, dfd = 48, P [corrected for multiple comparisons] ≫ 0.5). This is also the case for the T_b on T_a regressions for embryos in internally pipped and pip-holed eggs (F^* = 1.88, dfn = 1, dfn = 51, P [corrected for multiple comparisons] > 0.5). The regressions for embryos in externally and internally pipped eggs do differ significantly (F^* = 132.8, dfd = 1, dfn = 50, P [corrected for multiple comparisons] < 0.0001). An F-test based on an ANCOVA incorporating data for embryos at all four stages showed a highly significant interaction between developmental stage and T_a as predictors of T_b (F = 16.57, dfn = 3, dfd = 97, P < 0.0001).

HATCHLINGS

Mass and down. Hatchling Sooty Terns averaged 22.45 ± 2.35 g (n = 106) in body mass (including residual yolk). The mass of the musculature of the legs of nine chicks, 1.57 ± 0.22 g, represented 6.8% of body mass, approximately triple the proportion of the breast muscles, which weighed only 0.43 ± 0.09 g and represented only 1.8%. The hatchlings were completely covered with down weighing 0.86 ± 0.17 g (n = 6) and representing

TABLE 2. Equations for the relations of oxygen consumption ($\dot{V}O_2$), total evaporative water loss (TEWL), respiratory frequency (f), and body temperature (T_b) to ambient temperature (T_a) in embryonic and hatchling Sooty Terns.

Stage	T_a range (°C)	Equation[a]	Q_{10}[b]	Coefficient of determination (r^2)
Embryos in late incubation, unpipped eggs	28–38	1) $\dot{V}O_2 = -0.76 \pm 0.05T_a$	1.9	0.73
	28–38	2) $T_b = 5.01 + 0.89T_a$		0.99
Embryos in externally pipped eggs	28–38	3) $\dot{V}O_2 = -0.79 + 0.05T_a$	1.9	0.90
	28–38	4) $T_b = 4.99 + 0.90T_a$		0.99
Embryos in internally pipped eggs	28–38	5) $\dot{V}O_2 = -1.86 + 0.09T_a$	2.3	0.86
	28–38	6) $T_b = 1.39 + 1.03T_a$		0.99
Embryos in pip-holed eggs	28–38	7) $\dot{V}O_2 = -1.40 + 0.08T_a$	2.0	0.93
	28–38	8) $T_b = 2.41 + 1.00T_a$		0.99
Hatchlings	27.5–43	9) $\dot{V}O_2 = 16.30 - 0.77T_a + 0.01(T_a)^2$		0.58
	27.5–43	10) $T_b = 41.21 - 0.44T_a + 0.01(T_a)^2$		0.80
	15–27.5	11) $\dot{V}O_2 = -2.25 + 0.18T_a$		0.62
	15–27.5	12) $T_b = 2.64 + 1.23T_a$		0.82
	36–43	13) $f = 2{,}016.5 - 126.4T_a + 1.99(T_a)^2$		0.98
	36–43	14) $TEWL = 191.62 - 11.59T_a + 0.18(T_a)^2$		0.94
	36–43	15) $TEWL/\dot{V}O_2 = 9.56 - 6.00T_a + 0.10(T_a)^2$		0.87

[a] $\dot{V}O_2$, TEWL, $TEWL/\dot{V}O_2$, and f are in ml O_2 $(g \cdot h)^{-1}$, mg H_2O $(g \cdot h)^{-1}$, mg H_2O/ml O_2, and breaths $(min)^{-1}$, respectively. T_b and T_a are in degrees Celsius. All correlations are significant ($P < 0.01$).
[b] The Q_{10} listed are for the T_a interval of 28° to 38°C.

3.9% of body mass. The length of this down averaged 8.0, 7.0, and 4.0 mm on the back, sternum, and crown, respectively.

