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DETERMINATION OF HATCHING DATE FOR EGGS OF BLACK-CROWNED NIGHT-HERONS, SNOWY EGRETS AND GREAT EGRETS

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Abstract.—Flotation of eggs in water and specific gravity of eggs of Black-crowned Night-Herons (*Nycticorax nycticorax*), Snowy Egrets (*Egretta thula*) and Great Egrets (*Casmerodius albus*) were evaluated as methods to determine date of hatching. Length of incubation and duration of hatching period were also documented for each species. Although specific gravity was a better predictor of hatching date than egg flotation, both techniques were imprecise. The regression between specific gravity and the number of days before hatching differed among clutches, but not among eggs within clutches. Specific gravity of eggs predicted hatching date only to within 3.8 d for Snowy Egrets, and 4.7 d for Black-crowned Night-Herons and Great Egrets. The mean incubation period was 27.3 d for Great Egrets, 23.7 d for Snowy Egrets and 22.8 d for Black-crowned Night-Herons. For all three species, the A egg (first egg laid) had a longer incubation period than the B or C egg. For all three species, the number of days between hatching of A and B eggs was significantly less (median = 1 d) than between hatching of B and C eggs (median = 2 d).

DETERMINACIÓN DE LA FECHA DE ECLOSIONAMIENTO PARA HUEVOS DE *NYCTICORAX NYCTICORAX, EGRETTA THULA* Y *CASMERODIUS ALBUS*

Sinopsis.—Se evaluó el método de flotación en agua y gravedad específica para determinar la fecha de eclosionamiento de huevos de Yaboa Real (*Nycticorax nycticorax*), Garza Blanca (*Egretta thula*) y Garza Real (*Casmerodius albus*). El largo del período de incubación y duración del período de eclosionamiento fue también documentado para cada especie. Aunque el método de gravedad específica permitió predecir mejor la fecha de eclosionamiento que el método de flotación, ambos resultaron poco precisos. La regresión entre la gravedad específica y el número de días previos al eclosionamiento difirió entre camadas, pero no entre los huevos de una misma camada. La gravedad específica permitió predecir la fecha de eclosionamiento en la Garza Blanca sólo para un período de 3.8 d y de 4.7 d, para las otras dos especies. El período promedio de incubación resultó ser de 27.3 d para la Garza Real, 23.7 d para la Garza Blanca y de 22.8 d. para la Yaboa. Para todas las especies, el primer huevo puesto (A) resultó con un período de incubación más largo que los próximos dos (B y C). Para todas las especies el número de días entre el eclosionamiento del huevo A y B fue significativemente menor (mediana = 1 d) que el eclosionamiento entre los huevos B y C (mediana = 2 d).

The water immersion method (Hays and LeCroy 1971, Nol and Blockpoel 1983, Schreiber 1970, Van Paassen et al. 1984), the density index method (Collins and Gaston 1987, Furness and Furness 1981, O'Malley and Evans 1980, Saunders and Smith 1981, Wooller and Dunlop 1980), or a combination of both methods (Dunn et al. 1979, Westerskov 1950) have been used to predict the stage of incubation in bird eggs. These methods are based on evaporative water loss in eggs during incubation (Drent 1970, Rahn and Ar 1974). The methods have not been evaluated for the Ardeidae (bitterns, herons and allies).

Only qualitative information is available on the length of the incubation period and the time of hatching between first and last eggs of Black-crowned Night-Herons (*Nycticorax nycticorax,* Gross 1923), Snowy Egrets (*Egretta thula*) and Great Egrets (*Casmerodius albus,* Harrison 1978, Palmer 1962). Quantitative studies for other ardeids indicate that hatching of the clutch takes several days and that the incubation period is longer in early than in later laid eggs in the clutch (Fujioka 1984, Inoue 1985, Werschkul 1979).

Ardeids have recently been the focus of studies of sibling rivalry, primarily because asynchronous hatching is widespread in the family and results in chicks of different sizes within broods (Fujioka 1985, Inoue 1985, Mock and Parker 1986). In addition, because of their high trophic position, ardeids have been selected as indicators of environmental contaminants (Bellward et al. 1990, Custer et al. 1992, Ohlendorf et al. 1979). Therefore, methods of assessing dates of hatching for these species have both theoretical and practical significance for avian ecologists. The objective of this study was to evaluate the use of water immersion and density index methods to estimate hatching dates of Black-crowned Night-Heron, Snowy Egret and Great Egret eggs. We also documented the length of the incubation period and the duration of the hatching period for each species.

