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DEVELOPMENT OF VOCAL REGULATION OF TEMPERATURE BY EMBRYOS IN PIPPED EGGS OF RING-BILLED GULLS

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ABSTRACT.—Temperatures of Ring-billed Gull (*Larus delawarensis*) eggs during the hatching period indicate that parents allow their last-hatched embryos to cool to 36.8°C, about 2°C below first-hatched eggs. Temperatures of artificial last-hatched eggs, corrected upwards to take account of embryonic metabolic heat, averaged only 35.5°C, suggesting an active effect of live embryos on parental incubation. In the laboratory, embryos in pipped eggs vocalized strongly when body temperature dropped below 36°C. When cold-induced calls triggered 4-min periods of rewarming (vocal regulation), embryonic temperature stabilized. Vocally regulated temperature was independent of the duration of the rewarming periods over a range of 3 to 8 min, but increased significantly with embryonic development over the final day of incubation. During the final 12 to 18 h before hatching, most embryos were able to regulate vocally their body temperature at or above the level at which cold-induced hatching delays occur. Results are consistent with the interpretation that cold-induced vocalizations are honest signals of offspring need for warmth. Vocal thermoregulation by pipping embryos is a potentially adaptive mechanism facilitating the maintenance of safe embryonic temperature during the critical hours before hatching. *Received 31 March 1993, accepted 17 August 1993.*

BIRD EMBRYOS are functionally ectothermic, unable to regulate body temperature by metabolic heat production (Drent 1970, Freeman and Vince 1974, Whittow and Tazawa 1991). Recent evidence from American White Pelicans (*Pelecanus erythrorhynchos*), however, indicates that embryos exposed to a moderately low ambient temperature (20°C) at the pipped egg stage are able to maintain body temperature (T_b) by calling, provided calls result in rewarming by a cooperative parent or surrogate heat source (Evans 1988, 1990a, b). In the laboratory, American White Pelican embryos are able to maintain T_b near 36°C, sufficient to avoid cold-induced mortality or hatching delays (Evans 1990c). Within naturally incubated clutches, this vocal thermoregulatory response appears to be especially relevant for the last-hatched egg, which is subject to incubation neglect as parents begin to feed and care for the first-hatched chick (Evans 1989, 1990d).

Neglect of terminal eggs is known to occur in several other species where eggs within a clutch hatch asynchronously (e.g. Herring Gulls [*Larus argentatus*], Haycock and Threlfall 1975, Lee et al. 1993; Pied-billed Grebes [*Podilymbus podiceps*], Forbes and Ankney 1988; Green-rumped Parrotlets [*Forpus passerinus*], Beissinger and Waltman 1991; for review, see Evans and Lee 1991). The extent to which late-stage embryos of these species are able to maintain body temperature by vocally eliciting more effective incubation is unknown. We examined aspects of this issue in Ring-billed Gulls (*Larus delawarensis*), a ground-nesting species with modal clutch of three eggs that hatch over a period of up to 2.5 days (Woulfe 1989; for details of life history, see Ryder 1993).

The possibility that neglect of last-laid eggs could pose a problem in Ring-billed gulls was suggested by prior laboratory data (Evans 1990e), which indicated that hatching was significantly retarded when pipped eggs were chilled in a commercial incubator set at 33°C. Under natural conditions, cold-induced retardation selectively affecting last-laid eggs would be expected to increase the magnitude of hatching asynchrony, to the probable detriment of the last-hatched chick (Forbes and Ankney 1988). We hypothesize that cold-induced calling is a potentially important adaptive mechanism whereby chilled late-stage embryos elicit closer parental brooding and, thereby, maintain embryo temperature above the level at which they would be damaged or experience hatching delays. We first assessed the extent of terminal-egg neglect

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in the field, then tested the ability of chilled embryos at the pipped egg stage to regulate T_b vocally, at a safe level, in the laboratory.

To assess the ability of embryos to defend T_b against chilling in the laboratory, it was important to establish that the temperature attained by vocalizing embryos was not simply an artifact of the procedures used. The amount of time that a surrogate heat source was set to rewarm chilled embryos in response to their vocalizations was thought to be a likely confounding source of variation in the steady-state temperature attained by vocally regulating embryos. We examined this possibility by experimentally varying the duration of call-induced rewarming.

