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EFFECTS OF VARIABLE HUMIDITY ON EMBRYONIC DEVELOPMENT AND HATCHING SUCCESS OF MOURNING DOVES

GLENN E. WALSBERG AND CATHERINE A. SCHMIDT
Department of Zoology, Arizona State University, Tempe, Arizona 85287, USA

ABSTRACT.—Newly laid Mourning Dove (*Zenaida macroura*) eggs were held at a constant temperature of 37.5°C at one of three relative humidities: 0–5% ("arid"); 35–45% ("intermediate"); and 95–100% ("humid"). Samples in the humid treatment lost significantly less water than those in intermediate or arid treatments. There was significantly more water in embryos of the humid treatment than in the intermediate or arid treatments. Protein and carbohydrate content of samples in the humid treatment was significantly smaller than those in intermediate or arid treatments, whereas lipid and ash contents did not differ among the three treatments. Even in the worst case of constant exposure to near-saturation humidities, 50% of embryos succeeded to hatching. This is, however, significantly below the 85% or 90% hatching success characteristic of the arid or intermediate humidities, respectively. Of the doves that failed to hatch in the humid condition, 90% developed to pipping and initiated aerial respiration. Contrary to previous analyses that suggested that hatching failure is likely due to the embryo's failure to initiate pulmonary respiration or to maintain constant hydration, the failure of Mourning Dove embryos to hatch apparently was due to severe mechanical restriction upon movement within the rigid shell. This was produced by the lack of adequate internal space produced by evaporative water loss. *Received 4 April 1991, accepted 6 November 1991.*

MAINTENANCE of an appropriate hydric environment within avian eggs usually is assumed to be vital to embryonic development (Lundy 1969). Consequently, evaporative water loss from eggs has been the focus of substantial research (Ar et al. 1974, Rahn and Ar 1974, Paganelli et al. 1975, Morgan et al. 1978, Ar and Rahn 1980). Rahn and Ar (1974) found that an average of 15% of the mass of newly laid eggs is lost during incubation. An average of an additional 3% is lost at pipping (i.e. when the embryo first breaks through the shell; Rahn 1984). This loss of mass essentially equals loss of water, because the mass of the other materials exchanged through the shell, oxygen and carbon dioxide, are equal and in opposite directions (Drent 1970).

Two major functional consequences have been attributed to this egg water loss. Ar and Rahn (1980) argued that evaporative water loss balances metabolic water production by the embryo, thereby producing constant embryonic hydration. In addition, water loss also may facilitate the initiation of pulmonary respiration. Egg dehydration allows formation of an air cell within the shell (Simkiss 1974), which is used immediately prior to hatching when pulmonary respiration begins (Seymour 1984).

Though expected to be an important determinant of successful development, tolerance of avian embryos to variable water loss is poorly understood. Most data are for domestic chickens (*Gallus gallus*). Snyder and Birchard (1982) noted that excess water loss reduced hatching success in this species. However, Simkiss (1980) found that chickens hatched even when shell porosity was increased three to four fold and water loss increased. Davis et al. (1988) noted tolerance of chicken embryos to both high and low humidities, although considerable variation in relative hydration existed. In a study of wild birds, Carey (1986) found substantial tolerance to artificially induced alteration in water loss rate in Red-winged Blackbirds (*Agelaius phoeniceus*).

Finally, it is notable that maintenance of a given hydration state depends on the amount of metabolic water generated and lost. Metabolic water production varies with the relative ratios of proteins, lipids and carbohydrates being metabolized (Sotherland and Rahn 1987). Altering the relative use of these substrates might provide a mechanism to enhance tolerance to water stress. However, most studies on avian egg composition have focussed only on newly laid eggs (e.g. Carey et al. 1980, Williams et al. 1982, Warham 1983, Birkhead and Nettleship 1984, Ricklefs 1984) and little is known regarding changes in composition with time or varying hydric conditions.

