Determining and testing the accuracy of incubation stage of Ruddy Duck eggs by floatation

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An egg floatation model was developed for determining the incubation stage of Ruddy Duck (Oxyura jamaicensis) eggs, and the accuracy of this model was then tested by correlating actual incubation age against the incubation age predicted from the egg floatation model. A strong positive correlation existed between actual incubation age and predicted age suggesting that model accuracy was high. Also, predicted incubation age was ≤ two days of actual incubation age on 84% of the occasions and within three days 93% of the time. We consider floating Ruddy Duck eggs to be an accurate and practical alternative to field candling and encourage others to develop and test egg floatation models for difficult-to-candle avian species.

Key Words: candling, egg floatation, incubation, Ruddy Duck, *Oxyura jamaicensis*.

Accurate assessment of avian embryo age is important for determining nest initiation and expected hatch dates. Wildlife managers and researchers depend on these dates to: (1) determine nesting phenology for implementation of habitat management programmes, (2) synchronise embryo age in egg-swapping experiments, (3) establish embryo age in toxicological studies, and (4) select the optimum time to capture incubating adults. Egg candling (Hanson 1954; Weller 1956) and egg floatation (Westerskov 1950) are the most common techniques employed in the field, since other methods, such as measuring specific gravity or density loss (Westerskov 1950), are not practical for field studies. The ability to use egg floatation as a method of determining incubation age is related to the formation and enlargement of the air cell within the egg. As the egg loses weight due to water loss and the air cell develops, a concomitant decline in egg specific gravity occurs, from a specific gravity greater than water to a specific gravity less than water

(Westerskov 1950). When egg specific gravity is less than the specific gravity of water the egg is able to float.

Concerns about the effects of egg floatation on embryos exist, but hatchability appears not to be affected (Martin & Arnold 1991; Alberico 1995). However, Martin & Arnold (1991) reported that eggs immersed for 30 seconds during late incubation had non-significant increases in length of incubation period by over three hours. In contrast, eggs immersed for 30 seconds during early incubation had no effect on length of incubation period. Given these concerns, egg floatation is still a common and probably safe alternative to candling, especially for opaque or thick-shelled eggs, and individuals inexperienced with candling.

The Ruddy Duck *Oxyura jamaicensis* is a common breeding bird in the prairie pothole region of North America. Ruddy Ducks lay an average clutch of about seven white, thick-shelled eggs (Alisauskas & Ankney 1994; Brua 1999). Although white, the shell thickness makes it very difficult to candle in the field and it is only possible under very good light conditions. Thus, egg floatation presented the only practical option for determining nest initiation and hatch dates. Misterek (1974) described egg floatation patterns for Ruddy Ducks but used broad, discrete floatation categories, which have an inherent error built into the model (Walter & Rusch 1997), and Misterek did not validate his floatation model. Thus, our study had two objectives: (1) develop an egg floatation model of daily incubation stages of Ruddy Duck embryos, and (2) field test the power of our egg floatation model by comparing the actual incubation age against the predicted age of the clutch.

Methods

Development of the floatation model

The floatation model was developed by immersing 12 known-aged Ruddy Duck eggs from two clutches, consisting of seven and five eggs, collected near Minnedosa, Manitoba, Canada (50°10'N, 99°47'W). Eggs were incubated until hatch in a Petersime force-draft incubator maintained at a constant 38°C and a humidity of 70%, as measured from a wet bulb hygrometer (Ward & Batt 1973).

Eggs were floated in a clear, plastic container (van Paasen *et al.* 1984). On the outside front of the container, a clear, plastic protractor was glued in the middle to measure the angle between the longest egg axis and the level bottom of the container. Similarly, a clear, plastic millimetre ruler was attached to the front to measure the height of the egg above the water surface, once the egg started to float. Eggs were measured to the nearest five degree angle and nearest 1mm above the water surface in incubation temperature water. Only one observer measured eggs to provide measurement consistency and they were floated at approximately the same time every day. However, not all eggs were measured every day or on the same day of incubation, thus sample sizes for egg measurements vary for each day. Data for days two and 13 of the 23-day incubation period are lacking.