Methods

Our study was conducted during the summers of 1989 to 1992. Fieldwork and collection of most eggs were done at a stable colony of about 12,000 pairs of Ring-billed Gulls (Koonz and Rakowski 1985) located at Kaweenakumik (formerly Kawinaw) Lake, Manitoba (52°50'N, 99°29'W; map in O'Malley and Evans 1980).

Neglect of last-hatched eggs.—Neglect was assessed at nests with modal clutch size of three by measuring temperatures of embryos in live pipped eggs and surface temperatures of artificial eggs. For measurements of live embryo T_b, a 30-gauge thermocouple was inserted approximately 1 cm directly into the pip hole, away from the outer shell. The probe was held in position with porous adhesive tape ("Micropore") applied to the exterior of the shell. The thermocouple wire was led below the egg and rim of the nest to an 8-channel Grant Instrument (Cambridge) Model 1203 data logger positioned about 10 m from the nest. Parents returned rapidly to nests with instrumented eggs and resumed apparently normal behavior.

To assess the thermal consequences of neglect of last-hatched eggs, the mean temperatures of pipped eggs subject to neglect (both older siblings hatched; Beer 1962, Evans and Lee 1991) at 12 nests were compared with the mean temperature of first-pipped eggs from 12 control nests. To equate effects of variations in ambient temperature, natural disturbances, or other uncontrolled variables, pairs of eggs (one with both siblings hatched, the other with neither hatched) were measured simultaneously.

The colony was visited once a day to set up the thermocouples and to retrieve information from the data loggers. The gulls were not subjected to any other human disturbance. Temperatures were logged every 10 min throughout the day and night, then the logger was switched to other nests and the procedure repeated. To maintain statistical independence, all temperature records over the entire daily measurement period (range 112 to 143 10-min readings per egg) were averaged to provide a single mean value for each egg. All statistical tests were calculated using Statistix (version 3.5, Analytical Software, St. Paul, Minnesota). Nonparametric tests were employed where data were not normally distributed. All probabilities are based on two-tailed tests.

Temperatures of artificial eggs.—These temperatures were taken to assess the magnitude of terminal-egg neglect in the absence of any embryonic input to the parents. Artificial eggs were constructed by sawing through the pointed end of fresh Ring-billed Gull eggs, then strengthening the inner surface of the empty shell with fiberglass. Two thermocouples were inserted from inside the egg through small holes drilled on opposite sides of the blunt end of the egg, at a plane approximating the location of external pipping. Each probe was positioned so that its outer edge was flush with the external egg surface. A 16-g weight was glued to the inner surface of the egg behind one of the thermocouples, creating a mass asymmetry to ensure that one probe would usually be up, the other down (Drent 1970). The thermocouple leads exited the egg on the weighted side of the shell. The eggs were filled with 1.5% agar and sealed with epoxy (see Evans 1989 for further details).

Temperatures of artificial eggs subject to natural levels of neglect were obtained at 12 nests containing two hatched chicks and a pipped egg, which was exchanged for an instrumented artificial egg. Control readings for artificial eggs not subject to neglect associated with the presence of older siblings were obtained at 12 nests during the late incubation period, up to and including the onset of pipping. One of the three eggs in each control clutch was removed at random, and replaced with an instrumented artificial egg. All other procedures were the same as those described for live pipped eggs.

Egg collection and laboratory incubation.—Unpipped Ring-billed Gull eggs were collected one per nest, without reference to laying order. Eggs were incubated at 37.8 ± 0.5°C and 65 ± 5% relative humidity in a forced air Petersime Model No. 1 commercial poultry incubator. Eggs were turned automatically every 2 h, and were examined at least three times daily to determine the onset of external pipping. Pipped eggs were moved to a smaller, relatively silent circulating air incubator with the same temperature and humidity conditions. Median time between external pipping and hatching of Ring-billed Gull eggs incubated under these conditions in the laboratory is 77.5 h (Evans 1990e:table 3). Eggs in the present experiments were not tested until at least 48 h after pipping, usually later.

