How far is an egg through incubation?

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From the linear measurements and mass of an egg, a method is devised to estimate the starting date of incubation. The method is based on the approximately 15–16% decrease in egg mass that occurs during incubation. The method is illustrated using data collected on African Black Oystercatchers *Haematopus moquini*. For this species, we provide an approximate standard deviation of the estimated starting date. We show that the earlier an egg is found during incubation, the smaller the standard deviation of the estimated starting date. This is a consequence of the fact that eggs lose mass at varying rates.

INTRODUCTION

When one finds a nest with eggs, it is useful to have a quick and simple method to estimate the stage of incubation of the eggs. This information can be used to estimate the period for which the eggs have already been incubated so that you can estimate when incubation started. Similarly, it can be used to estimate the length of the remainder of the incubation period and thus the expected date of hatch. In this paper, the focus is on the date on which incubation started, because this provides the first step in the production of probability distributions of incubation starting dates in a population (Matanyaire *et al.* 2002). This enables nesting phenology to be described quantitatively, and strong comparisons to be made between years or places. We describe a statistical approach to achieve this.

Currently, incubation stage is probably most frequently estimated in the field by "floating" the eggs (van Paassen *et al.* 1984). This method is commonly used, for example, on the breeding grounds in Russia (Tulp *et al.* 2000, Tulp & Schekkerman 2001). Eggs are placed in a small container of water, and the angle at which the eggs "float" is measured. A fresh egg sinks to the bottom so that the long axis of the egg is horizontal. As incubation progresses, eggs lose mass through the evaporation of water through the pores on the egg shell, the air sac increases in size, and an angle develops between the long axis of the egg and the water surface until the long axis is vertical. Once the eggs float, with the blunt end of the egg lifting out of the water, the distance from the tip of the egg to the surface of the water is measured. The flotation method depends on creating a relationship for eggs of known laying and/or hatching date. The angle and distance measurements can then be used to determine developmental stage of the egg. Both measurements, the angle and the distance, are subject to a fair degree of subjectivity: measuring the angle through the water with a protractor is biased by refraction; measuring the distance above the water's surface is bedeviled by the surface tension of the water.

In this note, we develop an alternative approach, which does not involve immersing the eggs in water. The procedure described here, based on weighing and measuring eggs, is not new; it is built on a method proposed by Furness & Furness (1981), and further developed and implemented by Green (1984), Galbraith & Green (1985) and Grant (1996). We develop not only an estimate of the date of start of incubation, but also an estimate of the reliability of this date, by providing an approximate standard deviation.

The biological basis of both approaches, floating and measurement, is the fact that eggs lose approximately 15–16% of their mass between the start of incubation and the point in the hatching process when the internal egg membranes are broken (Ar & Rahn 1980). During this period the rate of mass loss is, to a first approximation, linear; once the membranes are broken, the rate of mass loss increases rapidly. The membranes are broken at some point between "starring", which starts when the first roughness can be felt on the surface of the egg, and "pipping", which starts when the egg-tooth removes the first fragment of shell from the egg.

To illustrate this mass loss measurement method for determining the start of incubation, we collected data over two breeding seasons on African Black Oystercatchers *Haematopus moquini* on Robben Island, South Africa. The relatively large size of oystercatcher eggs (average mass *c*.58.5 g on laying, with mean lengths and breadths of 61.3 mm and 42.0 mm respectively; KMC unpubl. data) provides a good demonstration of this method.

METHODS

Egg length *L*, and two breadth measurements, at right angles to each other, B_1 and B_2, were made on each egg with vernier or dial calipers, and recorded to 0.1 mm precision. Two breadth measurements were taken, because eggs are frequently not circular when viewed end on (see Discussion). The mass was recorded to 0.1 g, using an electronic balance (Tanita model 1479V). The egg-dimensions and mass

Table 1. Standard deviations (SD) associated with each percentage mass loss. Observed percentage mass losses should be rounded to the nearest whole percent. The standard deviations were estimated by simulation; see Methods.