STUDY AREA AND METHODS

We studied nesting Black-crowned Night-Herons, Snowy Egrets and Great Egrets on a dredge-material island in Lavaca Bay near Port Lavaca, Texas (28°36′N, 96°34′W; colony 609-121, Texas Colonial Waterbird Society 1982) from 14 Apr. to 8 Jul. 1988. We visited the colony every 2–3 d during the laying and incubation period and every day during the hatching period; some nests we visited daily during the laying and incubation period. Nests were flagged with ribbons, and eggs were individually marked with a felt-tipped permanent ink pen when first observed.

We determined laying order by hatching order (Custer and Frederick 1990) or by observation. Date of hatching was determined from observation or was based on eggs cracking 2 d before hatching and pipping 1 d before hatching (T. W. Custer, pers. obs.). Date of laying was determined by observation or the assumption that one egg was laid every other day (Fujioka 1984, Inoue 1985, Tremblay and Ellison 1980). The incubation period of an individual egg was defined as the time (days) between laying (day 0) and day of hatching.

Eggs were placed in a beaker of water, and the angle between a horizontal plane and the long axis of the egg was measured to the nearest 5° with a protractor. If the egg floated, the diameter of the portion above the surface of the water was measured with dial calipers (0.01 mm).

Specific gravity was determined by dividing the mass (g) of the egg by its volume (cm^3). Egg volume was determined in the field by subtracting the mass of the suspended egg in water from its mass in air (Custer and Frederick 1990, Evans 1969, Hoyt 1979). A battery-powered electronic balance accurate to 0.1 g was used to determine mass of eggs. Each egg's volume was determined twice on separate days. If the estimate of volume differed by more than 0.1 ml, a third measurement was taken and either one of the three values was discarded or an average volume calculated from the three volumes.

We used multivariate analysis of variance (MANOVA) to test whether regressions between specific gravity and days before hatching of individual eggs differed among clutches or by laying order (first, second or third eggs laid). As a result of limited data, only three-egg clutches, the most frequent clutch size (Custer and Frederick 1990), were used in these tests. Prediction of days before hatching based on angle or diameter were evaluated with linear regression. The significance level for all tests was 0.05.

We used linear regression to estimate the relationship between specific gravity and days before hatching. We used PRESS methodology (Draper and Smith 1981) when evaluating the utility of the regression equations for predicting days before hatching and to reduce bias caused by using the same data to develop and test a methodology. For the PRESS technique, a linear regression between specific gravity and days before hatching was calculated for each egg with more than one observation. One egg was excluded and an average regression was calculated from the remaining eggs. Prediction of days before hatching for each of the specific gravity measurements of the excluded egg were calculated with the average regression. This process was repeated excluding one egg at a time. Ninety-five percent confidence intervals of the difference between predicted and observed days before hatching were calculated as the remaining smallest and largest differences after elimination of the smallest and largest 2.5% of the observed differences.

Incubation periods were compared among species and among A (first egg laid), B and C eggs of three-egg clutches with two-way analysis of variance (ANOVA). Tukey's multiple comparison procedure was used to separate means. Differences in the time for all eggs in a clutch to hatch (either ≤ 1 d or ≥ 2d) were compared among species (n = 3) and laying order (n = 3) with a three dimensional contingency table. Hypotheses about the proportion of observations in each category were tested with log-linear models (Bishop et al. 1975).

RESULTS

The relationship between days before hatching and angle or diameter of the eggs was less precise than the relationship between days before hatching and specific gravity (Table 1, Figs. 1, 2). Therefore, angle and diameter of the eggs were not used in developing predictive equations.

An almost perfect linear relationship was found between specific gravity and days before hatching for individual Black-crowned Night-Heron, Snowy Egret and Great Egret eggs ($R^2 > 0.97$ for all but one Black-crowned Night-Heron, two Snowy Egret and one Great Egret eggs). This relationship varied so greatly among eggs, however, that methods attempting to exploit directly the individual egg relationships for prediction were unsuccessful (unpubl. data). For all three species, the regressions of specific gravity and days before hatching differed ($P < 0.0001$) among clutches but not among laying order within clutches. Therefore, laying order was not used in further predictive equations.

Ninety-five percent of the predicted hatching dates, based on specific gravity measurements, were within −2.8–3.8 d of the actual date of hatching for Snowy Egrets, −3.2–4.7 d for Great Egrets and −3.8–4.7 d for Black-crowned Night-Herons. These intervals were shortened by about 1 d at each end when only specific gravity measurements from early in the incubation period were used (Table 2).