Embryonic heat production.—Although gull embryos are not able to regulate body temperature physiologically, they do produce metabolic heat that can raise body temperature above ambient, especially during

the last few days before hatching (Drent 1970). Since this embryonic heat source was lacking in artificial eggs, direct comparison of their temperatures with live embryos required the addition of a correction factor to the artificial egg temperatures. Heat production by pipped Ring-billed Gull eggs ($n = 27$) was estimated in the laboratory. A thermistor probe was inserted about 1 cm into the pip hole, as was done in the field, and read with a YSI Thermistemp thermometer (± 0.05°C). Instrumented eggs were placed in a 33°C incubator and left for 3 h to reach a steady state. Three temperature readings of both the egg and the incubator were then taken at hourly intervals without opening the incubator. The mean elevation of T_b over incubator temperature provided an estimate of embryonic heat production (Drent 1970). An incubator temperature of 33°C was close to the mean temperature of neglected artificial eggs measured in the colony (see results).

Effects of continuous chilling.—To examine the effects of continuous chilling on embryo T_b and calling, pipped eggs were individually placed horizontally, pip hole uppermost, in a close-fitting coil of copper tubing within an insulated chamber (Evans 1990b). Water from one of two insulated tanks (picnic coolers) was pumped through the coil to control the egg's incubation temperature. Water tanks were thermostatically regulated to produce coil temperatures of either the normal commercial incubator temperature of 37.8 ± 0.5°C, or moderate chilling at 20.0 ± 0.5°C. A thermocouple inserted approximately 1 cm directly into the pip hole of the egg, as done for field measurements, was connected to a Sensortek BAT-12 digital thermometer (± 0.05°C). To monitor calls, a microphone connected to a tape recorder and earphones was positioned through a hole in the plexiglas top of the coil chamber, about 2 cm immediately above the pip hole.

For each egg ($n = 10$), the coil temperature was held at 37.8°C for the first 5 min, then lowered to 20°C for 60 min to produce moderate chilling, followed by a return to 37.8°C for a final 5-min period. Body temperature was recorded manually from the BAT-12 thermometer at the end of each successive 5-min period. The number of vocalizations emitted during each 5-min period was monitored through the earphones and noted manually.

Vocal regulation of temperature.—An initial test for embryonic vocal temperature regulation under standardized laboratory conditions was conducted with 10 pipped eggs. The apparatus used in the above-described chilling experiment was modified slightly to conform to the method used previously for vocal regulation of temperature in American White Pelican embryos (diagram and full description in Evans 1990b). In the modified apparatus, T_b was recorded automatically by connecting the analog output of the BAT-12 thermometer to a strip-chart recorder. Embryonic calls were recorded automatically by connecting the microphone to a sound-operated relay and event recorder. Sensitivity of the relay was set to be triggered by calls reaching a sound level of 78 dB or greater (GenRad model GR 1565-B sound-level meter, B fast scale) as measured at the position of the microphone, approximately 2 cm above the pip hole.

The sound-operated relay was also used to control water flow from the two temperature-regulated water tanks. At the start of a test, the egg coil was held at 20°C by a continuous flow of water from the chilled tank. When the embryo began to vocalize, the relay triggered a timer and pump system that switched the source of circulating water to the warm tank, thereby raising the coil temperature to 37.8°C. To ensure that on average more than a single call or spurious noise would be required to initiate rewarming, a delay counter (one to five calls) was inserted between the sound-operated relay and the timer. Rewarming was pre-set on the timer to last for an arbitrary period of 4 min, then the coil automatically reverted to 20°C and chilling continued until another call-induced rewarming cycle began. Thus the default condition, in the absence of embryonic calling, was continuous chilling. However, by calling in response to the onset of chilling, embryos could start a rewarming cycle, thereby controlling the ratio of chilling to rewarming time and hence their own temperature.

Vocal regulation tests lasted for 2 h, then the embryo was subjected to a further 30 min of continuous chilling. This period of chilling provided an additional control to establish whether the temperature attained during vocal regulation could be maintained metabolically in the absence of vocally produced rewarming periods.

Effect of varying duration of rewarming.—To assess the possibility that T_b attained by embryos in the above-described vocal regulation test was a function of the duration of the rewarming periods rather than a true indication of embryonic temperature regulation ability, we conducted an additional series of vocal regulation tests in which the duration of rewarming cycles was set at 3, 5, 7, or 8 min. Four separate batches of eggs ($n = 9$ or 10 per batch) were used. Time of hatching was recorded to the nearest hour for those that hatched during the normal daily working period. All other procedures were the same as for the vocal regulation experiment using 4-min rewarming periods described in the preceding section.