In our analysis, therefore, we addressed the following questions. (1) What is the tolerance level of avian embryos to extreme hydric conditions during development? (2) To what phase of development do such embryos successfully develop and what does this indicate about major functional consequences of dehydration? (3) What effect do extreme hydric conditions during development have on organic composition of avian embryos?

The Mourning Dove (*Zenaida macroura*) is an excellent species on which to conduct such analyses. It ranges widely south from Canada to Panama and east from California to the West Indies (Goodwin 1967) and, therefore, encounters widely differing humidities. In Arizona, they are locally abundant and nest repeatedly in the Sonoran Desert from February through August. Clutches typically consist of two eggs (Nice 1922).

MATERIALS AND METHODS

Sample collection.—Newly laid eggs were collected during June and July from Mourning Doves nesting in orchards in Maricopa County, Arizona. Eggs were packed in cotton, placed in plastic vials, and taken to the laboratory within 3 h of collection. Clutches were kept together in the event that one egg was infertile or broken during handling, but data were recorded from only one egg from a particular clutch.

Experimental conditions.—Eggs were held at T_{AIR} = 37.5°C during all treatments, mimicking natural incubation temperatures (Walsberg 1983). Humidity was measured daily using a Vaisala humidity probe calibrated using salt solutions (Winston and Bates 1960). Eggs were randomly assigned to one of the following relative humidity treatments that approached the maximum range of humidities possible at an air temperature of 37.5°C.

(1) For the arid treatment, eggs were exposed to a relative humidity of 0–5% by placing then on a platform in a chamber over a layer of Drierite. The chamber was continuously flushed with air dried by passing through a column of Drierite. (2) For the intermediate treatment, eggs were exposed to a relative humidity of 35–40% by placing them in a commercial incubator that allowed control of humidity. This humidity parallels that experienced by Mourning Dove eggs in the Sonoran Desert (Walsberg 1983). (3) In the humid treatment, eggs were exposed to a relative humidity of 95–100%, representing essentially the highest humidity possible at average incubation temperatures. This condition was produced by placing eggs on a platform in a 40-L container over a layer of distilled water. Air was pumped continuously into the container after humidification by bubbling serially through two 0.75-m columns of water.

Aging embryos.—At the time of collection in the field, the age of each egg was estimated by floating in water. Floating indicates increased age associated with air cell formation. Only clutches in which both eggs failed to float, and therefore apparently were laid recently, were collected. In addition, all single-egg clutches were collected. Single-egg clutches were assumed to be about one day old as doves lay one egg a day. Oxygen consumption values for the first week of development for the 15 single-egg clutches were combined and mean values quantified for each day of development. Other individuals were aged by matching their oxygen consumption to these mean values. No differences in any result could be detected associated with these aging methods (analysis of variance, ANOVA, $P > 0.05$).

For such determinations of embryonic age, egg oxygen consumption was measured daily using 50-ml syringes as metabolic chambers. Eggs were held in the chambers for 30 min daily for the first week of development and 10 to 15 min daily during the second week of development to avoid exposure to hypoxic conditions. Oxygen content of chamber and room air was determined using an Applied Electrochemistry S3A O_2 analyzer. Tests were conducted with eggs held at 37.5°C. Eggs not consuming any oxygen after three days were presumed infertile ($n = 2$), and those that ceased oxygen consumption for two days within the first week of development were presumed dead and omitted from further analyses ($n = 2$). We assumed that treatment did not adversely affect the ignored samples. Eggs cracked during handling also were disregarded.

Mass determination.—Eggs were weighed daily to the nearest 0.01 g and the data normalized for a 24-h loss rate. The first measurement was omitted as inaccurate because less than 12 h had passed since collection and treatment had not yet had a substantial effect. Final measurements in those samples where chicks had broken through the shell were omitted, as this greatly increases water loss. Twenty individuals were used in each treatment, with mean initial mass not differing significantly between treatments (ANOVA, $P = 0.38$).