Days before hatching, based on the average of all the specific gravity vs days before hatching regressions from the PRESS methodology, can be predicted from specific gravity as follows:

Black-crowned Night-Heron	DBH = 207.5 (SG) − 199.5
Snowy Egret	DBH = 173.5 (SG) − 162.9
Great Egret	DBH = 228.9 (SG) − 218.3,

where DBH = days before hatching and SG = specific gravity (gm/cm^3).

The period of incubation was longest in Great Egrets and longer in Snowy Egrets than in Black-crowned Night-Herons ($P < 0.0001$, Table 3). In all species, A eggs were incubated 1 d longer than B or C eggs. There was no significant interaction ($P = 0.69$) between species and laying order for the length of incubation.

The number of days between hatching of sequential eggs (Table 4) did not differ among species for three-egg clutches of Black-crowned Night-Herons, Snowy Egrets and Great Egrets. The number of days between hatching of A and B eggs was more often ≤ 1 (range −1–3 d) and significantly different than the number of days between hatching of B and C eggs, which was more often ≥ 2 d (range 0–3 d, $P < 0.0001$). The median number of days for the entire brood to hatch for all species was 3 d and varied from 0 to 4 d.

DISCUSSION

Our predictions of hatching date by specific gravity are comparable to results from studies of other species. Ninety-five percent of the predicted hatching dates were within 4.7 d of the observed date of hatching for

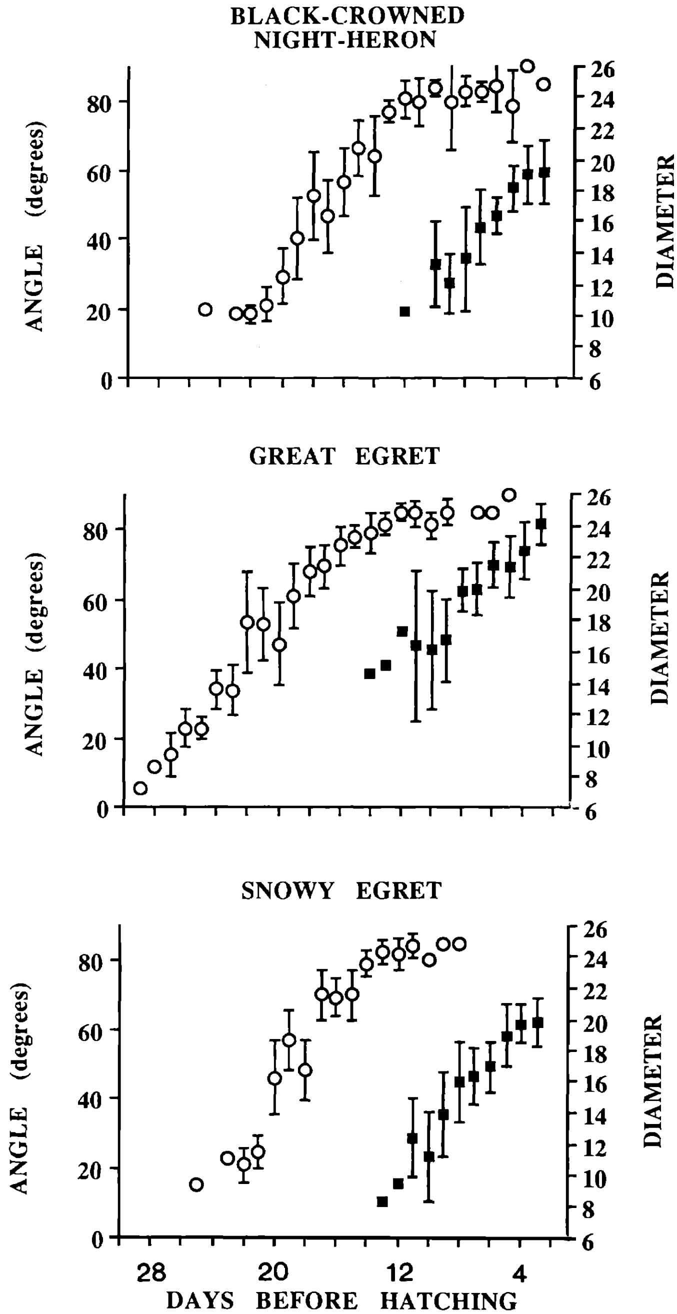

FIGURE 1. Mean angle (°) ± 2 SE (open circles) between a horizontal plane and the long axis of an egg and mean diameter (mm) ± 2 SE (darkened squares) above the water surface of immersed Black-crowned Night-Heron, Snowy Egret and Great Egret eggs in relation to days before hatching.