Oxygen consumption. Hatchlings were tested at single T_a between 15° and 43°C. The relation of their $\dot{V}O_2$ to T_a (Fig. 3) contrasts with the essentially ectothermic response noted in Sooty Terns in the final stages of incubation (Fig. 1). Metabolic rate of these hatchlings varied inversely with T_a between 36°C (the temperature at which the eggs and hatchlings were maintained in the laboratory) and 27.5°C, where summit metabolism was attained. Values averaged 1.73 ± 0.39 ($n = 9$) and 2.84 ± 0.21 ml $O_2(g \cdot hr)^{-1}$ ($n = 15$) at 36° and 27.5°C, respectively. The regression line fitted to the data between 27.5° and 43°C (equation 9, Table 2) indicates a rise in $\dot{V}O_2$ above 36°C, due largely to two outlier values. However, the $\dot{V}O_2$ of nine individuals tested above 36°C were in the lower half of the range of rates observed at this temperature (Fig. 3), suggesting that the birds do not generally increase their metabolic rate appreciably at T_a up to 43°C. In tests at cooler temperatures, we were primarily interested in metabolic values at the end of 2-hr test periods. However, we did follow some hatchlings throughout tests at T_a below 27.5°C. These animals showed a transient rise in $\dot{V}O_2$ to as high as the summit level, but they were unable to sustain this. Their inability to maintain high T_b at T_a between 15°C and 27.5°C (see below) was accompanied by the end of 2-hr tests with a 6% decline in $\dot{V}O_2$ (relative to summit metabolism) per degree reduction in experimental T_a (Fig. 3; see equation 11, Table 2).

Body temperature. Body temperature of hatchlings at 36°C was 38.6 ± 0.6°C. Values measured at the end of 2-hr experiments indicated a direct relation between T_b and T_a between 43° and 27.5°C (equation 10, Table 2), but even at the lower temperature all of the T_b matched or exceeded 35°C (Fig. 4). Vigorous evaporative cooling at T_a near 43°C (see below) allowed the hatchlings to maintain T_b at 41.0 ± 0.4°C ($n = 5$), 2°C below their surroundings. The control of T_b evident at T_a above 27.5°C contrasted with the results obtained at cooler temperatures, where a strong dependence of the former variable on the latter was apparent (Fig. 4; equation 12, Table 2).

Respiratory frequency and total evaporative water loss. Between 36° and 43°C, the respective relations of f and TEWL to T_a are curvilinear (Figs. 5, 6; equations 13 and 14, Table 2). Respiratory frequencies of hatchlings at T_a of 40° and 43°C differed from that at 36°C (incubation

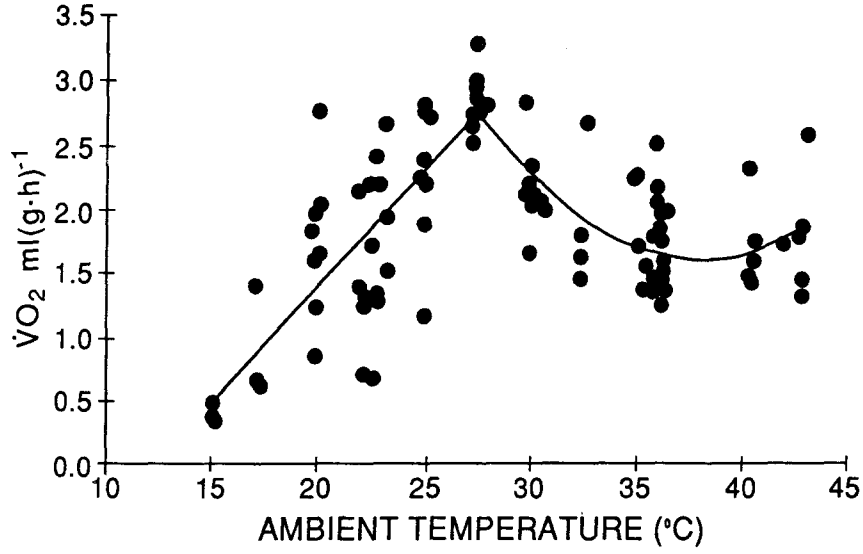

FIGURE 3. Oxygen consumption ($\dot{V}O_2$) of hatchling Sooty Terns at different ambient temperatures. The regression lines meeting at 27.5°C are described by equations 9 and 11 in Table 2.

temperature) by factors of 3.6 and 5.7, respectively. The factors relating TEWL at 40° and 43°C to that at 36°C are 3.1 and 4.6, respectively.