Percentage mass loss	SD of start date of incubation (days)
0	1.7
1	1.8
2	1.9
3	2.0
4	2.1
5	2.3
6	2.5
7	2.7
8	2.9
9	3.1
10	3.3
11	3.6
12	3.8
13	4.1
14	4.4
15	4.7
16	5.1

measurements were usually made on the first visit to a nest. For a sample of eggs, mass was recorded on each visit to the nest, usually at intervals of 2–6 days. The mass recorded on the ith visit was denoted M_i. Date and time (hours and minutes) were recorded on each visit; the time of ith visit was denoted t_i, in days since some convenient calendar date:

$(t_i =$ whole days + (hours + minutes/60)/24).

The initial mass M_0 of the egg at the time of laying was estimated as $M_0 = k L B_1 B_2$, where k is regarded as a random variable with a mean μ_k and standard deviation σ_k, and for simplicity is considered to have a normal distribution. Values for the mean and standard deviation of k were calculated from a sample of eggs which were first weighed in the period between laying and the start of incubation, during which the loss of mass is small. For this sample of fresh eggs, individual values of k were calculated from the formula:

$k = M_0/L B_1 B_2$.

The mean μ_k and the standard deviation σ_k were estimated by the sample mean and sample standard deviation of these values. The mean of k should be regarded as species-specific; it depends on the shape of the egg, the overall specific gravity of the egg, which in turn depends on the thickness of the eggshell, and the relative composition of the albumen and the yolk. Likewise, the standard deviation of k depends on how variable these factors are within the species.

The rate of mass loss for each egg is closely linear until the egg "pips" a day or two before hatching, after which the rate of mass loss increases rapidly (Ar & Rahn 1980). For each egg, the rate of mass loss was estimated as the slope coefficient of a linear regression of mass M_i on date of measurement t_i. We excluded masses of pipped eggs from the regressions. The unit of the slope coefficient is grams per day. The slope coefficient was divided by the estimated initial mass, $M_0 = k L B_1 B_2$, to obtain the daily rate of mass loss per gram initial mass, denoted r. We regard r as a random variable with mean μ_r and standard deviation σ_r, and we estimated these by the sample mean and standard deviation of the set of eggs for which we were able to calculate linear regressions of the rate of mass loss. We assume that r has a normal distribution.

For an egg first measured and weighed t days after the start of incubation, the expected mass would be:

$M_1 = k L B_1 B_2 - t r k L B_1 B_2$.

In general, t is unknown, and is estimated as

$t = (k L B_1 B_2 - M_1)/(r k L B_1 B_2)$,

by substituting the first observed measurement of mass, M_1, into this equation. This provides a point estimate of the number of days t for which the egg has been incubated. We now want an estimate of the standard deviation of t. Both k and r show random variation; we have estimates of their standard deviations. If we measure and weigh a fresh egg (without knowing that it is a fresh egg), then the error in our estimate of t depends on the variability of k. Because the rate of mass loss varies between eggs, the later we weigh an egg during incubation, the more variable the amount of mass loss will be, and the less certainty we can have in the estimate of t. Thus, intuitively, we expect the standard deviation of t to be larger for large values of t.

There are various ways to estimate the standard deviation of t. We did it by simulation. For mass losses of 0%, 1%, …, 16%, we estimated the standard deviation of t by simulating 4,000 pairs of random values of k and r, using a random value generator for the normal distribution, calculated t from the equation above, and found the standard deviation of the resulting t values. This approach, though approximate, is adequate for the purposes required.

RESULTS AND DISCUSSION

105 eggs from 68 clutches from the 2001–2002 breeding season and 146 eggs from 85 clutches from the 2002–2003 breeding season were used in the analysis (KMC unpubl. data). For African Black Oystercatcher eggs, the estimated mean and standard deviation of k (g mm^{-3}) were 0.0005330 and 0.0000051 respectively, and the estimated mean and standard deviation of r (days^{-1}) were 0.00502 and 0.00096 respectively.

The results of the simulation of standard deviations showed that the standard deviation of the estimated starting date of fresh eggs which had lost no mass was 1.7 days (Table 1). This is attributable to the variability of k, the conversion factor for converting the length and breadth measurements to the estimated fresh mass; not all fresh oystercatcher eggs have the same shape, density and egg shell thickness. Once an egg had lost 10% of its fresh mass, the standard deviation of the estimated starting date was 3.3 days. The steady increase of the standard deviations with increasing mass loss is a reflection of the fact that not all eggs lose mass at exactly the same rate.