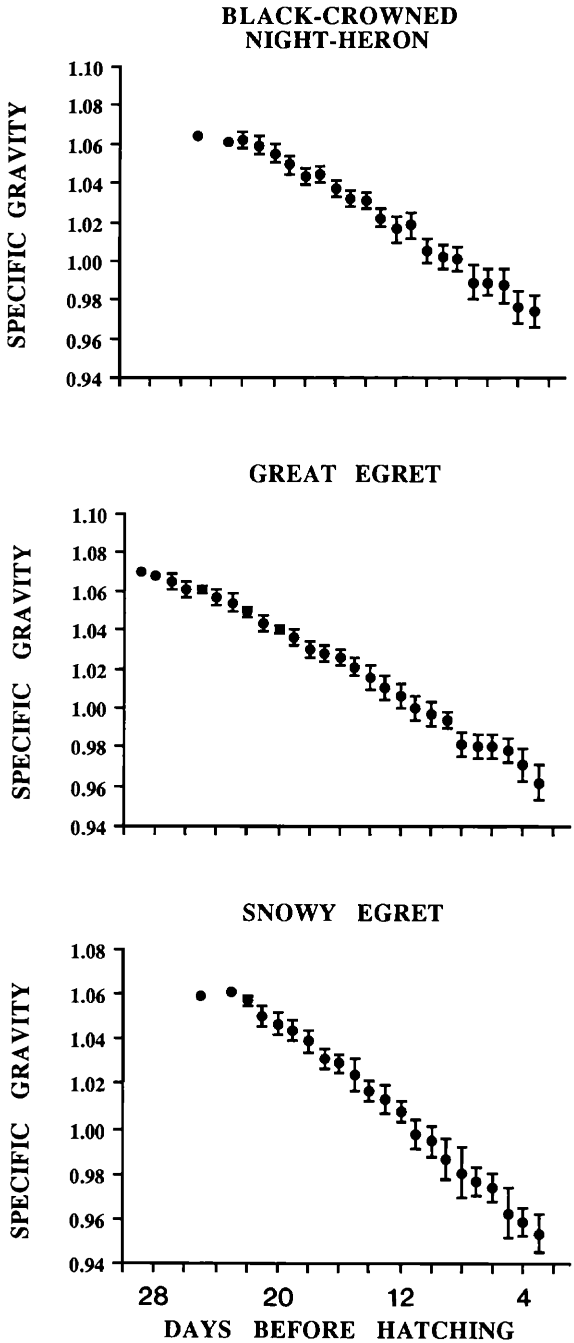

FIGURE 2. Mean specific gravity (gm/cm^3) $\pm$ 2 SE of Black-crowned Night-Heron, Snowy Egret and Great Egret eggs in relation to days before hatching.

TABLE 1. Relationships, determined by linear regression, between days before hatching and angle of eggs submerged in water, diameter of eggs exposed above water and specific gravity of eggs in Great Egrets, Snowy Egrets and Black-crowned Night-Herons.

Species	Explanatory variable	R^2	Sample size		
			Nests	Eggs	Records[a]
Great Egret	Angle[b]	0.742	22	54	113
	Diameter[c]	0.279	21	50	115
	Specific gravity[d]	0.872	22	56	244
Snowy Egret	Angle	0.720	28	73	245
	Diameter	0.587	30	79	158
	Specific gravity	0.896	30	80	431
Black-crowned Night-Heron	Angle	0.646	26	62	217
	Diameter	0.319	27	61	112
	Specific gravity	0.830	27	68	353

[a] Number of measurements.
[b] Angle of egg submerged in water.
[c] Diameter of egg exposed above water.
[d] Specific gravity (g/cm^3) of egg.

Black-crowned Night-Heron eggs, 3.8 d for Snowy Egret eggs and 4.7 d for Great Egret eggs. The hatching dates of 90% of Great Skua (*Catharacta skua*) eggs were estimated to within 4 d with specific gravity measurements (Furness and Furness 1981). Specific gravity was used to age 87% of Silver Gull (*Larus novaehollandiae*) eggs to within 2 d (Wooller and Dunlop 1980) and 69% of cockatoo (five species combined) eggs to within 3 d (Saunders and Smith 1981). In contrast, for European Starlings (*Sturnus vulgaris*), the 95% prediction interval for the regression of egg age and specific gravity was ±2.8 d (Dunn et al. 1979), but, because the incubation period of the starling is less than one-half that of the species in this study, a narrower confidence limit is expected.

Data from our study support the suggestion of Nol and Blokpoel (1983) that variability of incubation behavior among adults may account for a large portion of the variability of specific gravity of eggs. In our study, the regression of specific gravity and days before hatch varied significantly among clutches but not among eggs within clutches.

