Adaptations of Black Tern (*Chlidonias niger*) Eggs for Water Loss in a Moist Nest

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Eggs of Black Terns (*Chlidonias niger*) are laid in damp, spongy nests 3–5 cm above water line. The daily mass loss of 31 tern eggs in their nests was 70.9 ± 20.3 mg/day (mean ± SD), resulting in a 14.7% loss of mass during the 22-day incubation period. Eggshell water vapor conductance was 28.13 ± 7.52 $mg \cdot day^{-1} \cdot$ kilopascal $(kpa)^{-1}$, a value 1.5 times greater than predicted for bird eggs with similar mass and incubation period and 1.4 times greater than predicted for other tern eggs in particular.

The increased eggshell conductance, relative to other eggs laid in drier circumstances, is the result of an increased number of pores in the shell. The water vapor conductance per pore of the Black Tern egg is not significantly different from the pore conductance of eggs of 7 other tern species and of eggs of other bird species. This observation is consistent with the argument that all bird eggs may have a similar water vapor conductance per pore (Ar and Rahn 1979). The adaptation of shell conductance to a humid nest microclimate is accomplished by increasing the number of pores in the shell rather than by altering the ratio of pore surface area to pore length.

Favorable conditions for the development of avian embryos are achieved through species-specific combinations of nest-site selection, nest construction, parental behavior, and eggshell structure. Eggs must be maintained at a high, relatively constant temperature throughout incubation (Drent 1970, 1975; White and Kinney 1974). In addition, the rate at which the eggs lose water is regulated so that total mass loss during incubation amounts to between 10 and 20% of initial egg mass (Ar and Rahn 1980). Egg temperature and water loss are intimately related. If a typical bird egg is to lose an appropriate fraction of its mass, its average temperature must exceed 35°C (Ackerman and Seagrave 1984) and, on the average, the vapor pressure difference between the inside and the outside of the eggshell must be about 3.33 kpa (Rahn and Ar 1974). Because the vapor pressure inside the egg is fixed by the egg temperature, systematic variation in the nest vapor pressure so as to alter the vapor pressure difference away from 3.33 kpa must be compensated for by changes either in egg temperature or in the eggshell water vapor conductance. Pied-billed Grebe (*Podilymbus podiceps*) eggs incubated in wet, floating nests lost 16% of their initial mass. Egg water loss in a humid microenvironment occurs because the eggshell water vapor conductance of the grebe egg is 2.7 times higher than eggs of similar mass and incubation period in dry nests (Davis et al. 1984).

Black Terns belong to a family of birds (Laridae) that may be characterized as nesting on dry substrate (Harrison 1975, Rahn et al. 1976). However, Black Terns build crude, spongy nests of algae and bits of damp plant material on floating mats of vegetation (Provost 1947, Cuthbert 1954, Weller and Spatcher 1965, Bergman et al. 1970). The location and construction of the nests may produce a microclimate around the eggs that is more humid than that found in dry nests (Rahn et al. 1976). Vleck et al. (1983) reported that some species of terns and herons nesting on the ground appear to have a higher nest humidity than other similar species nesting in trees. However, the floating, moist nest of Black Terns should have a nest microclimate that is even more humid than the exposed scrapes of sand or gravel used as nests by most other species of ground-nesting terns. This should decrease the water vapor concentration difference between the inside and the outside of the egg and, therefore, either an increased egg temperature or an increased eggshell conductance is expected if Black Tern eggs are to lose about 15% of their initial mass during incubation. On the other hand, Black Tern eggs may lose less water during incubation than other tern and other bird eggs.

Black Tern eggs and nests were studied in marshes near West Lake Okoboji, Dickinson County, Iowa. The nests were found by searching likely areas in May and June. The brown-spotted eggs were marked with white waterproof ink and weighed every 3–4 days to obtain daily mass loss ($\dot{M}_{H_2O}$: mg/day). Nests were revisited until hatching occurred. Egg mass was measured with either a 30-g Pesola spring balance (to 0.1 g) or a Torbal torsion balance (to 0.02 g). The calibration of the balances was checked against a set of standard weights. Eggs found in nests floating over deep water were weighed with a torsion balance in a boat using the apparatus described in Fig. 1.