Field Methodology

Ruddy Duck nests were found by systematically searching the emergent fringe of wetlands. When a nest was found and during subsequent nest checks every 7-10 days, we recorded clutch size and

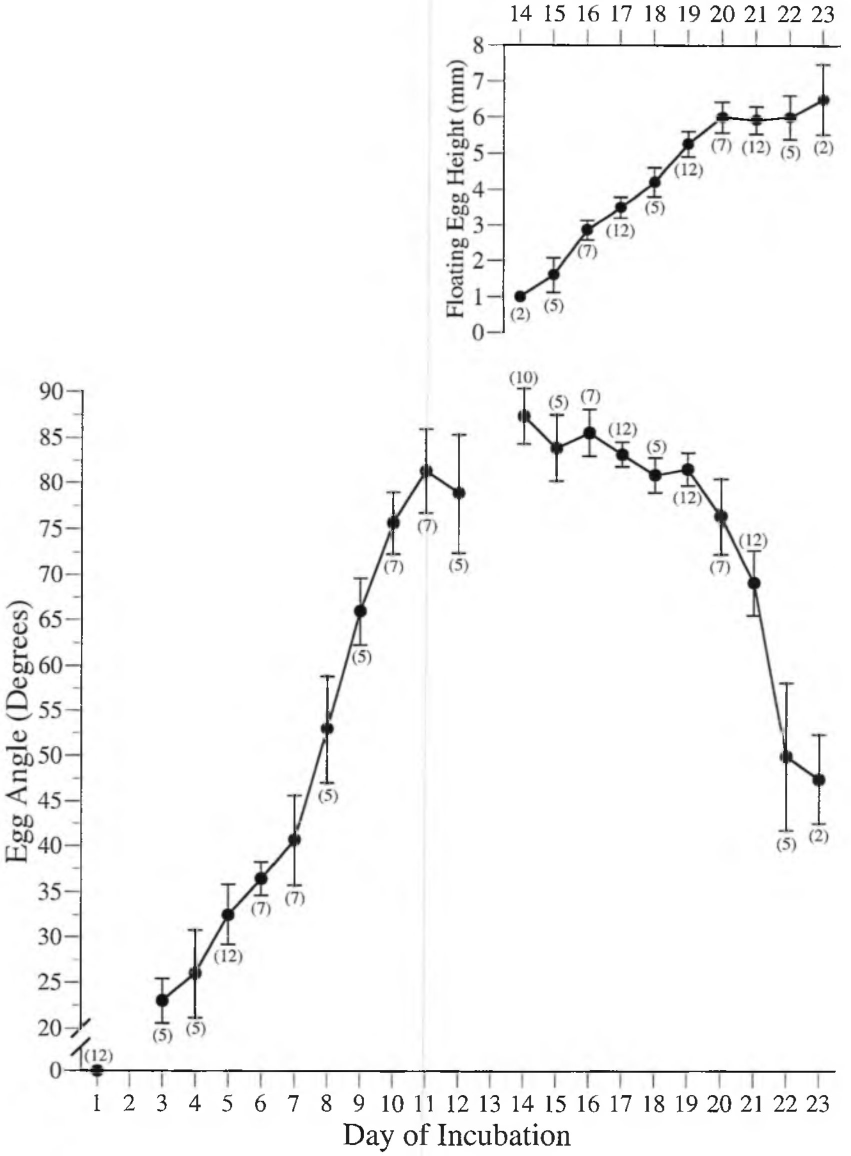

Figure 1. Mean angle and 95% confidence interval of Ruddy Duck eggs at various stages of incubation. Eggs from day one to 14 sink to the bottom, except for two eggs at 14d began to float. Eggs from day 15 to 23 float and the corresponding mean height above the water is shown in the upper chart. Sample size is shown for each day in parentheses.

determined stage of incubation by floatation. Because it was impractical to carry and maintain incubation temperature water during long hours of nest searching, we floated eggs in pond water and determined the nearest five degree angle and noted if the eggs sank or floated. Two to four eggs/clutch were floated to assign an angle. However, if large discrepancies in the estimated angles within the clutch existed, we took the average angle for that clutch to assign the predicted age. Nests were revisited at or near hatch to assign accurately incubation age based on a 23-day incubation period, the most common incubation period during a three year study (Brua 1999). We recorded the observed hatch date as the day of the nest visit if ducklings were present or the day after visitation if the eggs were pipped internally (tapping) or externally.

Assessing accuracy of the floatation model

To determine the predictive power of the egg floatation model, the angle derived from eggs in the nest when first found was compared and assigned the predicted age of that clutch based on our model. For nests found during laying, the angle determined during the first nest check was used. Thus, nests were used only once in assessing the predictive power of our floatation model. The predicted incubation age was assigned to the closest mean value in the model and by the degree of overlap with the 95% confidence interval for that age. Only nests with known hatch date were used to test the model. The incubation stage of 83 individual Ruddy Duck nests ascertained in the field, were compared, with our incubation model to predict incubation age. Pearson Correlation was used to test actual incubation age against predicted incubation age. Values reported are means ± one standard deviation, and alpha values were $P=0.05$.

