The Condor 89:364–368

INTRACLUTCH VARIATION IN THE SIZE, MASS AND COMPOSITION OF RING-BILLED GULL EGGS[1]

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Abstract. In 1984, 94 complete three-egg clutches of Ring-billed Gull (*Larus delawarensis*) eggs were collected on Granite Island, northern Lake Superior. The fresh eggs (i.e., collected within 12 hr of being laid) were weighed, measured and frozen for later chemical analysis to determine if relationships existed between egg size, composition, and sequence. Egg size and mass decreased through the laying sequence. The average composition of fresh eggs was 35% yolk, 56% albumen, and 9% shell. Water accounted for 69% of fresh egg mass. Approximately 8% of fresh egg mass was yolk lipid. Dry yolk consisted of approximately 40% protein and 60% lipid. Caloric content averaged 1.55 kcal/g fresh egg mass. Within clutches, third-laid eggs contained absolutely less albumen and nutrients than earlier laid eggs. Albumen increased in proportion to the 1.2 power of egg mass, and the yolk in proportion slightly greater than the 0.66 power of egg mass. Results agreed well with egg composition studies of other semiprecocial species.

Key words: Ring-billed Gull; Larus delawarensis; *egg composition; egg variation; egg size; egg sequence.*

INTRODUCTION

The quality of eggs has been defined in terms of their relative size, weight, and composition (Ricklefs 1977a). In Lesser Black-backed Gulls (*Larus fuscus*), Houston et al. (1983) found that egg quality was positively correlated with the condition of the laying female. If the body condition of females is depressed through the laying sequence in response to the stresses of egg laying and food acquisition, so might egg quality deteriorate as subsequent eggs are laid (Meathrel 1986). Therefore, within a clutch, the first-laid egg might be expected to be larger, heavier, and contain absolutely more nutrients (lipid and protein) than eggs laid later.

It has been widely documented that larids typically lay three eggs, the third egg usually being the smallest (Coulson 1963; Parsons 1976; Coulter 1977; Schreiber et al. 1979; Meathrel et al., in press). They have shown that this results from reduced albumen deposition during egg formation.

This study was designed to examine intraclutch relationships between the size, mass, and nutrient content of Ring-billed Gull (*L. delawarensis*) eggs.

[1] Received 19 May 1986. Final acceptance 1 October 1986.

METHODS

This study was conducted on Granite Island, Black Bay, northern Lake Superior, Canada during the summer of 1984. Newly-initiated Ring-billed Gull clutches (n = 233) were chosen randomly. Daily nest checks were performed to collect first- (a-egg), second- (b-egg) and third-laid (c-egg) eggs. All eggs removed were replaced with surrogates. At collection (i.e., within 12 hr of being laid), eggs were marked in sequence laid with indelible ink and weighed to ±1 g with a 100-g Pesola spring scale. Egg length and width were then measured to ±0.1 mm with Vernier calipers.

In total, 282 eggs were collected from complete three-egg clutches. After being weighed to ±0.1 g on an Ohaus digital scale, the fresh eggs were boiled for 10 min so that yolk, shell, and albumen could be easily separated (Jones 1979, Ricklefs 1982). They were allowed to cool in air to room temperature, and were then tightly wrapped in cellophane, sealed in a jar, frozen, and returned to the laboratory for chemical analyses.

In the laboratory, eggs were thawed for 24 hr, then weighed whole to the nearest 0.0001 g on an Oertling balance. Eggs were then separated into yolk, albumen, and shell (with corresponding membranes) and each constituent was weighed again. Any water loss caused by boiling (as reported by Nisbet 1978 and Ricklefs and

Montevecchi 1979) or freezing was corrected for by adding the difference between fresh egg weight and thawed weight to wet albumen weight (Williams et al. 1982). The constituents were then dried for one week to a constant weight in a Gallenkamp Incubator (56°C), and weighed again to determine water content.