Our ability to predict hatching dates decreased as the incubation period

TABLE 2. Ninety-five percent confidence limits for differences between predicted and actual days before hatching based on deleting the smallest and largest 2.5% of the differences of eggs of Black-crowned Night-Herons, Snowy Egrets and Great Egrets.

Incubation period	Black-crowned Night-Heron	Snowy Egret	Great Egret
≤29 d before hatch	−3.8–4.7	−2.8–3.8	−3.2–4.7
12–29 d before hatch	−2.6–3.1	−1.5–2.8	−2.1–3.1

TABLE 3. Length of incubation by laying order of eggs for three-egg clutches of Black-crowned Night-Herons, Snowy Egrets and Great Egrets.

Species	Laying order	Number of eggs	Number of days of incubation Mean ± SE
Great Egret	A	6	28.3 ± 0.4
	B	6	27.0 ± 0.3
	C	6	26.5 ± 0.6
	Overall		27.3 ± 0.3 A[1]
Snowy Egret	A	6	24.3 ± 0.3
	B	5	23.2 ± 0.2
	C	4	23.3 ± 0.3
	Overall		23.7 ± 0.2 B
Black-crowned Night-Heron	A	10	23.5 ± 0.3
	B	9	22.6 ± 0.3
	C	9	22.3 ± 0.3
	Overall		22.8 ± 0.2 C

[1] Interspecific means not sharing the same letter are significantly different (two-way ANOVA: Species, Laying order, Species*Laying order, $\alpha = 0.05$). A-eggs had significantly longer incubation periods than B- or C-eggs, regardless of species.

increased. For example, for Snowy Egrets, 95% of the predicted days before hatching were −1.5–2.8 of observed days before hatching when specific gravity was measured more than 12 d prior to hatching, whereas the interval was −2.8–3.8 d when specific gravity was measured on any day before hatching. This difference occurred because of the large variance in specific gravity late in the incubation period. Westerskov (1950) also observed this trend and stated that the use of specific gravity to age Ring-necked Pheasant (*Phasianus colchicus*) eggs was not recommended for the later part of the incubation period.

As a result of the greater precision of specific gravity, we did not explore the use of flotation of eggs in water to predict hatching date. Dunn et al.

TABLE 4. Difference (d) in hatching date between laying order of eggs in three-egg clutches of Black-crowned Night-Herons, Snowy Egrets and Great Egrets.

Species (No. clutches)	Difference (d) in hatching date among laying order: B–A					C–B				C–A				
	−1[a]	0	1	2	3	0	1	2	3	0	1	2	3	4
Black-crowned Night-Heron (n = 13)		5	3	5			2	9	2	1		3	6	3
Snowy Egret (n = 13)		2	7	3	1	1	4	8			1	3	7	2
Great Egret (n = 19)	1	3	11	4			5	12	2		2	4	9	4
Overall (n = 45)	1	10	21	12	1	1	11	29	4	1	3	10	22	9

[a] In one case the B-egg hatched before the A-egg.

(1979) included flotation of eggs in water in their key for aging European Starling eggs, but placed more quantitative emphasis on specific gravity. Likewise, Nol and Blokpoel (1983) discounted flotation of eggs in water as an aging technique for Ring-billed Gull (*Larus delawarensis*) eggs. In contrast, the hatching date of 95% of Northern Lapwing (*Vanellus vanellus*) eggs were aged to within 3 d from the angle of the submerged egg in water and to within 4 d from the diameter of the egg exposed above the surface (Van Paassen et al. 1984). Hays and LeCroy (1971) estimated age of Common Tern (*Sterna hirundo*) eggs to within 2 d using flotation of eggs in water, but their sample size was small (two eggs for each of nine categories of embryo development).

In our study, eggs within clutches hatched over several days (range = 0–4 d) and the hatching interval between A and B eggs was shorter than between B and C eggs. These same patterns have been observed in Cattle Egrets (*Bubulcus ibis,* Fujioka 1984), Little Egrets (*Egretta garzetta,* Inoue 1985) and Little Blue Herons (*Egretta caerulea,* Werschkul 1979), and result from increased incubation through the egg laying period (Fujioka 1985, Gross 1923, Inoue 1985, Milstein et al. 1970).

We conclude from this study that specific gravity of eggs is a better predictor of hatching date than angle of the egg when submerged in water or diameter of the egg exposed above water. Even though the ability to predict hatching date from specific gravity of eggs was higher earlier in incubation, the confidence limits around the prediction of hatching date were so large (±3–5 d) that we consider this method to be only a rough estimate of hatching date in eggs of the three species studied here.

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