RELATIONSHIPS AMONG FRESH MASS, INCUBATION TIME, AND WATER LOSS IN JAPANESE QUAIL EGGS[1]

PAMELA A. MARTIN[2]
Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

TODD W. ARNOLD[2]
Department of Zoology, University of Western Ontario, London, Ontario N6A 5B7, Canada

Abstract. Egg size is commonly assumed to increase the fitness value of hatchlings, and yet, most intraspecific variation in egg size appears to be highly heritable. Because traits strongly associated with fitness are expected to have low heritabilities, these two observations are not mutually compatable unless other factors act to offset the fitness value of large eggs. In this paper, we assess the influence of intraspecific egg-size variation on incubation periods and rates of egg water loss among Japanese Quail (*Coturnix japonica*) eggs. In contrast to interspecific studies of these relationships, we observed very low correlations between the logarithms of fresh egg mass and incubation time ($r^2 = 0.016$, $P = 0.01$, $n = 378$) and between fresh egg mass and daily water loss ($r^2 = 0.028$, $P = 0.03$, $n = 163$). Analysis of fresh eggs and newly hatched chicks confirmed that heavier eggs lost proportionately less water during incubation. These observations suggest that statistical relationships derived from interspecific comparisons of egg size and egg physiology are not generally applicable to studies of intraspecific egg-size variation.

Key words: Coturnix japonica; egg composition; egg size; egg water loss; incubation; Japanese Quail.

INTRODUCTION

Egg size (mass and/or volume) varies considerably within many bird species, with a large component of this variation being attributable to additive genetic variance (reviewed in Boag and van Noordwijk 1987, Lessells et al. 1989). Highly heritable characters are theoretically presumed to have a low association with fitness (Falconer 1981). However, egg size has been positively correlated with several components that are likely related to fitness, including greater hatchability, larger structural size and/or nutrient reserves of hatchlings, higher initial growth rates, enhanced thermoregulatory capabilities, greater fasting endurance, and greater survival to fledging (e.g., Parsons 1970; Schifferli 1973; Nisbet 1978; Ricklefs et al. 1978; Krapu 1979; Lundberg and Väisänen 1979; Ankney 1980; Furness 1983; Rhymer 1983, 1988). These observations present an enigma; egg size should not be highly variable, highly heritable, *and* highly correlated with reproductive success. If these three obser-

vations are generally true among birds, then there must also be certain disadvantages associated with large eggs, otherwise strong directional selection should have acted to fix the egg size of each species (or population) at some "optimally" large size.

Lack (1967) hypothesized that nutrients may be limiting for laying female waterfowl, and thus, species producing relatively large eggs (for their body size) must compensate by laying fewer eggs. This notion of a trade-off between egg size and clutch size has figured prominently in theoretical life-history models (e.g., Smith and Fretwell 1974, Brockelman 1975); however, empirical studies generally have not supported such an hypothesized trade-off (e.g., Parsons 1976, Rohwer 1988, Lessells et al. 1989).

Nest predation can exert considerable selection pressure on the length of laying and/or incubation periods, thereby acting to reduce the total amount of time eggs and chicks remain vulnerable in the nest (Lack 1948, Arnold et al. 1987). Among species of birds, incubation periods are positively correlated with egg size (Worth 1940), and some ornithologists have assumed that a similar relationship applies to intraspecific egg-size variation (Parsons 1972). If clutches of large eggs required longer to incubate,

---

they would also be at greater risk of predation, and selection might therefore favor smaller eggs.

Egg water loss during incubation is a function of egg shell conductance and the incubation environment (e.g., temperature, humidity, atmospheric pressure). Because larger eggs have a lower surface area to volume ratio, they should also experience lower rates of water loss, given similar characteristics of the shell (e.g., pore diameter, pore density, and shell width). Such variation in water loss might have important consequences for birds breeding at high altitudes (Carey et al. 1983) or in extremely wet or dry environments (Lomholt 1976).

