DETERMINATION OF FRESH EGG MASS DURING NATURAL INCUBATION

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TABLE 1. Comparison of an egg's fresh mass and its mass after 30-64 days of natural incubation when the air cell is filled with water.

	n	Mass of the egg (g) ^a		
Species		When laid	After 30–64 days of incubation ^b	
Laysan Albatross Black-footed Albatross	22 16	$\begin{array}{r} 282.4 \pm 21.7 \\ 301.5 \pm 16.0 \end{array}$	$\begin{array}{c} 282.7 \pm 21.9^{\circ} \\ 302.3 \pm 15.7^{\circ} \end{array}$	

^a Values in the table are $\bar{x} \pm SD$. ^b Mass after the air cell was filled with water.

^e Means are not significantly different (P > 0.9).

Groebbels (1932) was probably the first to make a systematic attempt to predict the mass of a freshly laid egg (initial egg mass). He weighed 54 eggs, representing 15 species, then removed their contents, refilled them with water, and reweighed them. The second weighing showed a reduction in egg mass ranging from 2.6 to 3.3%. This deficit presumably reflects the difference between the density of water and that of the egg's contents, which averages 1.031 $g \cdot cm^{-3}$ (Rahn et al., in press). In recent experiments Ar and Rahn (1980) carefully filled the air cells of fertile and infertile chicken (Gallus gallus) eggs with water after 18 days of incubation and compared their mass with the mass at laying. For fertile eggs, the mean difference \pm SD was 0.014 ± 0.006 g. For infertile eggs, it was only 0.003 ± 0.011 g.

Thus, the technique of refilling the air cell with water and then weighing the egg appears to be a reasonably reliable method of obtaining the egg's initial mass during artificial incubation. However, the validity of this method under conditions of natural incubation had not been examined. In this paper, we present results of such a study on eggs of the Black-footed Albatross (Diomedea nigripes) and the Laysan Albatross (D. immutabilis) which nest on Sand Island, Midway Atoll, Hawaii (28°13'N, 177°23'W). Many predictive equations are based on fresh egg mass. This technique obviates the need to intensively watch nests to determine when an egg is laid. Albatross eggs were chosen because they lose considerable mass during their long incubation period and because we were able to measure their metabolism simultaneously (Pettit et al. 1982).

We weighed eggs on the day they were laid and after 30-64 days of incubation. We then filled the air cell of each egg with distilled water and weighed it again. Between laving and the time of collection, the eggs lost 20.2 to 42.4 g. Their mass at the time of laying and that determined by filling of the air cell appear in Table 1. The mass of an incubated egg whose air cell is filled with water is essentially the same as that of the egg when freshly laid. The method overestimates the egg's fresh mass by 0.1% for Laysan and 0.3% for Black-footed albatross. The maximum error is 1.2%. This error is probably related to (1) our occasional failure to completely fill the air cell with water, and (2) the presence of a small air cell in some eggs at the time of laying (such spaces sometimes develop when an egg cools after it is laid). Furthermore, we examined nesting sites only once a day. Consequently, a maximum of 24 h may have elapsed between the time when some eggs were laid and subsequently weighed, during which as much as 674 mg of water may have been lost (Grant et al. 1980). In spite of these drawbacks, this field technique gives an accurate estimate of the egg's mass at laying.

Simkiss (1974) and Prinzinger et al. (1979) estimated that 21-25% of the overall mass lost during incubation

was due to metabolism and the remainder to the loss of water. Our estimates, based upon differences between CO₂ and O2 exchange during development in eggs of the Laysan Albatross, are considerably less. Table 2 gives mean values for the oxygen uptake and the respiratory quotient (RQ) of eggs at various stages of development (Pettit et al. 1982). The CO₂ output of an egg can be calculated from such information because $RQ = 0.78 P_{A_{CO2}} / \Delta P_{O2}$ (Rahn et al. 1974), where $P_{A_{CO_2}}$ is the partial pressure of CO_2 in the air cell and ΔP_{O_2} is the difference in the partial pressure of O_2 between the external environment and the air cell. One can estimate changes in the egg's mass associated with the uptake of O_2 and loss of CO_2 by multiplying the volume of each gas by its density (1.429 and 1.978 g·l-1, respectively). The net change in mass depends upon the rate of metabolism (which increases continuously during embryonic development) and the RQ (which decreases continuously from 0.8 at 30 days to 0.71 at 59 days of incubation). At about day 56 of incubation, when the RQ is 0.722, no net metabolic change of mass occurs because the O₂-CO₂ mass exchange is zero. When the RQ falls below 0.722 (after day 56), metabolism produces a gain in mass.

For the Laysan Albatross, the mean daily loss in the egg's mass during natural incubation was 674 mg day-(Grant et al. 1980). Thus, for example, on day 40 of incubation, approximately 51 of the 674 mg, or 8% of the total mass loss, is due to metabolism. When the RQ is below 0.722, embryonic metabolism increases the egg's mass. The increment is about 5% of the total mass loss on day 59 of incubation. Consequently, the largest error that will occur if one equates daily mass loss with water loss is only 5-8%. Over the entire 59 days of incubation prior to external pipping, the mass difference due to metabolism amounts to only about 2.5% of the total mass lost by the albatross egg. This is considerably less than the

TABLE 2. Changes in mass of the Laysan Albatross egg caused exclusively by O2 and CO2 exchange during various stages of embryonic development (calculated from Pettit et al. 1982).