RESULTS

Neglect of last-hatched eggs.—At nests where both older siblings had hatched, embryonic temperatures of the remaining last-hatched pipped eggs averaged 36.8°C, significantly below the mean value of 38.7°C for embryos in pipped eggs at nests where no young had yet hatched. Lower mean temperature of the ne-

TABLE 1. Effect of incubation neglect on temperature of live pipped and artificial Ring-billed gull eggs ($\bar{x} \pm$ SE; for each cell, $n = 12$ eggs).

	Neglected (sibs hatched)	Not neglected (none hatched)	Statistic[a]
A. Live pipped eggs			
Mean	36.8 ± 0.47	38.7 ± 0.14	$t_{12.9} = 3.90$**
CV[b]	4.43	1.25	$F_{11,11} = 11.43$***
B. Artificial eggs (top surface)			
Mean	33.9 ± 0.74	36.5 ± 0.28	$t_{14.0} = 3.33$**
CV	7.51	2.64	$F_{11,11} = 6.95$**
C. Artificial eggs (bottom surface)			
Mean	32.5 ± 0.65	32.6 ± 0.34	$t_{16.5} = 0.11$
CV	6.94	3.60	$F_{11,11} = 3.71$*

[a] *F*-values test for equality of variance. Two-tailed *a priori* *t*-tests for independent samples with unequal variance; df (shown as subscript) according to Satterthwaite's approximation (Snedecor and Cochran 1980). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; others ns. ($P > 0.05$).

[b] Coefficient of variation.

glected eggs was accompanied by a highly significant increase in temperature variability (Table 1). The upper-surface temperatures of artificial eggs at nests where the first two chicks had hatched (neglected eggs) were also significantly lower and more variable than at nests where none had hatched (not neglected; Table 1). The presence of two hatched chicks also increased variability at the lower surface of artificial eggs, but there was no difference in mean temperature. At the bottom of artificial eggs, mean temperature was always below the temperature at the top of the same eggs, but the magnitude of the top-to-bottom difference was significantly less for neglected eggs owing to the significant lowering of their upper surface temperature compared with those that were not neglected (unpaired $t = 5.65$, df = 22, $P < 0.0001$). All mean temperatures at artificial egg surfaces were lower than those within live pipped eggs (Table 1).

To permit a realistic comparison of neglect between artificial and live eggs, an estimate of the internal temperature of artificial eggs (see Evans 1990d) was obtained by taking the average of the temperatures recorded from the top and bottom probes, and adding to it our measured estimate of embryonic heat production. Based on surface temperatures listed in Table 1, calculated internal temperature of neglected artificial eggs exclusive of embryonic heat production was 33.2°C. Embryonic heat production, calculated in a laboratory incubator set at

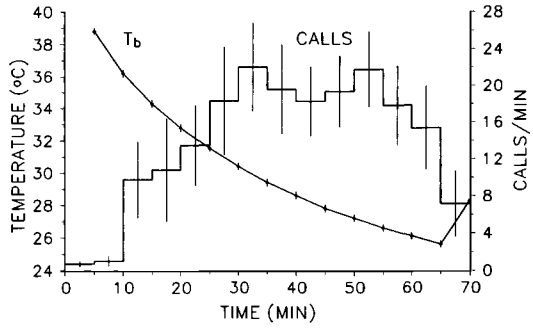

Fig. 1. Body temperature (T_b) and rate of calling of Ring-billed Gull embryos in pipped eggs held for 5 min at control temperature (37.8°C), then exposed to continuous moderate chilling (20°C) for 1 h, followed by 5 min rewarming ($\bar{x} \pm$ SE; $n = 10$).

33°C, averaged 2.3 ± SE of 0.1°C (embryo T_b minus incubator temperature). Summing these values gives an estimated internal temperature for neglected artificial eggs, corrected upwards for embryonic heat production, of 35.5°C.