Among species of birds, incubation time (I, in days) is proportional to egg mass (W, in g) raised to the 0.22 power ($I = 12.03W^{0.22}$), and daily water loss (M_{H_2O}, in g/day) is proportional to egg mass raised to the 0.74 power ($M_{H_2O} = 0.015W^{0.74}$) (Rahn and Ar 1974). Rahn and Ar (1974) combined these two relationships, assuming that $W^{0.22} \cdot W^{0.74} = W^{0.96} \approx W^{1.00}$, to obtain $F = M_{H_2O} \cdot I \cdot W^{-1}$, thereby demonstrating that eggs of most species lose a constant fraction (F) of their fresh mass as water vapor during incubation (F = 18%; later amended to 15% in Ar and Rahn 1980). These equations have proved to be highly robust, changing little with the addition of new data (Ar and Rahn 1980, 1985). Although there appear to be taxonomic differences, the general relationships are also apparent within individual orders of birds (Ar and Rahn 1985: Table 6). However, these relationships have received little attention intraspecifically. In order to better understand the relationships among egg mass, incubation time, and rate of water loss within a species, we examined these factors under controlled incubation conditions using eggs of the Japanese Quail (*Coturnix japonica*).

METHODS

Unincubated Japanese Quail eggs were obtained from the Quail Genetic Stock Centre at the University of British Columbia. Eggs were of unknown age when we received them, and incubation was further delayed for four days after we obtained them. Prolonged pre-incubation storage is known to affect hatchability and incubation time in domestic chickens (*Gallus domesticus*) (McLaury and Insko 1968), but we could not control for this potential source of variation in our study. Eggs were systematically numbered (n = 450), weighed (±0.01 g), and set to incubate

in two forced-draft incubators which rotated the eggs through 90° every 2 hr and kept environmental conditions constant at 35°C and 56% RH.

The eggs used in this study were part of an experiment to assess potential effects of grasshopper-control insecticides on developing embryos (Martin 1990); however, only control eggs from that experiment are included in the present study. Control eggs were immersed in distilled water at room temperature (approx. 24°C) for 30 sec on incubation day 0, 4, or 14 and allowed to drip-dry (approx. 20 min at room temperature) before being returned to the incubators.

We reweighed 200 eggs on incubation day 13 to determine rates of water loss (M_{H_2O}, mg·d^{-1}). Because the incubation environment was controlled and essentially constant among eggs, variation in water loss was primarily a function of variation in egg shell conductance. Eggs were quickly returned to their incubators after weighing. Only eggs which subsequently hatched were used in analyses comparing daily water loss to fresh egg mass.

Eggs were moved into a hatching unit on day 14 of incubation. Because of space constraints, we placed four to six eggs in each individual holding compartment; however, we positioned eggs so that they had minimal contact with each other so that between embryo synchronization would not affect hatching times (e.g., Vince 1966). Eggs typically hatched asynchronously within individual holding compartments, but 43 chicks could not be matched with certainty to their respective eggs (these 43 eggs were excluded from analyses involving attributes of the chicks). The hatching unit was monitored approximately once per hour, from onset of pipping in the first egg (day 15), until 6 hr after the last successful egg hatched (day 20). During the peak hatching period (days 16–17) we maintained 24 hr vigilance over the incubators, but on days 18–20 we did not check the incubator at 01:00, 02:00, 04:00, or 05:00. We subjectively categorized the wetness or dryness of the plumage of newly-hatched chicks using five categories to help interpolate hatch times between the two most recent incubator checks (Batt 1969). Using these criteria, the incubation period of individual eggs could usually be determined to the nearest ½ hr. Newly-hatched chicks were allowed to dry for 1 hr in the hatcher before being weighed (±0.01 g) and measured (tarsus length, ±0.05 mm).

A subsample of 123 chicks and a separate sam-

TABLE 1. Variation in egg and chick composition (g), tarsus length (mm), incubation period (d), and daily egg water loss (g) of the Japanese Quail.

	n	x̄ ± SD	CV (%)	Range
Egg mass[a]	378	9.54 ± 0.69	7.0	7.18–11.25
Egg mass[b]	100	9.48 ± 0.85	8.9	7.09–11.28
Shell, dry		0.76 ± 0.10	12.8	0.52–1.41
Total protein, dry		1.30 ± 0.13	10.1	0.91–1.62
Total lipid		0.95 ± 0.12	13.0	0.70–1.26
Total water		6.47 ± 0.60	9.3	4.62–7.57
Chick mass[a]	369	6.64 ± 0.60	11.4	4.78–8.32
Chick tarsus[a]	369	16.67 ± 0.78	4.7	13.40–18.80
Chick mass[c]	123	6.68 ± 0.78	11.6	4.65–8.95
Total protein, dry		1.18 ± 0.15	12.9	0.78–1.61
Total lipid		0.46 ± 0.12	26.2	0.21–0.74
Total water		5.04 ± 0.59	11.7	3.58–6.75
Chick tarsus[c]	123	16.48 ± 0.87	5.3	13.40–18.70
Incubation period[a]	378	16.58 ± 0.61	3.7	15.09–18.99
Incubation period[c]	123	16.35 ± 0.48	3.0	15.09–17.88
Daily water loss[d]	163	0.077 ± 0.012	15.6	0.050–0.126