Since Romanoff and Romanoff (1949) and Ricklefs (1977b) found that the lipid content of the albumen and the carbohydrate content of both yolk and albumen represented less than 1% of fresh egg weight, the levels of these contents were not determined. Ryder et al. (1977) found that carbohydrates averaged 0.6% of wet yolk weight for Ring-billed Gulls. The nutrient content of the dried yolks was determined using Soxhlet-extraction with petroleum ether (B.P. = 30° to 60°C). Dried post-extraction remains were assumed to consist of only protein. Lipid content was determined by the difference between dry yolk weights before and after ether extraction (Ricklefs 1977b). The energy content of eggs per g of fresh weight was calculated using the caloric equivalents of 9.5 kcal/g lipid and 5.65 kcal/g nonlipid dry (Ricklefs 1977b).

Statistical analyses were performed using one-way analysis of variance (ANOVA) and allometric regressions (Sokal and Rohlf 1981). Significance was assumed at $P \leq 0.05$.

RESULTS

One-way ANOVA indicated that fresh egg size and mass differed within clutches (Table 1). C-eggs were significantly lighter ($F = 35.90$, $P < 0.001$) and shorter ($F = 18.02$, $P < 0.001$) than a- or b-eggs. Egg width ($F = 60.06$, $P < 0.001$) decreased through the laying sequence.

The average composition of Ring-billed Gull eggs (a-, b-, and c-eggs pooled) was 35% yolk (14% dry matter, 21% water), 56% albumen (10% protein, 46% water), and 9% shell (7% dry shell, 2% water), with a wet yolk to albumen ratio of 0.63. In total, 69% of the fresh egg was water. Dry yolk consisted of approximately 40% protein and 60% lipid. The average energy content of eggs was about 1.55 kcal/g fresh egg weight.

The composition of fresh eggs varied within clutches (Table 2). Fresh egg mass decreased through the laying sequence, as did wet albumen mass. C-eggs contained absolutely less dry yolk, yolk lipid, fresh shell, and dry shell mass than a- or b-eggs. B-eggs had absolutely more wet yolk and yolk protein than a- or c-eggs. First-laid eggs contained proportionately less yolk water and more albumen water than c-eggs. Additionally, a-eggs contained absolutely more albumen protein, and had lower wet and dry yolk to albumen ratios than either b- or c-eggs. Although the percent lipid in both wet or dry yolks did not change through the laying sequence, a-eggs had less energy per gram than b-eggs.

Allometric regressions examined relationships between fresh egg constituents and fresh egg mass (after Ricklefs 1984). Yolk size and nutrient content were poorly correlated with egg mass (Table 3). The constituent most strongly related to egg mass was fresh albumen. Therefore, whole egg mass was strongly correlated with the mass of water in the egg, moderately correlated with caloric content and the mass of egg protein; but was not correlated with the mass of lipid in the egg. Shell weight increased in direct proportion with egg mass. Albumen increased in proportion to the 1.2 power of egg mass, and the yolk in proportion slightly greater than the 0.66 power of egg mass.

TABLE 1. Size and weight of fresh Ring-billed Gull eggs in relation to laying sequence, Granite Island, 1984.

Variable	n	$\bar{x}$	SD	CV (%)
Pesola weight (g)[a]				
Egg a	103	60.2	3.7	6.1
b	103	59.2	3.3	5.6
c	101[b]	56.3	3.3	5.9
Length (mm)				
Egg a	197	59.0	2.4	4.1
b	197	58.8	2.2	3.7
c	195	57.8	2.2	3.8
Width (mm)				
Egg a	197	42.4	1.1	2.6
b	197	42.1	1.0	2.4
c	195	41.3	1.0	2.4

[a] Not all eggs were weighed on the colony in order to decrease disturbance.
[b] Two c-eggs were destroyed when measured.

DISCUSSION AND CONCLUSIONS

The production of eggs induces a physiological stress on the laying female (King 1972). Various researchers have emphasized that the nutritional status of female gulls at the time of laying may determine egg size and mass (Schreiber and Lawrence 1976, Mills 1979, Schreiber et al. 1979, Murphy et al. 1984). Both intraspecific and intraclutch variation in egg size have been attributed to the amount of albumen deposited around

TABLE 2. Composition of fresh Ring-billed Gull eggs in relation to laying sequence, Granite Island, 1984.