Effects of continuous chilling in laboratory.—When live pipped eggs were continuously chilled at 20°C, T_b dropped steadily, with little variation, from the initial incubation temperature near 38.8°C down to 25.6°C by the end of 1 h (Fig. 1). Few vocalizations were emitted before or during the first 5-min period of chilling (Fig. 1; second bar). Calls increased significantly (Wilcoxon matched-pairs signed-ranks test, $T = 1$, df = 9, $P < 0.01$) during the next 5-min period. Calling continued to increase gradually over about the first 25 min of chilling, then remained at high but variable levels until the final 5-min period of rewarming. Comparison of T_b and call rates indicates little effect of temperature on calling until T_b had dropped below 36°C, with maximum vocal effect being reached at approximately 30 to 31°C. Calling decreased significantly ($T = 1$, df = 10, $P < 0.01$) during the final 5-min period of rewarming, although it did not reach the low level present before the onset of chilling (Fig. 1; compare first and last bar).

Vocal regulation of T_b.—During vocal regulation sessions, when chilling was ameliorated by 4-min periods of vocally-induced rewarming, average T_b initially dropped to approximately 33°C (Fig. 2). The rate of decline then slowed and after 30 min T_b became relatively stable, averaging 31.9°C during the final 1.5 h of vocal regulation. There was an immediate steep drop in T_b when eggs subsequently were exposed to

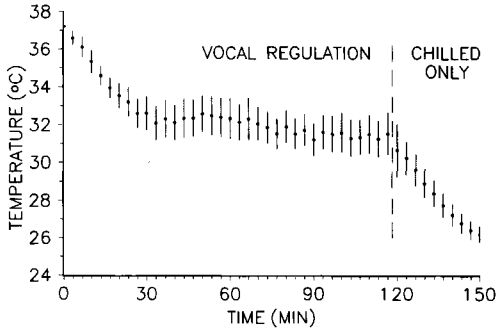

Fig. 2. Body temperature of Ring-billed Gull embryos in pipped eggs during 2-h period of vocal regulation in which eggs chilled at 20°C were given 4-min periods of rewarming at 37.8°C in response to cold-induced vocalizations. Embryos exposed to continuous chilling at 20°C during final 30 min of testing ($\bar{x}$ ± SE; n = 10).

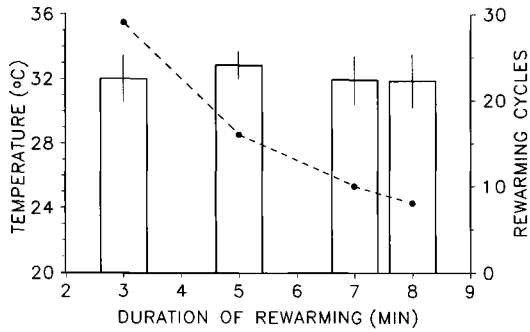

Fig. 4. Body temperature (open bars) and number of vocally-elicited rewarming cycles (filled circles and dashed line) in relation to pre-set durations of rewarming cycles ($\bar{x}$ ± SE for temperature, $\bar{x}$ for number of cycles; n = 9 or 10 at each duration).

continuous chilling at 20°C during the final 30 min of testing (Fig. 2).

The rapid drop in T_b during the first 30 min of vocal regulation corresponded to a relatively low rate of calling (1.44 ± 0.41 calls/min) in response to the onset of periods of chilling. Mean call rate in response to chilling was significantly greater (5.04 ± 1.05 calls/min; paired t = 4.03, df = 9, $P < 0.005$) during the final 1.5 h of vocal regulation, when T_b was maintained at a relatively stable level.

Call rate declined rapidly during the first minute of call-induced rewarming (Fig. 3; Friedman ANOVA, X^2 = 16.13, df = 2, $P <$ 0.001). By the end of the 4-min periods of rewarming, mean call rate had dropped to 0.46 ± 0.20 calls/min, about 10-fold below call rates occurring in response to chilling during the final, relatively stable 1.5 h of vocal regulation (see above), and 3-fold below call rates that were ineffective in maintaining T_b during the first 30 min of testing.