Values of $\dot{V}O_2$ and TEWL from tests in which both were measured were converted to rates of heat production and heat dissipation (see Fig. 7 for conversion factors) to assess the capacities of hatchlings for evaporative heat loss in and above the zone of thermal neutrality. The percentage of heat production dissipated by evaporation increased six-fold between 36°C and 43°C, reaching 150% at the latter T_a (Fig. 7; equation 15, Table

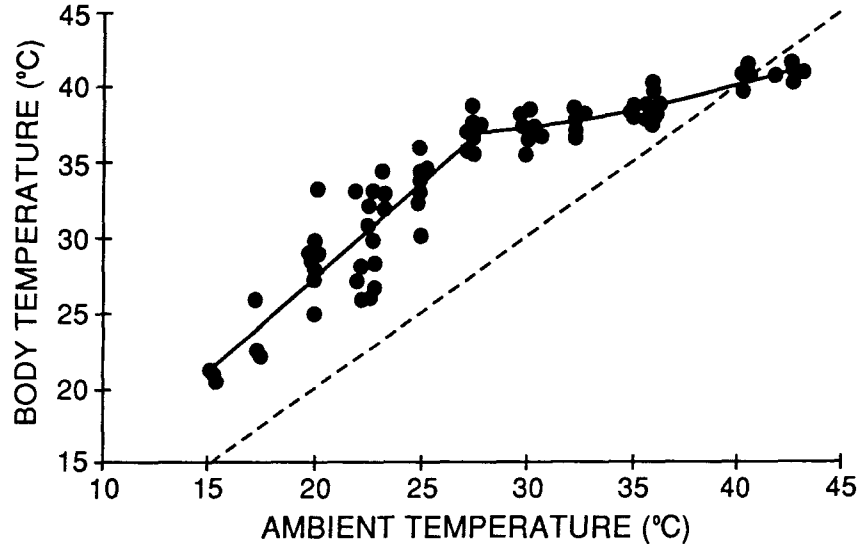

FIGURE 4. Body temperature of hatchling Sooty Terns at different ambient temperatures. The regression lines meeting at 27.5°C are described by equations 10 and 12 in Table 2. The lower diagonal line (– – –) links equivalent values of body and ambient temperature.

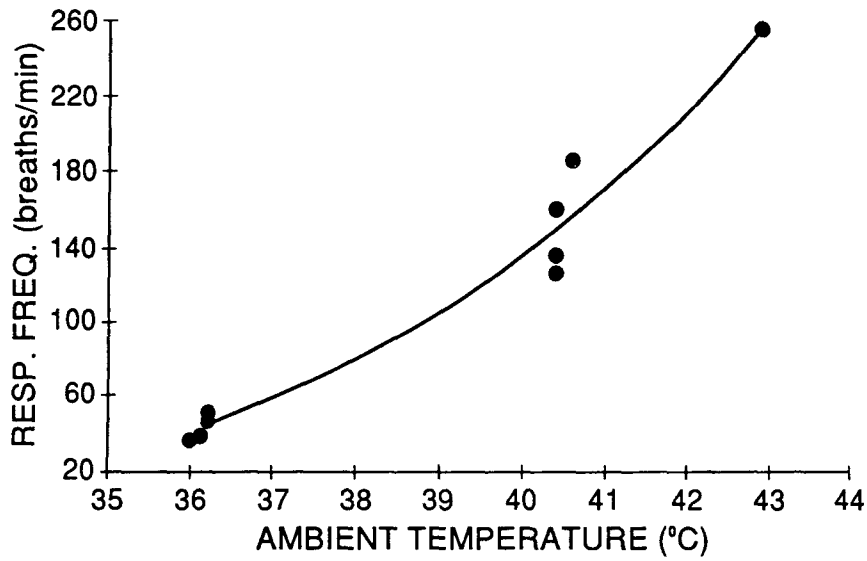

FIGURE 5. Respiratory frequency of hatchling Sooty Terns at different ambient temperatures. The three points near 43°C are tightly clustered and appear as one. The solid line fitted to the data represents equation 13 (Table 2).