[a] Overall sample of hatching eggs.
[b] Sample used to analyze egg contents.
[c] Sample used to analyze chicks.
[d] Sample includes only those eggs (of 200) that subsequently hatched.

ple of 100 unincubated eggs were collected for analysis of nutrient composition (only chicks which could be unambiguously assigned to an egg were included in this sample). Eggs were boiled for 5 min, chicks were killed by decapitation, and eggs and chicks were individually sealed in plastic bags and frozen at −17°C. In the laboratory, eggs were thawed and separated into yolk, albumen, and shell (including shell membranes) and wet masses were determined for each component. Egg components and whole chicks were dried at 80°C for 48 hr to determine dry masses (Kerr et al. 1982). Lipid content of yolk and whole chicks was determined by extraction with petroluem ether for 4 hr in a Soxhlet apparatus (Dobush et al. 1985). Remaining dry matter in yolks and chicks, and the dry mass of egg albumen, were assumed to be primarily protein (Ricklefs et al. 1978).

All statistical tests were performed using the SAS statistical package (SAS Institute Inc. 1985). Hatchability as a function of fresh egg mass and daily water loss was analyzed using logistic regression (SAS CATMOD procedure). Ordinary least squares (OLS) regressions of $\log_{10}$ nutrient component masses on $\log_{10}$ fresh egg mass, $\log_{10}$ fresh chick mass, or $\log_{10}$ tarsus length were used to determine allometric relationships among egg and chick components (SAS GLM procedure). We recognize two potential problems with this technique. First, we were regressing the components of an object on the entire object, so frac-

tionally large components necessarily had high correlations and slopes near unity (e.g., Hochachka 1988). Second, OLS regression assumes that all variation (lack of fit) resides in the ordinate (McArdle 1988), a rather unlikely assumption. Nevertheless, we proceeded with this technique because alternative methods (e.g., principal components analysis, reduced major axis regression) have their own interpretational problems, and because all previously published studies with which we wished to compare our results had also used OLS regression.

RESULTS

Japanese Quail displayed a considerable range of variation in egg mass, chick mass, incubation time, and rate of incubation water loss, with water loss exhibiting the most relative variation and incubation time exhibiting the least relative variation (Table 1: CVs). For both egg and chick composition, lipid content had the highest coefficient of variation and water content had the least (Table 1). Eggs and chicks collected for nutrient analysis were similar in mean mass to the larger samples of controls, but samples collected for analysis tended to be more variable (Table 1). This reflects our attempt to sample the widest possible spectrum of egg- and chick-size variation by actively selecting very large and very small eggs and chicks for nutrient analysis. Chicks collected for nutrient analysis had significantly shorter incubation periods than the entire sample

TABLE 2. Variation in hatchability and incubation period (d) of Japanese Quail eggs associated with 30 sec immersion in distilled water.

Treatment day	Hatchability[a]	Incubation period[b]
0	0.847	16.53 ± 0.62
4	0.832	16.53 ± 0.60
14	0.847	16.67 ± 0.61

[a] Fraction of all eggs hatching, based on 150 eggs for day 0 and day 14, and 149 eggs for day 4.
[b] $\bar{x} \pm 1$ SD.

FIGURE 1. Allometric relationship between incubation time and egg mass of Japanese Quail eggs. Axes are in log scale.

(by about 5.5 hr; Table 1); this difference was an inadvertent consequence of non-random sampling.