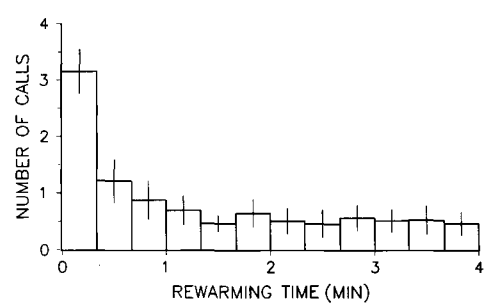

Fig. 3. Number of calls emitted during successive 20-s intervals following onset of vocally-elicited rewarming cycles of 4-min duration ($\bar{x}$ ± SE; n = 10).

Effect of varying duration of rewarming.—Varying the duration of rewarming cycles from 3 to 8 min had no effect on T_b averaged over the second hour of vocal regulation ($F_{3,33}$ = 0.10, P = 0.96). T_b means were all near 32°C (Fig. 4), similar to the value of 31.9°C obtained for the preceding experiment that used 4 min of rewarming.

The similarity in average T_b obtained despite variations in the duration of the rewarming cycle appeared to be a direct result of a corresponding change in the number of rewarming cycles produced by the vocalizing embryos. As shown in Figure 4, there was a significant increase in the number of rewarming cycles produced when they were of shorter duration (Kruskal-Wallis ANOVA, X^2 = 14.74, P = 0.002, df = 3). As a result of this inverse relationship, the cumulative duration of rewarming did not differ among the four treatments (X^2 = 0.40, P = 0.94, df = 3); hence, mean T_b was also the same among groups.

Developmental effects.—Examination of individual records for live pipped eggs measured in the field indicated that, at the time the thermocouples were implanted, 4 of the 12 eggs had well-developed pip holes indicative of a relatively advanced developmental state. Mean T_b of the four advanced eggs was 37.4°C, compared with a mean of 36.5°C for the other eight eggs measured. While sample size was too small for a meaningful statistical comparison of this difference, the result raised the possibility that

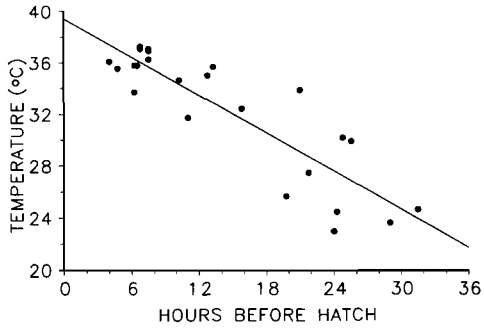

Fig. 5. Vocally regulated body temperature of Ring-billed Gull embryos in pipped eggs, plotted against development measured in hours before hatch ($\bar{x}$ for individual embryos; $n = 24$).

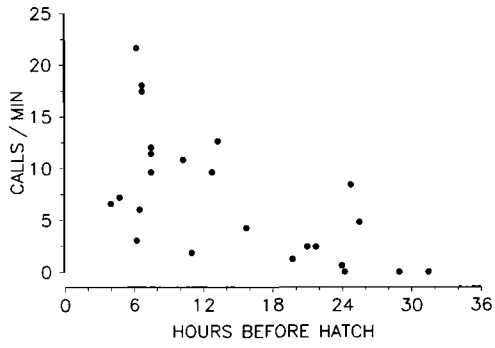

Fig. 6. Rate of cold-induced calling by vocally thermoregulating Ring-billed Gull embryos in pipped eggs, plotted against developmental stage measured in hours before hatch ($\bar{x}$ for individual embryos; $n = 24$).

vocal temperature regulation develops progressively over the final one to two days before hatching. Alternatively, the effects noted in the field could have been due to greater embryonic heat production in the older embryos (Drent 1970). To examine this issue more closely, we compared vocal regulation performance in relation to time until hatching in 24 eggs from the preceding laboratory experiment for which hatching times were known.

There was a strong inverse correlation between the number of hours before hatching that an egg was tested and its mean T_b during the final hour of vocal regulation (Fig. 5; $r = -0.88$, df = 22, $P < 0.01$). This developmental effect reflected a tendency for embryos closer to hatching to call more when chilled (Fig. 6; $r = -0.64$, df = 22, $P < 0.01$). A correspondingly greater number of rewarming cycles and a significantly greater total rewarming time ($r = -0.86$, $df = 22$, $P < 0.01$) were also produced by embryos tested closer to hatching.