Compositional analyses.—Embryos that died after at least one week of development and birds that broke a hole through the shell (pipped externally), but did not hatch, were preserved by freezing. Hatchlings were killed by exposure to carbon dioxide and frozen until dissection.

Water content of yolk and embryos was determined separately by dissecting the remaining yolk out of hatchlings. Fully developed embryos that pipped externally, but failed to hatch, were dissected from their shells and their yolks removed. Embryos that died during development were dissected from the egg and their yolk removed. Samples were weighed to the nearest 0.1 mg and dried to constant mass at 65°C to determine water content. Embryos and their yolks then were combined and ground with a mortar and

pestle. Lipids were extracted using chloroform in a Goldfisch fat-extraction apparatus.

After lipid extraction, samples were dried and ground in a Wiley mill. Ash content was determined by burning 10-mg aliquots for 3 h at 550°C in a muffle furnace. The difference between the dried lipid-free fraction and the ash fraction was used to estimate the protein and carbohydrate fraction.

Statistical analyses.—Unless otherwise noted, mean values were compared using an analysis of covariance (ANCOVA) followed, when appropriate, by a Tukey test (Zar 1984). Differences are accepted as significant at $P < 0.05$. *P*-values cited are for ANCOVA if no significant difference was detected, and for the Tukey test if differences were detected.

Results

Water loss.—Analysis of covariance with initial egg mass as a covariate reveals no significant effect upon water loss of initial egg mass differing between treatments ($P = 0.29$). Prior to pipping, however, significantly less water was lost from eggs incubated in the humid treatment than from eggs incubated under either the arid ($P = 0.012$) or intermediate conditions ($P = 0.0012$; Fig. 1). Mean values for pre-pipping water loss did not differ significantly between the arid and intermediate treatments ($P = 0.11$; Fig. 1), nor did post-pipping water loss of those embryos that hatched successfully differ between humidity treatments ($P = 0.14$; Fig. 1). Embryos that pipped but did not hatch in the humid condition lost 3.8% ± 1.2% ($\bar{x}$ ± SE, $n = 9$) of their initial mass by post-pipping water loss.

Body composition.—Varying the humidity significantly altered the body composition of full-term embryos ($P = 0.04$). There was no significant effect of initial egg mass between treatments when the data were analyzed using ANCOVA with initial egg mass as a covariate ($P = 0.43$; Fig. 2). Fractional water content was significantly higher in embryos incubated under very humid conditions compared to either the intermediate (Tukey test, $P = 0.010$) or arid conditions ($P = 0.018$), but did not differ significantly between the arid and intermediate treatments ($P = 0.07$). When water content was analyzed for the embryo and yolk separately, the only significant effects were on the water content of the embryo and not on the yolk (Table 1). An *a posteriori* use of the Tukey test indicated that yolk-free embryos from the humid treatment contained significantly more water than those from the arid or intermediate treatments. Water content of yolk-free embryos exposed to the latter two treatments did not differ significantly, using ANCOVA with yolk-free embryo mass as a covariate. No statistically significant differences were detected in the water content of the yolk ($P = 0.28$), indicating that the differences in whole embryos are due primarily to differential water content of the yolk-free embryos.

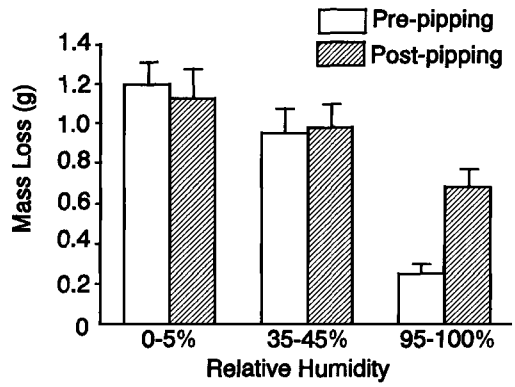

Fig. 1. Total water loss from Mourning Dove eggs incubated in three humidity treatments. Values expressed as percentage of initial egg mass. Sample size of 20 for each treatment. Values are means with 1 SE indicated.