Our sham exposure treatment (immersion in distilled water on incubation day 0, 4, or 14) had a slight effect on incubation time (ANOVA: $F_{2,375}$ = 2.75, $P = 0.07$). Eggs treated on days 0 and 4 hatched synchronously; however, eggs treated on day 14 required an additional 0.14 d (3 hr and 20 min) to hatch, on average (Table 2). There was no interaction between treatment day and egg size (i.e., egg size·treatment day interaction: $F_{2,372} = 0.83$, $P = 0.43$), nor did treatment day influence hatchability (Table 2: $\chi^2 = 0.16$, $P = 0.92$). The effect of egg immersion on incubation time was controlled for in the following statistical analyses by including treatment day as a class variable in the regression model (i.e., ANCOVA).

Hatchability was slightly affected by egg mass ($\chi^2 = 3.19$, $P = 0.07$); examination of frequency distributions for hatched and failed eggs revealed that this was a function of lower hatching success among eggs that were smaller than the median. Egg mass was weakly correlated with incubation time (Fig. 1; $r^2 = 0.016$, $P = 0.01$, $n = 378$) and with daily water loss (Fig. 2; $r^2 = 0.028$, $P = 0.03$, $n = 163$ hatched eggs). The relationship between egg mass and daily water loss, $M_{H_2O} = 0.034W^{0.364 \pm 0.170}$, indicated that larger quail eggs lost absolutely more, but proportionately less, water during incubation than did small eggs (i.e., the 95% CI for the exponent, $0.029 < b < 0.699$, includes neither 0 nor 1). Daily water loss had no effect on total incubation time ($F_{1,158} = 1.39$, $P = 0.24$, partial $r^2 = 0.008$, $n = 163$) or on hatchability ($\chi^2 = 2.06$, $P = 0.37$, $n = 200$).

Egg composition analyses revealed that the water, protein, and lipid components were directly proportional to egg mass, although there was considerable unexplained variation in the lipid relationship (Table 3). Shell mass did not
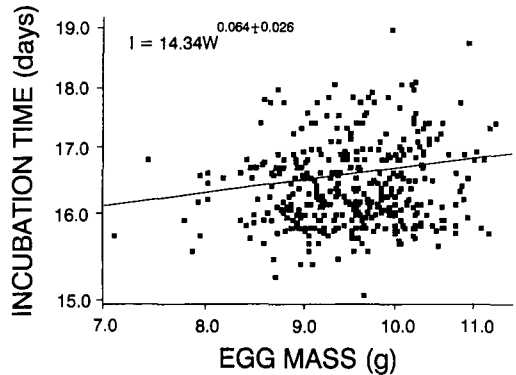
increase proportionately with egg mass ($b = 0.82$); however, the exponent for this relationship was not quite statistically different from one ($P < 0.08$).

Fresh mass, water content, and lipid content of analyzed chicks increased disproportionately with egg mass (b's > 1.00), but chick protein increased at a rate proportional to egg mass (Table 3). Within the total sample of hatchlings, chick mass was a constant proportion of egg mass ($b = 0.96$) and tarsus length increased at the 0.30 power of egg mass (Table 3). These relationships were further affected by incubation time: for a given egg mass, fresh mass of chicks and chick components were negatively correlated with total incubation time (Table 4). This effect was pronounced for lipid and protein content, but rather small for total water. Tarsus length was positively correlated with egg mass and incu-
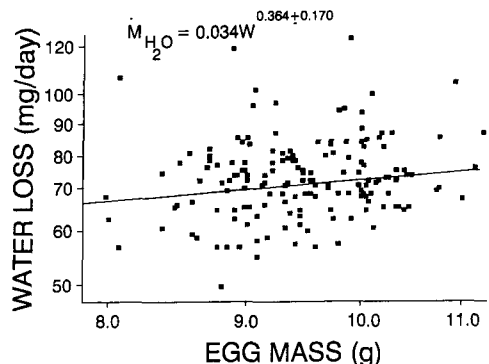

FIGURE 2. Allometric relationship between daily water loss and egg mass of Japanese Quail eggs. Axes are in log scale.