Discussion

Terminal-egg neglect in the field.—Live embryos in pipped Ring-billed Gull eggs at nests where two siblings were already hatched were subjected to significantly lower incubation temperatures than first-pipped embryos, but it is doubtful that the level of neglect was sufficient to cause significant temperature-induced developmental damage or hatching delays. It is known that hatching delays occur in a commercial incubator when pipped embryo temperature falls to about 35.3°C (corrected upwards for embryonic heat, eggs incubated at 33°C; Evans 1990e). Temperatures of live embryos in pipped eggs subject to natural levels of neglect in the present study approached this critical temperature, but were still about 1.5°C above it. In agreement with this result, hatch retardation of viable last-hatched eggs is rare in Ring-billed Gulls at Kaweenakumik Lake (Evans unpubl. data).

The field results for Ring-billed Gulls are also in general agreement with earlier findings for Herring Gulls (Lee et al. 1993). In the latter species, terminal-egg neglect resulted in lower mean embryo temperatures (33°C), but the level of neglect did not produce hatching delays or cause increased mortality of pipped eggs during either natural or laboratory incubation. Incubation of pipped eggs at 30°C or lower caused both hatch retardation and reduced hatchability in Herring Gulls (Lee et al. 1993). Evidently parents of both species usually maintain last-hatched pipped eggs at developmentally safe temperatures, with Herring Gull eggs being slightly more cold hardy than the smaller eggs of Ring-billed Gulls. There is evidence that some passerines also allow their eggs to cool but still maintain them at safe temperatures by limiting the duration of nest absences (Haftorn 1988). Other investigators (Beer 1962, Drent 1970, Haycock and Threlfall 1975; for review, see Evans and Lee 1991) have noted mortality in pipped eggs of gulls and attributed it to incubation neglect, but unfortunately temperatures of the neglected eggs were not reported.

The potential importance of a live embryo in maintaining a safe incubation temperature dur-

ing the period of neglect was suggested by the relatively low, potentially damaging temperatures reached by artificial eggs (Table 1). The discrepancy in temperature between live and artificial eggs was presumably due, at least in part, to probe location and lack of embryonic heat production in the artificial eggs. When these variables are taken into account (see results), our estimate of artificial egg temperature was 35.5°C, which was still 1.3°C below the mean T_b of neglected live pipped eggs, and close to the temperature (T_b = 35.3°C in an incubator set at 33°C) at which hatching delays are induced in this species (Evans 1990e). Results thus suggest that vocal or other relevant stimuli produced by live pipped eggs decrease their risk of experiencing low, potentially harmful temperatures. A direct test of this interpretation under field conditions awaits further study, as does examination of the opposite effect of neglect overheating, which could be a potential problem in warmer climates (Grant 1982).

Vocal temperature regulation in laboratory.—Like American White Pelicans (Evans 1990b), late-stage embryos of Ring-billed Gulls exhibited an ability to regulate body temperature in the laboratory when a surrogate heat source was provided briefly in response to cold-induced vocalizations. The overall form of the vocal regulation temperature curve for Ring-billed Gulls (Fig. 2) was in large part a reflection of the compensatory relationship between T_b and vocalizations as described in the results and shown in Figure 1. Low levels of calling during initial chilling would cause few if any periods of rewarming in the vocal regulation apparatus, thereby allowing T_b to drop. As T_b dropped farther, however, calling rate increased, which in the vocal regulation apparatus would trigger an increasing number of rewarming cycles and a stabilization of T_b.

The initial drop in vocally regulated T_b in Ring-billed Gulls (Fig. 2) is similar to, but more extreme than, that noted previously in American White Pelicans. When tested under the same conditions in the same apparatus, pelican T_b stabilized at or near 36°C (Evans 1988, 1990b), about 4°C warmer than Ring-billed Gulls. A consistent species difference of this magnitude seems unlikely to be due merely to constraints imposed by the apparatus. The interpretation that the temperature attained during vocal regulation was directly related to the pattern of cold-induced calling was supported by: (1) the increasingly strong embryonic vocal response to chilling as T_b dropped below 36°C (Fig. 1); (2) the significant increase in call rate during cold cycles after T_b had dropped during the first 30-min in the vocal regulation apparatus; and (3) the rapid decrease in call rate during rewarming cycles (Fig. 3).