Eggs of various ages were collected from tern nests during the first half of the incubation period. The water vapor conductances (G_{H_2O}: $mg \cdot day^{-1} \cdot kpa^{-1}$) of these eggs were measured in desiccators over silica gel at 25°C (Ar et al. 1974). Subsequently, the initial mass of the eggs was estimated by injecting the air cell with distilled water and weighing the eggs in air (Grant et al. 1982). Dimensions of the eggshells were measured thereafter. Pores in the eggshells of Black Terns were counted according to methods described by Rahn and Hammel (1982) and Davis et al. (1984). The area of the eggshell was estimated using the equation for surface area as a function of initial mass presented by Paganelli et al. (1974).

The mean $\dot{M}_{H_2O}$ of Black Tern eggs in the nest was 70.9 ± 20.3 mg/day (n = 33, Table 1), which resulted in a 14.7 ± 1.85% loss of initial mass during incuba-

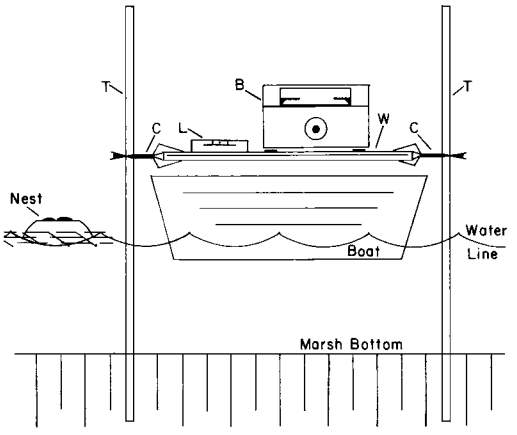

Fig. 1. Apparatus for weighing eggs found in nests floating over deep water. Two 3-m long, 2-cm diameter sections of galvanized conduit tubing (T) are pushed into the marsh bottom, with one on each side of the boat. A three-fingered thermometer clamp and a screw clamp (C) attach the wood plank (W) to the tubing. The clamps can be adjusted vertically and horizontally. The wood plank, which acts as the measurement table, is leveled by a small carpenter's level (L). The torsion balance (B) is placed on the plank, and measurements are made. The balance was calibrated in the laboratory before each field measurement.

tion. The mean G_{H_2O} of the eggs analyzed in this study was 28.12 ± 7.52 mg·day^{-1}·kpa^{-1} (n = 31, Table 1). No systematic change in G_{H_2O} was observed with time of measurement. An average of 114 pores/cm^2 of shell surface, or 2,622 pores/egg, was counted (Table 1). The average G_{H_2O}, 28.12 mg·day^{-1}·kpa^{-1}, was divided by the total number of pores to obtain a G_{H_2O}/pore of 1.07 × 10^{-2} mg·day^{-1}·kpa^{-1}·pore^{-1}. Internal egg temperature averaged 34.5 ± 2.1°C (n = 3).

Black Tern eggs are incubated just above water line in damp, spongy, floating nests. The eggs lose 14.7 ± 1.85% (n = 11) of their initial mass during incubation and appear to follow the general rule that all bird eggs lose about 15% of their initial mass during incubation. Rahn et al. (1976) reported an average incubation water loss of 14.3 ± 1.38% for 7 other tern species. Thus, water loss by Black Tern eggs is similar to that of other tern eggs.

The G_{H_2O} for avian eggs with a mass of 10.6 g and an incubation period of 22 days can be estimated to be 18.57 (± SEE 8.87) mg·day^{-1}·kpa^{-1} using the equation (Ar and Rahn 1978):

$$G_{H_2O} = 38.54\ W/I, \quad (1)$$

where W = initial mass (mg) and I = incubation period (days) and the units of G_{H_2O} have been converted to mg·day^{-1}·kpa^{-1}.

TABLE 1. Initial mass (M), average daily water loss in the nest ($\dot{M}_{H_2O}$), water vapor conductance (G_{H_2O}), egg dimensions, and shell characteristics of Black Tern eggs. Water vapor conductance is expressed as an average value of all individual eggs measured. All values are expressed as means ± SD (sample sizes in parentheses). G_{H_2O}, expressed as kilopascals (kpa), was converted from units in torr, where 1 torr = 0.133 kpa.