TABLE 3.   Regressions of $\log_{10}$ egg and chick component masses versus $\log_{10}$ egg mass in the Japanese Quail.[a]

Component	a ± SE(a)	b ± SE(b)	r^2
Egg shell, dry	−0.93 ± 0.10	0.82 ± 0.10	0.40
Egg water	−0.19 ± 0.02	1.03 ± 0.02	0.96
Egg protein, dry	−0.88 ± 0.05	1.02 ± 0.05	0.81
Egg lipid	−0.99 ± 0.10	0.98 ± 0.11	0.47
Chick wet mass[b]	−0.12 ± 0.04	0.96 ± 0.04	0.60
Chick tarsus[b,c]	0.93 ± 0.03	0.30 ± 0.03	0.20
Chick wet mass[d]	−0.32 ± 0.03	1.17 ± 0.04	0.90
Chick water	−0.42 ± 0.04	1.15 ± 0.04	0.86
Chick protein, dry	−0.99 ± 0.07	1.08 ± 0.07	0.65
Chick lipid	−1.98 ± 0.21	1.67 ± 0.22	0.33
Chick tarsus[d]	0.90 ± 0.04	0.32 ± 0.04	0.34

[a] $\log_{10}$ component mass = a + b($\log_{10}$ egg mass); all regressions significant at $P < 0.0001$.
[b] Sample of all known-egg chicks ($n = 335$).
[c] Units are mm, all other units are g.
[d] Sample of chicks analyzed for nutrient composition ($n = 123$).

bation time, and both of these factors seemed equally important (Table 4). Within the subsample of eggs for which water loss was measured, fresh mass of the chick was a positive function of egg mass, and a negative function of incubation time and daily water loss (Table 5). The interaction effect between total incubation time and daily water loss was marginally significant ($P = 0.07$), but explained only 0.5% of the variation in chick mass. The coefficient for this interaction was positive, presumably indicating that eggs with high shell conductance lost less water than expected when their incubation periods were longer than average.

Lipid content of chicks displayed positive allometry with respect to total chick mass and tarsus length (Table 6). Protein content was proportional to tarsus length, but tended to be negatively allometric with respect to chick mass (Table 6: $P = 0.10$), whereas water content increased disproportionately with respect to tarsus length, but was proportional to chick mass. Because water content was such a large fraction of total chick mass, it was not surprising that this relationship was isometric (a similar criticism could be applied to our regression of egg water content on total egg mass; e.g., Hochachka 1988). We therefore regressed chick water content on chick dry mass (protein + lipid) and compared the residuals from this regression (i.e., relative water content) to initial egg mass. Relative water content of newly hatched chicks was positively correlated with fresh egg mass ($r^2 = 0.137$, $P < 0.0001$).

For relationships involving hatchability, egg mass, chick mass, tarsus length, and incubation time, we verified the preceding results by repeating these analyses on all 1800 quail eggs used in the pesticide experiment (pesticide exposure had minimal effect on these variables; e.g., Martin 1990). We obtained quantitatively similar results in every case.

TABLE 4.   Effects of egg mass and incubation time on size and composition of chicks in the Japanese Quail.

Component:	Egg mass effect[a]				Incubation time effect[a]			
	b ± SE	F	P	r^2	b ± SE	F	P	r^2
Chick mass[b]	1.02 ± 0.04	779.1	0.0001	0.60	−0.86 ± 0.07	146.7	0.0001	0.12
Chick tarsus[b,c]	0.27 ± 0.03	80.1	0.0001	0.20	0.52 ± 0.06	81.3	0.0001	0.16
Chick mass[d]	1.20 ± 0.12	1,603.9	0.0001	0.90	−0.74 ± 0.10	54.6	0.0001	0.03
Chick water	1.17 ± 0.17	811.9	0.0001	0.86	−0.44 ± 0.14	10.5	0.002	0.01
Chick protein	1.14 ± 0.06	317.8	0.0001	0.65	−1.26 ± 0.21	35.6	0.0001	0.08
Chick fat	1.77 ± 0.21	72.7	0.0001	0.33	−2.56 ± 0.69	13.7	0.0003	0.07
Chick tarsus[c,d]	0.30 ± 0.04	60.9	0.0001	0.34	0.52 ± 0.13	16.3	0.0001	0.08

[a] Data from multiple regression of $\log_{10}$ component on $\log_{10}$ egg mass and $\log_{10}$ incubation time; b is the partial regression coefficient, r^2 is the partial coefficient of determination.
[b] Sample of all known-egg chicks ($n = 335$).
[c] Units are mm, all other units are g.
[d] Sample of chicks analyzed for nutrient composition ($n = 123$).