Examples of the arithmetical calculations are given in Table 2. The first line of the table shows an egg with $L = 64.0$, $B_1 = B_2 = 42.0$, and an observed mass M_1 on 16 December when first weighed of 56.9 g. The estimated fresh mass is:

$M_0 = k L B_1 B_2 = 0.0005330 \times 64.0 \times 42.0 \times 42.0 = 60.2$ g.

Assuming that the egg will hatch when it loses 16% of fresh mass, the estimated hatch mass is 50.5 g. The equation to estimate the number of days for which the egg has been incubated:

$t = (k L B_1 B_2 - M_1)/(r k L B_1 B_2)$

can be simplified to:

$t = (M_0 - M_1)/rM_0$,

so that $t = (60.2 - 56.9)/(0.00502 \times 60.2) = 10.9$ days. The egg has lost 5.48% of its initial mass. Reference to Table 1 indicates that the standard deviation of the estimate of date of

Table 2. Examples of calculations to estimate the date of start of incubation and its standard deviation from observed egg measurements at nests of African Black Oystercatchers.

Time and date of observation	Days since 1 Nov.	Measurements $L\ B_1\ B_2$ (mm), observed mass M_1 (g)	Fresh mass (hatch mass) (g)	Percentage mass lost	Days incubated	Day, since 1 Nov., of start of incubation	Date of start of incubation	SD
11h15 16 Dec	46.5	64.0 42.0 42.0, 56.9	60.2 (50.5)	5.5%	10.9	35.6	5 Dec	2.3
13h30 16 Dec	46.6	66.9 43.0 42.9, 60.8	65.8 (55.3)	7.6%	15.1	31.5	1 Dec	2.9
14h00 23 Dec	53.6	64.4 38.9 39.1, 52.0	52.2 (43.9)	0.4%	0.8	52.8	22 Dec	1.7
14h15 23 Dec	53.6	61.8 39.3 39.2, 51.1	50.7 (42.6)	0%	0	53.6	23 Dec	1.7
17h55 11 Jan	72.7	64.3 40.3 40.2, 49.3	55.5 (46.6)	11.2%	22.3	50.4	20 Dec	3.6

start of incubation is 2.3 days. This information can be used as input into usage of the "kernel method" (e.g. Wand & Jones 1995) to describe the overall distribution of the start of incubation in a population, as done by Matanyaire et al. (2002). This method was applied, in a preliminary form, by Underhill et al. (1993) and was developed further by Calf & Underhill (submitted). Most oystercatcher nests had two eggs, and the differences between the dates were generally within two days of each other.

Egg lengths are easy to measure, and results are usually both accurate and precise. Measuring egg breadth is less simple, requiring judgment about the appropriate position to make the measurement, and results tend to be less precise (more variability in the measurements), and less accurate (biases due to making the measurement incorrectly on the egg). In addition, implicit in making a single breadth measurement is the assumption that eggs are perfectly circular when viewed end on; in reality, a proportion of eggs are slightly elliptical. However, the ellipticity is small, and if these two measurements differed by more than 0.2 mm in oystercatcher eggs, we checked them. In the conventional formula to estimate egg mass, $M_0 = k\ L\ B^2$, using a single breadth measurement B, the breadth enters as a squared quantity; therefore, any errors in its measurement are exaggerated.

We did not use this method to forecast the date of hatching, although clearly we could have. However, we anticipate that "backcasting" the date of the start of incubation is more precise than forecasting the date of hatching. This is because (1) the percentage of mass lost until pipping varies between eggs (KMC pers. obs), and (2) the duration of the hatching process, after the point at which the membranes are broken, is variable (KMC pers. obs). We have not attempted to estimate standard deviations of point estimates of hatching dates; these would be substantially larger than estimates of the standard deviations of the date of start of incubation.

The backcasting approach tends to be more accurate for nests found fairly soon after the start of incubation and less accurate for nests found late in incubation. This is because the error involved in extrapolating from a regression line is larger when the extrapolation is over a long time period than over a short time period.

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