Variable	Egg a $n = 94$	Egg b $n = 94$	Egg c $n = 94$	F-ratio, significance[a] (ANOVA)
Digital weight (g)	55.9 ± 4.1	54.4 ± 3.8	51.8 ± 3.5	28.01***
Yolk: Wet yolk (g)	16.02 ± 1.28	16.69 ± 1.88	15.90 ± 1.85	5.94**
Dry yolk (g)	7.37 ± 0.61	7.57 ± 0.61	7.08 ± 0.72	13.90***
% water	53.95 ± 2.30	54.41 ± 2.80	55.17 ± 4.30	3.47*
Lipid (g)	4.41 ± 0.45	4.51 ± 0.45	4.26 ± 0.40	7.45***
% lipid	27.59 ± 2.18	27.12 ± 2.26	26.96 ± 2.31	1.99NS
Lipid (% dry)	59.87 ± 2.89	59.45 ± 2.80	60.60 ± 7.30	1.37NS
Nonlipid dry (g)	2.96 ± 0.31	3.07 ± 0.30	2.85 ± 0.31	11.32***
Albumen: Wet albumen (g)	32.74 ± 3.06	30.30 ± 3.02	28.42 ± 3.54	42.78***
Dry albumen (g)	4.49 ± 0.50	4.32 ± 0.46	4.08 ± 0.50	17.18***
% water	86.30 ± 0.60	86.03 ± 0.80	85.48 ± 2.80	5.29**
Shell: Wet shell (g)	4.70 ± 0.43	4.77 ± 0.49	4.31 ± 0.50	25.07***
Dry shell (g)	3.58 ± 0.31	3.54 ± 0.29	3.26 ± 0.29	31.35***
% water	23.73 ± 3.50	25.57 ± 4.40	23.98 ± 5.90	4.32*
Yolk/albumen (wet)	0.49 ± 0.05	0.56 ± 0.09	0.57 ± 0.13	18.37***
Yolk/albumen (dry)	1.66 ± 0.22	1.80 ± 0.22	1.76 ± 0.26	9.87***
Energy (kcal/g fresh)	1.51 ± 0.07	1.55 ± 0.07	1.54 ± 0.08	8.61***

[a] Significance: NS, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

the yolk in larids (Parsons 1976, Herring Gull, *L. argentatus*; Coulter 1977, Western Gull, *L. occidentalis*), as well as other groups (i.e., Ricklefs 1977a, European Starling, *Sturnus vulgaris*; Jones 1979, Great White Pelican, *Pelecanus onocrotalus*; and numerous Procellariiformes, Warham 1983).

In this study, the finding that c-eggs weighed less and had an absolutely smaller yolk containing less nutrients than a- or b-eggs indicated that the stored nutrient reserves of females, which are important to the production of eggs (Houston et al. 1983), were being depleted as subsequent eggs were laid. This observation, along with the finding that c-eggs had a larger wet yolk to albumen ratio than a- or b-eggs, contradicts Boersma's (1982) suggestion that yolk size is proportionately constant in eggs of different sizes. Allometric regressions of egg components on the mass of fresh eggs demonstrated that yolk size was poorly correlated with egg mass, and did not increase in direct proportion with increasing egg size. Large eggs apparently provided absolutely more albumen and water to the developing embryo.

The wet yolk to albumen ratios of Ring-billed Gulls (0.55) agreed with the 0.58 value reported by Ricklefs (1977b) for Laughing Gulls (*L. atricilla*). Carey et al. (1980) stated that the mean relative yolk content of semiprecocial eggs is 33%, similar to the results of this study (35%).