TABLE 5.   Effects of egg mass, incubation time, and daily water loss on hatching mass of Japanese Quail chicks.[a]

Effect:	b ± SE	F	P	r^2
Egg mass	1.13^b ± 0.05	480.4	0.0001	0.59^c
Incubation time	−0.98 ± 0.10	102.2	0.0001	0.14
Daily water loss	−0.14 ± 0.03	34.1	0.0001	0.05

[a] All variables $\log_{10}$ transformed for analysis.
[b] Partial regression coefficient.
[c] Partial coefficient of determination.

DISCUSSION

The relationship that we obtained between incubation time and egg mass in Japanese Quail ($I = 14.34W^{0.064±0.026}$, $r^2 = 0.016$) had a much shallower slope and was considerably weaker than the interspecific relationship described by Rahn and Ar (1974) ($I = 12.03W^{0.22}$, $r^2 = 0.74$) (see also Ar and Rahn 1985; $I = 11.61W^{0.223}$, $r^2 = 0.617$). The 99.9% CI of our regression exponent, $-0.219 < b < 0.152$, did not include Rahn and Ar's exponent of 0.22. Similarly, the intraspecific relationship that we obtained between daily water loss and egg mass ($M_{H_2O} = 0.034W^{0.364±0.170}$, $r^2 = 0.028$) was far weaker than Rahn and Ar's interspecific relationship ($M_{H_2O} = 0.015W^{0.74}$, r^2 not reported) (see also Ar and Rahn 1985; $M_{H_2O} = 0.0139M^{0.73}$, $r^2 = 0.906$). The 95% confidence interval for our regression exponent ($0.027 < b < 0.701$) does not include Rahn and Ar's exponent of 0.74. Rahn and Ar (1974) combined their two relationships and obtained the equation $F = M_{H_2O} \cdot I \cdot W^{-1}$ (assuming $W^{0.96} \approx W^{1.00}$), where F represents a constant fraction of fresh egg mass which is lost as water vapor during incubation. When we combined the two relationships derived from our data on Japanese Quail, we obtained $F = 0.034W^{0.364} \cdot 14.355W^{0.064} \cdot W^{-1} = 0.488W^{0.428} \cdot W^{-1}$. Unlike Rahn and Ar's (1974) interspecific relationship, the exponent of W for our data was substantially less than 1.00. Thus, fractional water loss was not independent of egg mass; small eggs lost proportionately more water than large eggs. From the extremes of egg mass encountered in this study (7.18–11.25 g), the estimated mean fraction of egg mass lost as water vapor ranged from 15.8 to 12.2% and the estimated mean incubation period ranged from 16.27 to 16.74 d.

Parsons (1972) found that larger than average a-eggs of the Herring Gull (*Larus argentatus*) took 16 hr longer to hatch than did a-eggs that were smaller than the mean; however, research with other larids has demonstrated weak or nonexistent correlations between incubation period and egg size (Nisbet 1975, Runde and Barrett 1981, Nol and Blokpoel 1983). In field studies of naturally breeding populations, correlations between these two variables could be confounded by variation in parental quality or timing of breeding (e.g., Parsons 1972, Davis 1975, Nisbet 1975), making the actual relationship between egg size and incubation time difficult to ascertain. Ricklefs and Smeraski (1983) conducted reciprocal egg exchanges between nests of European Starlings (*Sturnus vulgaris*) and found that most variation in incubation time was attributable to the incubating bird; only a small amount of variation could be assigned to the natural parent. Incubation time was not affected by egg mass, but it was related to the yolk content in eggs of both the parental and the incubating birds (Ricklefs and Smeraski 1983). Some studies of do-

TABLE 6.   Regressions of $\log_{10}$ chick component masses (g) on $\log_{10}$ chick mass (g) and $\log_{10}$ tarsus length (mm).[a]

Independent variable	Dependent variable	a ± SE(a)	b ± SE(b)	r^2
Chick mass	Total water	−0.12 ± 0.01	0.99 ± 0.02	0.97
	Total protein	−0.67 ± 0.05	0.90 ± 0.06	0.69
	Total fat	−1.52 ± 0.14	1.41 ± 0.17	0.36
Tarsus	Total water	−0.90 ± 0.20	1.32 ± 0.17	0.34
	Total protein	−1.08 ± 0.25	0.94 ± 0.20	0.15
	Total fat	−2.18 ± 0.56	1.50 ± 0.46	0.08