2). Thus hatchling Sooty Terns at high T_a can dissipate heat gained from the environment as well as from metabolism.

Behavior of hatchlings at various temperatures. The behavior of hatchlings varied with T_a during the 2-hr tests concerned with measurement of $\dot{V}O_2$ and T_b. At 36°C (where $\dot{V}O_2$ of normothermic individuals was minimal—see Fig. 3), they rested quietly or slept in the metabolism chamber. Panting was commonly observed at 40°C, but the chicks slept occasionally. At T_a near 43°C, all became restless and panted continuously. Exposure of the hatchlings to lower T_a resulted in signs of increased thermoregulatory activity. At

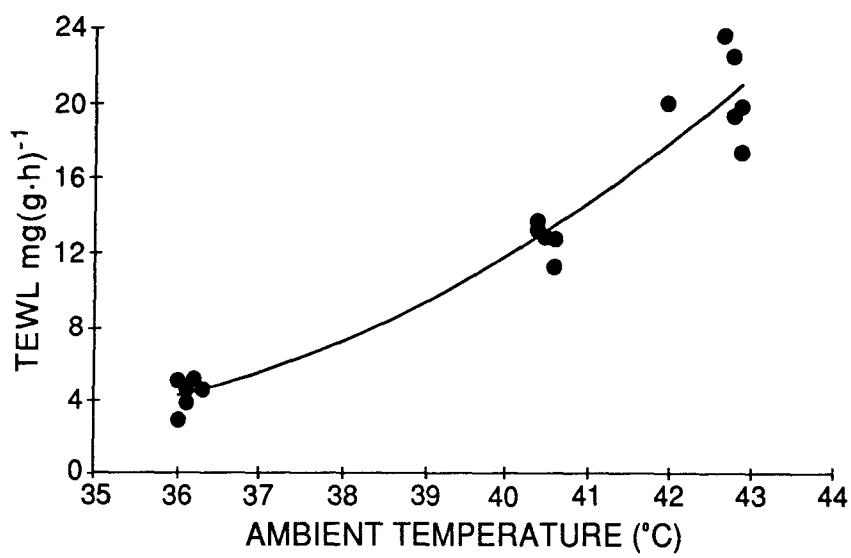

FIGURE 6. Pulmocutaneous evaporative water loss (TEWL) of Sooty Terns at various ambient temperatures. The solid line is the least squares regression line (see equation 14 in Table 2).