Day of incubation	(ml·day⁻¹)	RQ*	(ml · day⁻¹)	ΔW ^b (mg·day ⁻ⁱ)
30	150	0.80	120	-23
40	450	0.78	351	-51
50	1,020	0.73	745	-15
59	1,500	0.71	1,065	+37

 ${}^{*}\dot{M}_{o_{2}} = oxygen consumption; RQ = respiratory quotient; <math>\dot{M}_{co_{2}} = carbon dioxide production.$ ${}^{b}\Delta W = additions (+) \text{ or reductions } (-) \text{ in the egg's mass due to gas exchange } = (\dot{M}_{o_{2}})(1.429 \text{ g} \cdot 1^{-1}) - (\dot{M}_{co_{2}})(1.978 \text{ g} \cdot 1^{-1}).$ See text for further details.

21-25% estimated by Simkiss (1974) and Prinzinger et al. (1979).

In conclusion, the technique of refilling the air cell with water followed by weighing to obtain the initial or fresh egg mass is both a simple and a relatively accurate tool. Changes in mass due to embryonic metabolism (respiration) are small and can be ignored in most instances.

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HUMMINGBIRDS FEEDING ON AN EXCRETION PRODUCED BY SCALE INSECTS

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Reichholf and Reichholf (Bonn. Zool. Beitr. 24:7-14, 1973) reported that hummingbirds in the Bracaatinga (Mimosa bracaatinga) woodlands of the Serra do Mar in the state of Santa Catarina in southern Brazil, fed on the dilute sugar solution produced by coccids (some species of which are commonly called scale insects) living under the bark of the trees. Köster and Stoewesand (Bonn. Zool. Beitr. 24:15-23, 1973) reported similar observations of hummingbirds associated with the coccids of coffee-shade trees of the genus Inga (family Mimosaceae) in the Cordillera Oriental of Colombia, in the Department of Meta between Bogotá and Villavicencio. These authors also gave detailed descriptions and diagrams of the microscopic structure of the tube through which the "honeydew" of the coccids was excreted. Salas and Jirón (Brenesia 7:57-64, 1977) reported that hummingbirds fed on the excretions of "tailed coccoids" growing on trees of the genera Quercus, Ficus, and Pithecolobium.

On 4 June 1975, a field party based at Rancho del Cielo, Texas Southmost College's biological field station in southern Tamaulipas, Mexico, visited Agua Linda, an uninhabited locality in the highlands about 5 km west of Rancho del Cielo. The nearest major settlement is Gómez Farías, about 12 km SE of Agua Linda. At about 1,830 m elevation, Agua Linda is situated in pine-oak woodland in a humid valley near the summit of the Sierra de Guatemala range, in a zone of transition between the very wet eastern slope and the relatively dry western slope.

Holly Hobart and I observed the Bumblebee Hummingbird (*Atthis heloisa*) feeding on a sweet liquid produced by insects that were beneath the bark of many of the oak trees (*Quercus* sp.); most of these trees were about one foot in diameter and approximately 12 to 15 m tall.

We first noted that the hummingbirds hovered close to the tree trunks, apparently obtaining food from an unseen

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(by us) source. Close examination revealed many very slender, colorless filaments about 2 to 3 cm long projecting from the bark, reminding me of the hyphae of bread mold. Most of these filaments bore a tiny droplet of colorless, sweet-tasting liquid at the outer end. Cutting into the bark at the base of a filament disclosed a small living creature. A study of the papers by Reichholf and Reichholf (1973) and Köster and Stoewesand (1973) convinced me that this was a scale insect, excreting through the slender filament a "honeydew" similar to the sugary material excreted by some aphids.

After we were certain that the Bumblebee Hummingbird was actually feeding on this excretion. I recalled that I had earlier observed the Amethyst-throated Hummingbird (Lampornis amethystinus) behaving similarly as it hovered close to the bark of an oak tree. We did not see any other hummingbirds using the scale-insect excretions, nor notice any aggressive behavior between any hummingbirds at the infested trees. Neither did we note any insects using the sweet material during the approximately one hour of our observations. Reichholf and Reichholf (1973), in contrast, observed that various kinds of insects fed on the excretions, while the hummingbirds defended trees or groups of trees against other individual hummingbirds and against large insects. Köster and Stoewesand (1973) also noted that the hummingbirds in the vicinity of infested trees defended the trees against each other and against large wasps. Salas and Jirón (1977) observed that bees, wasps, flies, and other insects, as well as hummingbirds, fed on the excretions.

I recommend a closer study of other similar woodlands, in the southwestern U.S.A., for example, to determine whether this food source may be available and utilized by hummingbirds there.

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