M (g)	10.62 ± 0.37 (15)
Average $\dot{M}_{H_2O}$ (mg/day)	70.9 ± 20.3 (33)
Average G_{H_2O} (mg·day^{-1}·kpa^{-1})	28.12 ± 10.50 (31)
Incubation period (days)	22.1 ± 1.2 (10)
Egg length (cm)	3.48 ± 0.12 (7)
Egg width (cm)	2.44 ± 0.04 (7)
Egg volume (cm^3)	10.04 ± 0.37 (7)
Shell area (cm^2)	23.10 ± 0.53 (7)
Shell thickness (mm)	0.13 ± 0.008 (7)
Pores/egg	2,622 ± 338 (10)
Pores/cm^2 of shell surface	144 ± 16 (10)

The measured G_{H_2O} of Black Tern eggs, 28.12 ± 7.52 (n = 31), is 1.5 times the value reported for other birds with similar egg mass and incubation time. Although the water vapor conductance is not statistically different from the predicted value, this lack of significance is likely to be misleading because the equation used to predict G_{H_2O} (Eq. 1) was generated from data collected from eggs of many different bird species found in a wide variety of nesting conditions. Thus, eggs that may be adapted to widely divergent conditions of nest humidity have been treated collectively. It is important to note that if the Black Tern egg was characterized by a typical egg conductance (18.57 mg·day^{-1}·kpa^{-1}), we estimate the water loss to be 9.7% of initial egg mass. This value falls outside the 95% confidence interval for terns in general (14.0 ± 2.0%; see Rahn et al. 1976: Table 5) and for Black Terns in particular (14.7 ± 3.7%). If the Black Tern egg was characterized by a water vapor conductance at the lower 95% confidence limit of the estimate (Eq. 1; 9.7 mg·day^{-1}·kpa^{-1}), then the water loss of the egg in a Black Tern nest would be only 5% of initial mass.

The G_{H_2O} of an avian eggshell is proportional to the number of pores in the shell. The predicted number of pores (N) in the Black Tern eggshell can be estimated to be 1,696 pores/egg using the equation of Rahn and Ar (1980):

$$N = 3{,}520\ W/I, \quad (2)$$

where W = initial mass (mg) and I = incubation period (days). The Black Tern eggshell contains 2,622 pores/egg, which is an average of 114 ± 16 pores/cm^2 of shell surface (Table 1). This may be compared to 84 ± 14 pores/cm^2 reported for 9 other tern species (H. Rahn pers. comm.). Thus, Black Tern eggs have about 30 more pores/cm^2 of eggshell surface than similar-size eggs of other terns. Another wet-nesting

species, the Pied-billed Grebe, also has increased the number of pores per unit of eggshell surface of its egg (Davis et al. 1984).

The conductance per pore (mg $H_2O \cdot day^{-1} \cdot kpa^{-1} \cdot pore^{-1}$) may be calculated by dividing the G_{H_2O} of the egg by the number of pores:

$$G_{H_2O}/N = 1.07 \times 10^{-2} \text{ mg} \cdot \text{day}^{-1} \cdot \text{kpa}^{-1} \cdot \text{pore}^{-1}. \quad (3)$$

The pore conductance (95% confidence interval = 0.08) is not significantly different ($F = 1.05$) from values reported for 9 other tern species ($1.17 \times 10^{-2} \pm 0.12 \times 10^{-2}$ mg·day^{-1}·kpa^{-1}·pore^{-1}; H. Rahn pers. comm.). The Pied-billed Grebe had a G_{H_2O}/pore of $1.05 \times 10^{-2} \pm 0.24 \times 10^{-2}$ mg·day^{-1}·kpa^{-1}·pore^{-1} (Davis and Ackerman 1983), which also is not different from the Black Tern. The averaged G_{H_2O}/pore for 107 species of birds is $1.20 \times 10^{-2} \pm 0.45 \times 10^{-2}$ mg·day^{-1}·kpa^{-1}·pore^{-1} (Ar and Rahn 1979). These results suggest that avian species examined until now have essentially the same water vapor conductance per pore. As Rahn and Ar (1974) have shown, one can derive a relationship between the average G_{H_2O}/pore and the shell structure by rearranging the equations:

$$G_{H_2O} = (Ap/L) \cdot (D_{H_2O}/RT) \quad (4)$$

and

$$Ap = N\pi r^2, \quad (5)$$

where G_{H_2O} = water vapor conductance (mg·day^{-1}·kpa^{-1}), Ap = total pore area (cm^2), L = shell thickness (cm), D_{H_2O} = diffusion coefficient for water vapor (cm^3/s), R = gas constant, T = absolute ambient temperature (°K), N = number of pores per egg, and r = pore radius (cm). If we rearrange Eqs. 4 and 5, a relationship between water vapor conductance per pore, cross-sectional area, and length of the pore may be derived:

$$(G_{H_2O}/N) \propto (\pi r^2/L). \quad (6)$$

Because shell thickness increases with increasing egg mass (Ar et al. 1974), the cross-sectional area of the pore must increase at the same rate. This ratio is 0.73 μm for 107 species of birds (Ar and Rahn 1979). Adaptation of avian eggshells to environmental conditions has been accomplished by selecting for numbers of pores in the eggshells rather than for a geometrical ratio of pore radius to pore length.