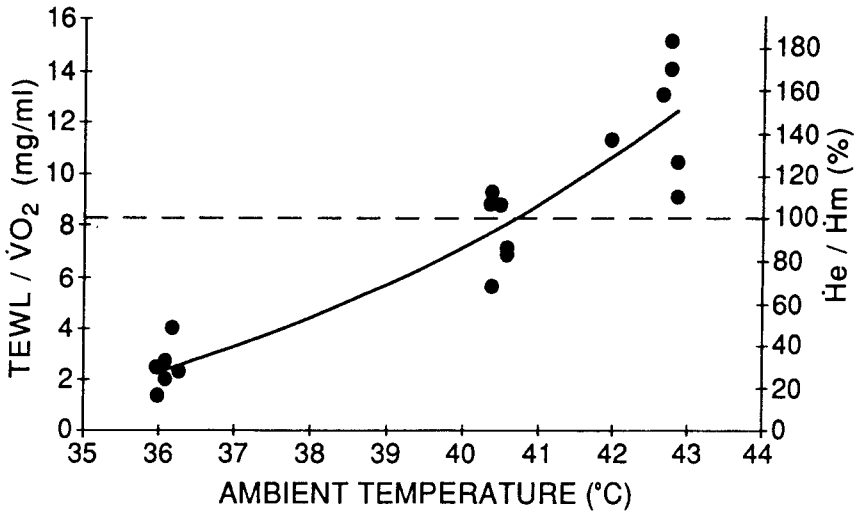

FIGURE 7. The ratio of pulmocutaneous water loss (TEWL) to oxygen consumption ($\dot{V}O_2$) and of evaporative heat loss ($\dot{H}_e$) to metabolic heat production ($\dot{H}_m$) in hatchling Sooty Terns at various ambient temperatures. The solid line is the least squares regression line fitted to the data (see equation 15 in Table 2). The horizontal line (— — —) marks equivalence between $\dot{H}_m$ and $\dot{H}_e$. Heat production and evaporative heat loss were estimated from $\dot{V}O_2$ and TEWL, respectively, by use of the following relationships: 1 ml O_2 consumed = 20.08 J and 1 mg H_2O evaporated = 2.43 J.

32°C they tended to adopt a more compact posture that left the limbs less exposed. These individuals often called repetitively. Tremors indicative of shivering were evident in hatchlings tested at 30°C and this assisted us in placing the lower boundary of the zone of thermal neutrality near 31°C. In some individuals these tremors alternated with quiescent periods in which the compact posture just described was assumed. In others in which the extent of the tremors indicated whole body shivering, this activity was occasionally interrupted by the chicks' standing quietly. All chicks tested at T_a between 27.5° and 20°C showed continuous tremors indicative of vigorous shivering. Hypothermia impaired this performance in birds exposed for 2 hr to T_a below 20°C and none was observed to shiver once T_b fell below 25°C.

DISCUSSION

CHANGES OF METABOLIC LEVEL DURING LATE INCUBATION

The latter stages in incubation of embryonic Sooty Terns encompass an important set of events involving growth, gas exchange, and, possibly, developmental changes involving maturation of neuroendocrine and effector systems. Based on adult scaling relationships for metabolism, intensity of metabolism ($\dot{V}O_2$/g) might be expected to decline with the increase in mass of the terns through most of these stages (Table 1). On the other hand, the initiation of pulmonary respiration with internal pipping and the establishment of direct access to atmospheric oxygen with the creation of the pip hole, might support an increase in metabolic intensity. Microscopic pores in the shell allow diffusion of oxygen from the surrounding air. The number and dimensions of these pores are established at shell formation and remain constant thereafter (Rahn et al. 1987, Tazawa 1987). Evidence from the domestic fowl, duck (presumably *Anas platyrhynchos*), and Turkey (*Meleagris gallopavo*) indicates that the rate of diffusion of this gas through the shell may limit embryonic growth, oxygen uptake, and oxygenation of the blood, especially just before pipping (Tazawa et al. 1971, Metcalfe et al. 1981, Tullett and Deeming 1982, Burton and Tullett 1983). However, external pipping by Sooty Terns did not significantly increase the general level of $\dot{V}O_2$ from that evident in advanced embryos in unpipped eggs (Fig. 1), even though shell oxygen conductance had presumably increased. Mass-specific $\dot{V}O_2$ of older embryos (Fig. 1) did rise significantly in the stages after external pipping. This is similar to the pattern noted in two other

semi-precocial species: the Brown Noddy, *Anous stolidus* (Mathiu et al. 1991), and the Wedge-tailed Shearwater, *Puffinus pacificus* (Mathiu et al. 1992). Internal pipping involves penetration of the air cell of the egg by the embryo's bill and the initiation of pulmonary ventilation, while the establishment of a pip hole, as noted above, provides direct access to atmospheric oxygen. The increases in metabolic level in embryos of the three species thus do coincide with improved opportunities for oxygen procurement. The intensification of mass-specific $\dot{V}O_2$ in the last stages of incubation occurs despite increasing embryonic body mass. For example, the mass-specific $\dot{V}O_2$ of embryos in internally pipped eggs at 36°C averaged 1.33× that of ones in externally pipped eggs, even though the former embryos were about 19% heavier than the latter. Tazawa (1987) has provided evidence from the domestic fowl that the internal shell membrane represents the major barrier to diffusion of oxygen into the blood of the embryo. The significant increase in $\dot{V}O_2$ of embryonic Sooty Terns between external and internal pipping (Fig. 1) could result in part from removal of this constraint. However, the establishment of pulmonary ventilation and, developmental changes should not be overlooked as possible factors in the increase.