Humidity treatment did not affect either the lipid or ash content of the embryo (ANCOVA using yolk-free embryo mass as a covariate, $P = 0.54$; Fig. 2). However, embryos in the humid treatment did contain significantly less carbo-

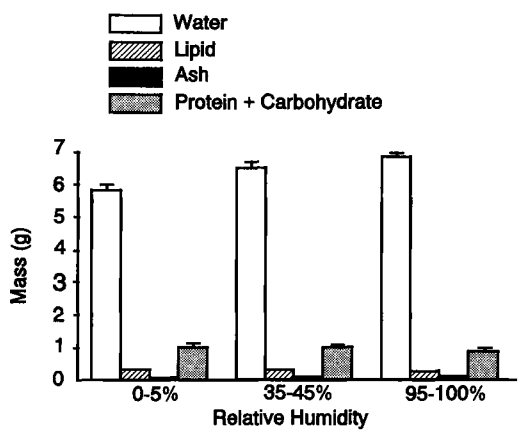

Fig. 2. Organic composition of full-term Mourning Dove embryos incubated in three humidity treatments. Sample size of 20 for each treatment. Values are means with 1 SE indicated. Standard errors not shown are too small to plot separately.

TABLE 1. Percentage water content for Mourning Dove embryos incubated at 37.5°C in three environmental humidities. Means, with standard errors in parentheses. Sample size of 20 for each treatment.

	Arid	Intermediate	Humid
Percent water in yolk-free embryo	83.2 (0.54)	84.2 (0.49)	86.5 (0.29)
Percent water in yolk	65.9 (1.08)	67.2 (1.15)	69.0 (0.78)

hydrate and protein than did embryos from either of the other two treatments (Tukey test, $P = 0.02$).

Hatching success.—Ambient humidity substantially affected hatching success (Fig. 3). Significantly more embryos (50% of total) died before hatching in the humid condition than in either the arid (15%) or intermediate (10%) conditions ($P = 0.006$, R×C test of independence using *G*-test; Sokal and Rohlf 1981).

Methods of hatching and apparent causes of hatching failure.—Birds hatching successfully in the arid and intermediate treatments followed the typical avian pattern of first breaking a hole near one end of the shell, then rotating inside the egg while perforating the shell. This formed a circular fracture around one end of the shell. The birds then pushed against the smaller end of the pipped shell, broke the shell apart at the circular fracture, and emerged.

In the humid treatment, only 5% died prior to pipping (Fig. 3). Rather, almost all (95%) of the birds made the initial hole in the shell and, in some cases, were heard vocalizing. These birds that pipped, however, did not rotate inside the shell and, therefore, did not form a circular fracture. Those that managed to hatch (50% of total) did so by pushing out through the small initial hole and simply shattering the shell. The remaining embryos (45% of total) pipped externally but failed to emerge (Fig. 3). The latter accounts for 90% of those birds that failed to emerge (Fig. 3). The latter accounts for 90% of those birds that failed to hatch. Thus, hatching failure of embryos in the humid treatment apparently involved mechanical restriction that prevented formation of a circular fracture around the egg.

DISCUSSION

Role of evaporative water loss in successful development.—Our results support the view that large-scale changes in environmental humidity can have important effects on the likelihood of successful development in embryonic birds. However, the major source of mortality apparently was mechanical restrictions on the embryo's ability to make the motions necessary to allow it to break the shell. This was imposed by the shell's rigidity and the failure to produce adequate internal space by evaporative water loss. Remarkably, this factor, which was of greatest importance in this experiment, has not been detected by previous workers.