The finding that vocally regulated embryonic temperature was not affected by the duration of individual rewarming periods provides further evidence that vocal temperature regulation was largely a function of the embryos themselves and not merely a reflection of the parameters imposed on the system by the experimenters. Instead of allowing temperature to drop because of shorter rewarming periods, as might be expected, the embryos compensated by increasing the frequency of vocally-elicited rewarming cycles, thereby maintaining mean T_b independent of the duration of the individual periods of rewarming (Fig. 4).

Developmental effects.—The significant improvement in vocal regulation ability as embryos approached the time of hatching (Fig. 5) points to the important conclusion that even in species with vocal embryos, external pipping and the onset of vocal temperature regulation do not necessarily occur at the same time. In American White Pelicans, embryonic vocalizations can be detected as vibrations in the egg at least two days before there are any signs of external pipping (O'Malley and Evans 1980), suggesting that species may differ significantly in the absolute and relative timing of these events. In addition, there is apparently no necessary developmental correlation between the onset of vocal thermoregulation and physiological thermogenesis, which occurs significantly later in altricial American White Pelicans (Evans 1984) than in semiprecocial Ring-billed Gulls (Dawson et al. 1976; for general review of endothermic development, see Whittow and Tazawa 1991). This developmental uncoupling of the vocal and metabolic thermoregulatory systems would appear to be critical to the adaptive development of low-cost vocal thermoregulation within the egg. The potential importance of the vocal thermoregulatory mechanism to embryos subject to terminal-egg neglect is highlighted by the fact that it appears to be the only way that an avian embryo, trapped within an egg shell, can do something about its own thermal environment; ectothermic bird embryos can call and inform a parent that they are

cold, but they cannot generate significant amounts of metabolic heat (Whittow and Tazawa 1991), and they cannot thermoregulate by moving to a warmer microhabitat.

Owing to the strong effect of development on vocal regulation, our hypothesis that Ring-billed Gull embryos can vocally maintain their temperature at a level sufficiently high to avoid hatching delays or other damage was supported only for embryos tested within about 15 to 18 h of hatching (Fig. 5). Only during the final 8 h before hatching were embryos able to maintain T_b at a level equal to that experienced by naturally incubated last-hatched embryos. If vocal temperature regulation functions as an adaptation to avoid cold-induced hatching delays, it is evident that this function is restricted, in this species, to the final few hours of embryonic development.

Whether pipped gull eggs are more susceptible to the effects of chilling during the last few hours before hatching than they are during the preceding two to four days is unknown, but seems plausible. Susceptibility of eggs to chilling commonly varies with development (Hunter et al. 1976, Webb 1987). An alternative, although not mutually exclusive, interpretation is that the embryonic age effects found in this study simply reflect the normal developmental processes needed to ensure the full functioning of the vocal temperature regulation system in newly-hatched young. Vocal regulation of body temperature is well developed in Ring-billed Gulls on the day of hatching (Wiebe and Evans in press), which lends credence to this interpretation. A developmental interpretation would seem especially relevant for the first and probably the second eggs to hatch, as these are less likely than the last-hatched egg to be subject to neglect during the hatching period (Lee et al. 1993). Further experimentation is required to assess the relative merits of these or other functional and developmental interpretations of vocal temperature regulation in gulls.

Honest signalling of offspring need for warmth.—The finding that calling by chilled Ring-billed Gull embryos increased when T_b dropped, then decreased upon subsequent rewarming, suggests that cold-induced vocalizations provided an honest indication of offspring need to be rewarmed by a brooding parent. That offspring can be selected to produce honest signals of need has recently received strong theoretical support (Godfray 1991, Maynard Smith 1991, Johnstone and Grafen 1992), but has rarely been tested experimentally (Litovich and Power 1992, Mock and Forbes 1992, Redondo and Castro 1992). In the context of embryonic vocal temperature regulation, offspring need in the form of lowered body temperature and resulting hatching delays can readily be measured in relation to the production of vocal signals, at least in gulls, pelicans, or other birds in which cold-induced calling is well developed (see Evans 1988). Parental responses to these signals can also be observed and experimentally quantified with the aid of playbacks (e.g. Evans 1992). Vocal thermoregulatory behavior of late-stage embryos or newly-hatched young thus provides a unique system for empirical investigation of this important aspect of animal communication.

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