Despite greater access to oxygen and increased metabolic rates with pipping, Sooty Terns, like other semi-precocial birds (Whittow and Tazawa 1991), including their fellow larids the Herring Gull, *Larus argentatus* (Drent 1970: Fig. 31), and the Brown Noddy (Mathiu et al. 1991), remain ectothermic until hatching (Figs. 1, 2). Mathiu et al. (1992) showed that hatching of Wedge-tailed Shearwaters, rather than abrupt biochemical maturation, is a proximate factor in the advancement of chicks of that species from a power-limited state (see Whittow and Tazawa 1991) in which sustained cold-induced thermogenesis and thus homeothermy are not possible. Hatching appears to be the key event in the attainment of endothermy by embryonic Sooty Terns as well, despite their having a shorter incubation period and a higher relative embryonic growth rate than either Wedge-tailed Shearwaters or Brown Noddies (44 and 19% shorter and 50 and 36% higher than comparable values for the shearwaters and noddies, respectively [calculations from data in Pettit and Whittow 1983, Rahn et al. 1984, Mathiu et al. 1992]). All these birds are dependent on parental attentiveness for temperature control until hatching. It would be interesting to determine whether, by communication with their parents, they can fine-tune the incubation response in late stages of pipping, as American White Pelicans, *Pelecanus erythrorhynchos,* do (Evans 1990).

METABOLIC LEVEL IN HATCHLING SOOTY TERNS

Some authors have assessed the degree of maturation of birds at hatching by comparing the metabolic rates of hatchlings at thermoneutral T_a (referred to subsequently as resting metabolic rate [RMR]) with estimates of standard metabolic rates (SMR) for hypothetical adults of similar size calculated from allometric equations (e.g., Aschoff and Pohl 1970). Bucher (1986) suggested that greater power in such comparisons requires either a) use of an allometric equation restricted to the particular order or lower taxonomic category that includes the species under consideration, or b) comparison of mass-independent metabolic rates, i.e., (SMR or RMR)/(mass)$^{0.67}$—see Heusner (1983, 1985), for both hatchlings and adults. We have used a compromise procedure involving extrapolation of the SMR for adult Sooty Terns to a value for a hypothetical adult of hatchling mass. The extrapolation involves use of one of two scaling factors, −0.33 (the mass-specific counterpart of the exponent advocated by Heusner [1983, 1985] for use in calculation of mass-independent metabolic rates) or −0.28 (the mass-specific counterpart of the exponent that Ellis [1984] obtained for the metabolism-mass relation for various adult seabirds). The hatchling and adult Sooty Terns to which this comparison pertains were both obtained from the Manana Island colony, so the method has the advantage of eliminating any latitudinal effects (see Ellis 1984, Klaassen and Drent 1991), unlike that depending on application of one of the general allometric equations. Extrapolations of the SMR of adult Sooty Terns (MacMillen et al. [1977] reported a mean rate of 0.97 ml $O_2[g \cdot hr]^{-1}$ for individuals that averaged 150.4 g in mass) to the hatchling mass of 22.5 g using scaling factors of 0.72 and 0.67 yields rates of 1.65 and 1.82 ml $O_2(g \cdot hr)^{-1}$, respectively. The mean RMR of the hatchlings (1.73 ml $O_2[g \cdot hr]^{-1}$, thus represents 105 and 94% of the respective extrapolated rates for a 22.5-g adult.