We give special thanks to Hermann Rahn for his correspondence and inspiration throughout the study, to Phyllis Parisi for her patience in counting pores, and to Mr. Mark Wehrspan, Dr. Richard Bovjberg, Dr. John Farmer, and the staff at Iowa Lakeside Laboratory for the use of their equipment and cooperation. We thank the members of the Iowa Conservation Commission for their cooperation at the study sites. The State of Iowa and the U.S. Fish and Wildlife Service granted permits to conduct this research. This study was supported by grants from the Chapman Fund of the American Museum of Natural History, Washington, D.C., the Okoboji Protective Association of Milford, Iowa, and the Graduate College at Iowa State University to T.A.D. and NSF PCM 79-16256 to R.A.A.

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Received 30 March 1984, accepted 6 February 1985.

Energetics of Free-living Nestling House Finches: Measurements with Doubly Labeled Water

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The total energy metabolism of nestling birds consists of anabolism (the building of body substance) and catabolism (metabolic heat production). Although anabolism has been measured directly as the accumulation of energy in body tissues with time (see Ricklefs 1974), most estimates of nestling catabolism have relied upon extrapolations of laboratory measurements of standard metabolism (based on oxygen consumption) to field conditions. An alternative to the latter technique uses doubly labeled water (DLW) to monitor directly the CO_2 production of nestlings in their natural physical and social environment. We have determined the total energy metabolism of free-living nestling House Finches (*Carpodacus mexicanus*) using DLW. Although the DLW method provides reliable measurements of CO_2 production and water flux in adult birds (see Nagy 1980, Nagy and Costa 1980, Williams and Nagy 1984), it has yet to be validated for use with growing animals. There is potential for error when DLW is used with rapidly growing animals. In growing animals, the total body water volume in which isotopes equilibrate changes regularly as mass changes. If the changes in body water volume are extreme, errors in calculation of isotope turnover can result (see Nagy 1980, Nagy and Costa 1980). In the present study, nestlings averaged 74% of adult mass, a size typically associated with a declining growth rate (Ricklefs 1969). In fact, nestling water volumes increased by <10% initial volume in our study. Such a change is within the limits that theoretically should permit accurate calculation of turnover rates (Nagy 1980, Nagy and Costa 1980). Therefore, we suspect that for nestlings of this size, errors in the DLW method should be small. However, in the absence of direct validation of the DLW method, our results must be regarded as preliminary.

All nestlings used in this study occupied natural nests constructed in and around buildings at the University of California's Philip L. Boyd Deep Canyon Desert Research Center near Palm Desert, California. All measurements were made in May 1980. Thirteen nestlings from 4 nests were studied. Mean nestling mass at the time of isotope injections (see below) was 15.0 ± 0.4 g ($\bar{x}$ ± SE) and did not differ between nests ($F = 1.93$, $P = 0.19$). Eight nestlings from 2 nests received intramuscular injections of tritiated water only (0.1 ml, containing 50 μCi ^3H), and 5 nestlings from 2 other nests received DLW (0.1 ml, containing 50 μCi ^3H in 95 atoms % ^{18}O-enriched water). These injection solutions provided sufficient isotope activity to assure final activities of both ^3H and ^{18}O that did not approach background activity after 48 h (general guidelines for suggested activities of injected isotopes are given in Nagy 1983).

Following injections, labeled nestlings were returned to their nests for 1 h to allow isotope equilibration in body water. At 1, 25, and 49 h postinjection, nestlings were removed from their nests and weighed. Blood (50 μl) was then drawn from a brachial vein, and nestlings were returned to the nest. Blood samples were stored in flame-sealed, refrigerated microhematocrit tubes until returned to the University of California, Los Angeles, for analysis. Blood was distilled according to procedures in Wood et al. (1975). Tritium activity of the distilled water samples was assayed by liquid scintillation spectroscopy (Beckman LS-230). Oxygen-18 was assayed using the proton-activation technique of Wood et al. (1975). Gamma emissions of the resulting fluorine-18 were quantified with a Packard-Gamma Rotomatic system. Carbon dioxide production and water influx were calculated using equations in Nagy (1980, 1983)

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