Results

Description of incubation stages

During the first two weeks of incubation, most eggs sank. The angle of the egg increased with age, starting at 0° for day one and gradually progressing to about 90° for embryos aged 14 days (**Figure 1**). At 14 days, two eggs began to float but eight of the remaining 10 eggs were at a stage that was termed 'want-to-float' or at nearly neutral density, where the egg would gradually sink back to the bottom of the container. Once an egg started to float there was a gradual decline in angle of the egg with a concomitant increase in height of the egg above the water surface up to 20 days (**Figure 1**). However, after day 20, there was little change in egg height above the water surface but a precipitous decline in egg angle from about 75° to 50° in two days. Ten of 12 eggs hatched on day 23, while the remaining two eggs hatched the next day ($\bar{x}=23.2 \pm 0.4$ days). This incubation period is similar to the incubation period for 19 known-aged Ruddy Duck clutches in the field ($\bar{x}=23.6 \pm 1.3$day, range=22-26 days, Brua unpubl. data).

Field test of incubation model

On 22 occasions (26.5%), our predicted incubation age matched exactly the actual incubation age. We predicted incubation stage within two days of actual age 84% of the time and 93% of our predictions were within three days of the actual age (**Figure 2**). Our model of egg floatation predicted

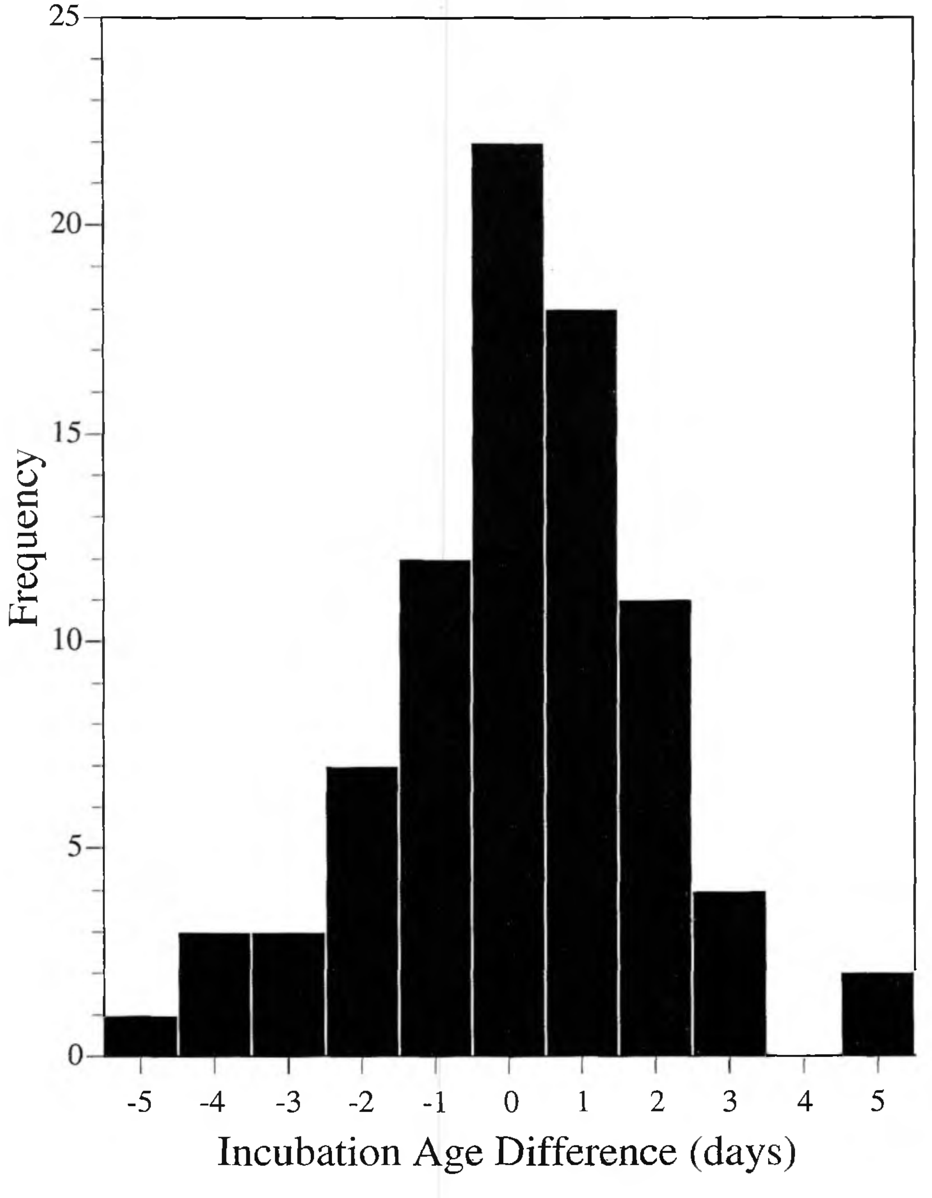

Figure 2. Frequency of nests in relation to days deviated from actual incubation age for 83 individual Ruddy Duck nests in southwestern Manitoba, Canada.

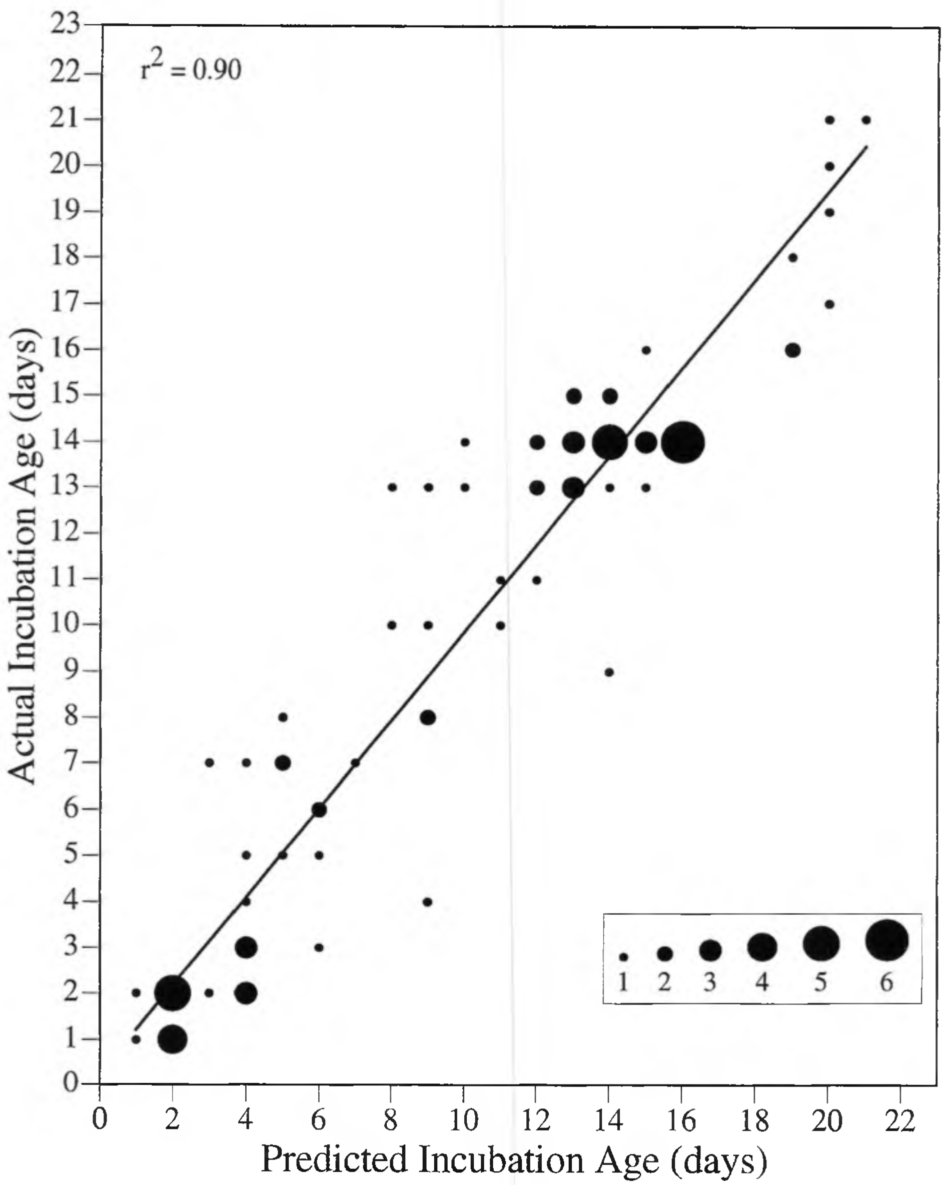

Figure 3. Relationship between predicted incubation age and actual incubation age for 83 individual Ruddy Duck nests in southwestern Manitoba, Canada. The dashed line signifies an exact match between predicted and actual incubation age. Size of filled circles in legend corresponds to number of samples at each point in the chart.