The percentages just referred to suggest that Sooty Terns hatch in a relatively mature state

TABLE 3. Thermoregulatory characteristics of hatchling and adult Sooty Terns.[a]

Age class	Body mass (g)	Thermoneutral Zone (TNZ) Width (boundaries)[b] (°C)	T_b in TNZ (°C)	$\dot{V}O_2$ in TNZ (ml [g·h]$^{-1}$)	Thermogenic capacity	Minimal c_{total} (mW [g·°C]$^{-1}$)	$\dot{H}_e/\dot{H}_m$ (%)	f (cycles/min)
Hatchling	22.5	<5 (ca. 31–36)	36.8–38.6	1.73	1.64 (27.5°C)	1.67	150 (43°C)	256 (43°C)
Adult	150.4	10 (24–34)	39.5	0.97	2.30[c] (10°C)	0.45	72 (45°C)	174 (44°–45°C)

[a] Data for the adults and hatchlings are from MacMillen et al. (1977) and the present study, respectively. Abbreviations or symbols are as follows: TNZ = zone of thermal neutrality; T_a = ambient temperature; T_b = body temperature; $\dot{V}O_2$ = rate of oxygen consumption; c_{total} = minimal mass-specific total or "wet" thermal conductance (includes cooling due to evaporation); $\dot{H}_e$ = rate of pulmocutaneous evaporative heat loss; $\dot{H}_m$ = rate of heat production; f = maximal respiratory frequency, which occurred at T_a indicated in parentheses. Thermogenic capacity is the factor by which the highest metabolic rate observed at cooler T_a (specified in parentheses) differs from the metabolic rate in the TNZ. Heat production and evaporative heat loss were calculated on the basis of 20.08 J per ml O_2 consumed and 2.43 J per mg H_2O evaporated.

[b] Estimated from figure 2.

[c] MacMillen et al.'s (1977) observations on $\dot{V}O_2$ for adult Sooty Terns only include T_a down to 10°C. Exposure of the birds to lower temperatures might well have produced results indicating a thermogenic capacity exceeding 2.3.

compared with many other semi-precocial birds (see Bucher 1986 for discussion). The relatively high RMR observed in Brown Noddies (Mathiu et al. 1991) and various gull hatchlings (Drent 1970, Palokangas and Hissa 1971, Dawson et al. 1976), which, like terns, are members of the Family Laridae, support a similar conclusion for these birds. However, a note of caution is necessary, for other authors have reported RMR for hatchling terns, including the Sooty Tern, substantially below levels for hypothetical adults of comparable size (Ricklefs and White 1981, Pettit and Whittow 1983, Klaassen et al. 1989). The discrepancy may reflect differences in the interval between hatching and metabolic measurement. Differences in thermoregulatory capacity also may be involved in certain cases. The earliest our Sooty Tern chicks were measured was approximately 5 hr after hatching, to allow their down to dry and become fully insulative. On the other hand, the youngest chick of this species studied by Ricklefs and White (1981), had "just hatched" and probably was less well insulated owing to the state of its down. Mass-specific metabolic level does appear to rise in tern chicks in the first few days and even first hours after hatching (see, for example, Fig. 3 in Klaassen et al.'s [1989] study of Arctic Terns, *Sterna paradisaea*) and Ricklefs and White (1981) found that the metabolic rate of a two-day-old Sooty Tern was 2.3× that of their recently hatched individual. Klaassen and Bech (1992) concluded that RMR is more closely correlated with body mass than with age in the Arctic Tern in the first 10 d after hatching. Ingestion of food can also substantially increase metabolic rate of tern chicks, judging by Klaassen et al.'s (1989) finding of an average increment of 1.0 ml O_2(g·hr)$^{-1}$ associated with feeding in young Arctic Terns. Metabolic level of tern chicks in the hours after hatching thus can be rather labile. This also is true for hatchling gulls (Kespaik and Davydov 1966) and the domestic fowl (Misson 1977). Consequently, standardization of variables in metabolic studies of hatchlings (dryness and fluffiness of downy insulation, T_b and T_a, time elapsed since hatching, body mass, nutritional status, etc.) appears critical for defining metabolic scaling factors, latitudinal effects, and the particular species' metabolic position in the altricial-precocial continuum.