When exposed to extremely high humidity, most doves developed to pipping and breathed with their beaks outside the shell. Thus, development is not stopped at an early stage, but almost always continues to full term and pulmonary respiration is initiated even in the absence of a substantial air cell. The slight water loss (3.2% of initial mass) exhibited by these doves exposed to near-saturation levels of water vapor is comparable to the 2 to 3% loss observed in Mallee Fowl (*Leipoa ocellata*; Seymour and Ackerman 1980). In the related Brush Turkey (*Alectura lathami*), Seymour (1984) found that embryos do not initiate pulmonary aeration be-

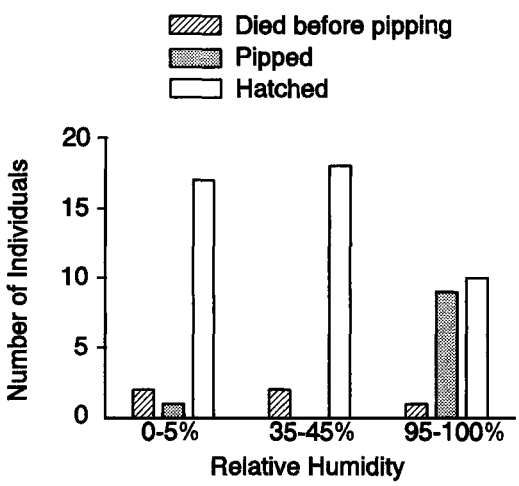

Fig. 3. State at death for Mourning Dove embryos exposed to three humidity treatments. Sample size of 20 for each treatment. "Pipped" indicates bird broke shell, but did not hatch.

fore pipping. Such observations and the present analysis suggest that the air cell is not crucial for initiation of pulmonary respiration.

A second important function suggested for evaporative water loss in avian eggs is that constant embryonic hydration is maintained by evaporation balancing oxidative water production (Ar and Rahn 1980). This view is not supported by our observation that Mourning Dove embryos almost always develop to full term even without substantial water loss.

Possible significance of differential use of metabolic substrates.—The significant depletion of protein or carbohydrates in eggs exposed to very humid conditions compared to less humid environments could indicate greater reliance on nonlipid metabolic substrates in the humid condition. It is not clear that this would be adaptive, however. Oxidation of a given mass of lipid generates more water than does oxidation of a similar mass of protein or carbohydrate. This might suggest the decreased oxidation of lipids would decrease the amount of metabolic water generated and, therefore, compensate for the decreased water loss occurring in humid environments. However, the amount of energy available from lipid oxidation is sufficiently large so that the ratio of oxidative water produced to energy liberated is typically lower when lipids are utilized compared to proteins or carbohydrates (Schmidt-Nielsen 1964). To supply a given amount of energy for embryonic metabolism, therefore, preferential reliance on lipid substrates should minimize oxidative water production in birds developing in humid environments. Our observations are contrary to this expectation. An alternative explanation for the lower protein and carbohydrate values in embryos developing in the humid condition is that it may result from the failure to utilize some albumin, which would reduce embryonic water content (Davis et al. 1988). This was, unfortunately, not quantified in the present analysis and merits further attention.

Tolerance to extreme ranges of environmental humidity.—It is striking that under the most extreme range of environmental humidities possible at normal incubation temperatures, a large fraction of the embryos developed to full term and were able to hatch. In the worst case of constant exposure to near-saturation humidities, one-half of the embryos succeeded to hatching. Previous studies that demonstrated avian sensitivity to humidity (Lundy 1969, Snyder and Birchard 1982) have used chickens as the experimental birds. However, domestic fowl have been artificially selected for egg characteristics for many generations and, thus, generalizations to other species from such data should be made with care. In contrast, our data and those of Carey (1986) indicate that considerable tolerance to widely varying water loss does exist in at least some naturally occurring species.

Our results indicate that Mourning Doves are remarkably tolerant of extreme ranges of humidity and wide variation in egg water loss. This suggests, therefore, that humidity effects on development are not an important selection pressure in this species. The degree to which similar patterns may be expected in other species is unclear. Development of embryonic tolerance to variable dehydration in a widely distributed form such as Mourning Doves may be selectively advantageous, and substantially different patterns may occur in species occupying a narrow range of climates.

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