Our results of Ring-billed Gull egg lipid (8%) and energy (about 1.6 kcal/g) content agreed well with other studies on egg energetics. Ricklefs (1977b) reported that yolks are large and contain 9 to 12% total lipids in precocial and semiprecocial species relative to altricial species (6%). The chemical energy of fresh eggs increases directly with yolk size because the yolk contains more high energy lipid relative to the protein content of albumen. The absolute energy content of eggs increased with increasing egg size since larger eggs contained more albumen and yolk. Carey et al. (1980) stated that, for semiprecocial species such as the Ring-billed Gull, yolk lipid accounted for 9.5% of fresh egg weight, and that larid eggs averaged 1.67 kcal/g. In this study, an average of 8.1% of fresh egg weight was accountable to the mass of yolk lipid; hence, these eggs contained relatively less energy per gram fresh weight (approximately 1.55 kcal/g) when compared to the energy level reported by Carey et al. (1980). Examined Ring-billed Gull eggs had dry yolks that consisted of 60% lipid and 40% protein. This agreed with Ricklefs' (1977b) results for the Laughing Gull, wherein yolk lipid accounted for 64% of dry yolk matter.

As was reported by Ricklefs and Montevecchi (1979) for Northern Gannett (*Sula bassanus*) eggs, egg mass was most strongly correlated with the water content of the egg, derived mainly from the albumen. Ricklefs (1977b) found that Laugh-

TABLE 3. Allometric regressions of egg constituents on fresh egg mass for Ring-billed Gulls nesting in 1984.[a]

Variable	Statistics[b]			
	a	b	s_b	R^2
Yolk: Wet yolk	0.060	0.662	0.070	0.240
Dry yolk	−0.445	0.755	0.062	0.338
Water	−0.134	0.622	0.102	0.116
Lipid	−0.506	0.662	0.070	0.238
Nonlipid dry	−0.762	0.710	0.072	0.254
Albumen: Wet albumen	−0.731	1.278	0.059	0.620
Dry albumen	−1.381	1.160	0.061	0.557
Water	−0.861	1.315	0.069	0.557
Shell: Wet shell	−0.935	0.921	0.066	0.405
Dry shell	−1.128	0.962	0.049	0.579
Water	−1.359	0.808	0.195	0.057
Whole egg: Water	−0.341	1.096	0.029	0.833
Nonlipid dry	−0.640	0.963	0.031	0.772
kcal	0.509	0.813	0.035	0.649

[a] a-, b-, and c-eggs were pooled for analyses ($n = 282$).
[b] a and b are the intercept and slope in the equation $\log Y = a + b \log X$; s_b is the standard error of b; R^2 is the coefficient of determination.

ing Gull eggs consisted of 75.4% water. Generally for semiprecocial species, water accounts for 76.5% of fresh egg weight (Carey et al. 1980). Ring-billed Gull eggs in this study averaged 69% water, falling just below the range given by Ricklefs (1977b) for semiprecocial species (70 to 75%).

This study has supported Sibbald's (1979) theory that egg quality cannot be measured wholly on the basis of egg energy content. Because egg weight was most strongly correlated with total water content, larger eggs did not necessarily contain more energy per gram than relatively smaller eggs. Allometric regressions revealed that increasing egg weight was a function of increasing albumen content. More albumen meant proportionately more water, and proportionately less yolk and energy, in larger eggs. Obviously, larger eggs with more albumen have absolutely more energy because they have more albumen protein than do smaller eggs. Boersma (1982) postulated that more albumen leads to a larger hatchling. In larids, increased amounts of albumen in eggs and the resultant larger hatchling size have been shown to increase hatchling survival (Parsons 1970, Lundberg and Vaisanen 1979). Hence, eggs with smaller yolks and lower energy by weight are not necessarily of lower egg quality (Sibbald 1979), especially if quality is measured in terms of hatchling survival.

ACKNOWLEDGMENTS

We thank Beatrice Termaat for her assistance in the field and laboratory, as well as her useful comments on the manuscript. Financial support for this study was obtained from a Natural Sciences and Engineering Research Council of Canada grant to JPR (A6520).

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