COMPARISON OF THE THERMOREGULATORY CHARACTERISTICS OF HATCHLING AND ADULT SOOTY TERNS

As noted above, the hatchling Sooty Terns we studied had RMR approaching values for the SMR of hypothetical adult terns of similar size. Their RMR also exceeded by 20% the value obtained from Klaassen and Drent's (1991) equation relating RMR to body mass for larid hatchlings. The chicks' T_b at thermoneutral T_a were at least 3°C lower than those of adults measured in the Sooty Tern breeding colony on Manana Island at a T_a of 30.9°C, but with intense insolation (MacMillen et al. 1977). However, these T_b for the hatchlings were only slightly below those reported by MacMillen et al. (1977) for three of four adults at the end of metabolic tests carried out near 30°C (Table 3).

Sooty Terns of the two ages do show some important differences in thermoregulatory characteristics. Adults have a wider zone of thermal neutrality (Table 3). Minimal total (includes

evaporative heat loss) thermal conductance (c_{total}) for the hatchlings is 1.67 mW(g·°C)$^{-1}$. This compares with values of 1.17 and 0.84 mW(g·°C)$^{-1}$ predicted for a hypothetical 22.5-g adult, using the nonpasserine allometric equations of Aschoff (1981) in the active and resting phases of their daily cycle. Evidently, a hatchling's down is less effective insulation than the mature plumage. Consistent with the difference in size (see Calder and King 1974), c_{total} is higher in the hatchlings than in the adult Sooty Terns (Table 3). The minimal value calculated from MacMillen et al. (1977) for the latter closely approaches the estimate for a 150.4-g adult bird obtained with Aschoff's (1981) equation for nonpasserines in the active phase of their daily cycles (0.44 [observed] vs. 0.45 mW[g·°C]$^{-1}$ [predicted]). Hatchling Sooty Terns also have more limited thermogenic capacity than adults; the ratio of summit to thermoneutral metabolic rate or SMR is only 1.64 for the younger birds, whereas it is at least 2.3 in the adults (Table 3). This appears related at least in part to the rudimentary state of the flight muscles in the younger birds, which, on the day of hatching, have a smaller mass than the leg musculature (Table 2). Flight muscles of various adult birds comprise as much as 15–25% of total body mass (Hartman 1961) and appear prominently involved in cold-induced thermogenesis (Marsh and Dawson 1989). Cold challenges probably do not constitute a major threat for hatchlings in the tropical and subtropical latitudes in which the Sooty Tern breeds. Nevertheless, the limited capacity of hatchling Sooty Terns for regulatory thermogenesis could lead to hypothermia if they were unprotected at night, particularly in wind. Parent birds are very attentive during the first three days after hatching while their chicks are confined to the nest site (Murphy 1936) and their brooding during this period undoubtedly compensates for any metabolic or insulative limitations of these young.

In contrast to their limited capacities for thermoregulation at cool temperatures, hatchling Sooty Terns have quite effective evaporative cooling. Their panting frequency at 43°C was 247 cycles/min (Fig. 5), which, not surprisingly, exceeds that of 174 ± 8 cycles/min noted in the much larger adult Sooty Terns (MacMillen et al. 1977). The hatchlings evaporated water at the rate of 20.33 mg H_2O(g·hr)$^{-1}$ at 43°C, resulting in the dissipation of heat at approximately 1.5 times the rate of heat production (Fig. 7), a performance that surpasses that of adult Sooty Terns (Table 3) and compares favorably with that of hatchling Brown Noddies (Mathiu et al. 1991) and hatchling gulls (Dawson et al. 1972, 1976; Dawson and Bennett 1981) under heat challenges. The enhanced breathing activity contributing to this performance in the hatchling Sooty Terns appears relatively effortless, for the $\dot{V}O_2$ of most individuals tested did not exceed rates at 36°C (Fig. 3). The fact that vigorous evaporative cooling appears earlier in their ontogeny than fully effective thermoregulation in cold is consistent with observations on other birds (e.g., Arad and Itsaki-Glucklich 1991). The capacity of Sooty Tern hatchlings for evaporative cooling could be important in protecting them from intense midday insolation in their exposed nests, should they be temporarily deprived of the shade provided by their normally attentive parents. Overheating from exposure to direct insolation is a serious threat for unshaded semi-precocial young even in environments with mild T_a (Barth 1951, Drent 1970, Dawson and Bennett 1981).

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