eggs to be 0.12 ± 1.87 days older than the actual age, which did not differ significantly from 0 ($t=0.59$, $df=82$, $P>0.5$). A strong positive association existed between predicted and actual incubation age (**Figure 3**), with 90% of the variation explained by the correlation ($R_p=0.95$, $n=83$, $R^2=0.90$ $P<0.0001$). When comparing actual ages with a perfect match, we tended to underestimate slightly embryo age from 8-14 days, but no consistent pattern existed from 1-7 days and 15-21 days (**Figure 3**).

Discussion

In general, the patterns of Ruddy Duck egg floatation produced are similar to other species and to the broad stages of incubation found for Ruddy Ducks in the field by Misterek (1974). At first, the egg sinks and lies horizontally but the egg angle increases gradually to become nearly vertical. Most of the eggs in this study were approximately neutral density on day 14, about 1.5 days earlier than found by Misterek (1974), and may be related to differences in humidity during incubation. Similar to van Paassen *et al.* (1984), we found that the height of the egg above the water surface increases linearly with little change in egg angle until shortly before pipping when an abrupt change in egg angle occurs with little change in egg height. Without the measurement of egg height above the water surface it would have been very difficult to determine nest age once the egg started to float, the time corresponding to greatest error in age determination (Westerskov 1950). It is believed that the height of the egg above the water surface is critical to determining incubation age. Others (Westerskov 1950; Hays & LeCroy 1971; Nol & Blokpoel 1983) have reported the diameter of the area of floating eggs but we agree with van Paassen *et al.* (1984) that the calculation of egg diameter is difficult, especially in the field, and the variability of egg shape may lead to high variability in measurement of the diameter.

Reliable estimates of incubation stages have been reported in a variety of species. Hays & LeCroy (1971) stated that egg floatation was accurate to within two days for Common Terns *Sterna hirundo*. Van Paassen *et al.* (1984) determined that egg floatation estimates for two species of shorebirds were accurate to ± three days >90% of the time. Carroll (1988) reported accuracy to within three days for Ring-necked Pheasants *Phasianus colchicus*, while Walter & Rusch (1997) stated that age estimates for Canada Goose *Branta canadensis* nests were within four days, 82% of the time. However, Nol & Blokpoel (1983) established that egg floatation was of limited success for Ring-billed Gulls *Larus delawarensis* and could only estimate age to within one week. In our study, we reliably estimated incubation age to within two days, 84% of the time.

Several possible sources of error may lead to imprecise incubation age determination. A significant source of error is probably related to the variation in shell porosity within and between clutches of eggs. Since weight loss is related to porosity, eggs with a greater porosity exhibit greater weight loss than eggs with lower porosity. Thus, an egg with a greater eggshell porosity will float sooner than an egg with a lower porosity. Also, two eggs with similar rates of water loss might differ in floatation since the smaller egg will float sooner than the larger egg (Westerskov 1950). Thus, a floatation model taking egg size into account may increase the precision of estimates of incubation age

(Westerskov 1950). A female's incubation efficiency may be affected by environmental, physiological, behavioural, individual, seasonal or clutch size factors that may produce variable incubation periods (Afton & Paulus 1992; Feldheim 1997), thus leading to incubation age error. However, if the egg floatation model is developed from the modal incubation period for that organism then the determination of embryo age should be within the range of incubation periods reported for that organism, and typically, only a few days error should occur, as in this study. Similarly, if the floatation model is based on broad, discrete categories (Westerskov 1950; Misterek 1974) instead of daily averages, an inherent error is built into the model (Watler & Rusch 1997). Egg parasitism, infertile eggs, and egg size may also cause large discrepancies among egg measurements within a clutch (Westerskov 1950; Nol & Blokpoel 1983; Carroll 1988). To reduce these discrepancies, we suggest using the average egg measurement of several eggs, and especially egg height above the water surface to determine embryo age. We consider egg floatation to be a practical and reliable method for determining incubation age of Ruddy Ducks, and encourage other investigators studying avian species with difficult to estimate incubation stages to develop species-specific egg floatation models based on the modal incubation period and daily averages, as opposed to broad incubation stage categories.

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