[a] $\log_{10}$ dependent variable = a + b($\log_{10}$ independent variable); all regressions significant at $P < 0.0001$ except total fat versus tarsus length ($P = 0.0015$).

mestic chickens and turkeys have shown positive correlations between egg size and incubation period (Hassan and Nordskog 1971; references therein), but these relationships have generally been very weak (e.g., $r^2 = 0.012$, $P = 0.05$; Bohren et al. 1961; $r^2 = 0.036$ [includes an effect of preincubation holding time], $P < 0.001$, McLaury and Insko 1968). These studies support our contention that intraspecific relationships between incubation time and egg size are very weak; however, none of these studies described the structural aspects of this relationship.

Studies of egg water loss have typically ignored the potential influence of intraspecific variation in egg mass (e.g., Rahn et al. 1976), or have inappropriately used ratios to adjust for this variation (e.g., Lomholt 1976; see Packard and Boardman 1988 for a critique of this method). However, rates of water loss from eggs of Barn Swallows (*Hirundo rustica*), Cliff Swallows (*Hirundo pyrrhonota*), and Black-billed Magpies (*Pica pica*) were not related to egg size (Packard et al. 1977, Sotherland et al. 1980, Taigen et al. 1980). Carey (1986) used fresh egg mass as a covariate in an analysis of egg water loss among Red-winged Blackbirds (*Agelaius phoeniceus*) and showed that egg mass was a significant predictor of daily water loss ($P = 0.001$), but it appeared to explain very little of the variation (Carey 1986: Table 1; partial $r^2 < 0.04$, assuming n of 63). As was the case for incubation period, none of these studies described the structural relationship between egg mass and daily water loss.

Daily water loss in this study was determined from mean water loss over the first 13 days of incubation, without taking into account daily variation associated with reduced rates of water loss during the initial stages of incubation (included in our 13 d period), or the often extensive water loss which occurs during hatching (not included in our 13 d period) (Carey 1979). Perhaps large eggs, which retained a higher proportion of water through the first 13 d of incubation, lost proportionately more water during hatching (e.g., Whittow et al. 1985). This seems unlikely with our data, however, because the pip-to-hatch interval in our quail eggs, though not quantified, appeared to be less than 2 hr. Furthermore, nutrient analyses of unincubated eggs and newly-hatched chicks suggested that disproportionately low water loss from large eggs gave rise to chicks with disproportionately high water content.

Intraspecific variation in fractional water loss, as recorded in this study, had no effect on hatchability or incubation time (see also Mather and Laughlin 1976, Burton and Tullett 1985), and only a slight effect on total mass of newly hatched chicks. Presumably, most of this variation in hatchling mass reflected differences in water content, although Burton and Tullett (1985) showed that dry mass of the chicken embryo was also influenced by variation in shell conductance (as indexed by rates of water loss). Carey et al. (1983) recorded fractional water loss from Red-winged Blackbird eggs being incubated at different altitudes, and thereby experiencing variable rates of water loss due to different partial pressures between eggs and atmosphere. Chicks hatched successfully from naturally incubated eggs that lost from 11.2 to 22.0% of their initial mass as water vapor, and some chicks also hatched successfully from eggs which lost up to 43.5% of their initial mass during incubation as a result of puncture manipulations to their shells (Carey 1986). In addition, chick water content was strongly correlated with rates of egg water loss (Carey 1986; r^2 ranged from 0.67 to 0.85).

Our results on nutrient composition of eggs and chicks of Japanese Quail differed little from earlier analyses conducted by Ricklefs (Ricklefs 1977, Ricklefs et al. 1978). Our data showed that protein and lipid content of eggs varied isometrically with egg size, whereas Ricklefs et al. (1978) suggested that protein content was disproportionately high in large eggs and lipid content was disproportionately low (only the protein relationship was significantly different from isometry). We also observed a weaker coefficient of determination between egg size and chick lipid content ($r^2 = 0.33$) than did Ricklefs ($r^2 = 0.73$). Most other relationships were remarkably similar between studies. We found, as did Ricklefs, that proportional lipid content of newly hatched chicks increased dramatically with egg size, despite the isometric relationships observed between egg lipid content and fresh egg mass. Alisauskas (1986) documented a similar relationship for newly hatched American Coots (*Fulica americana*), but proportional lipid content declined with egg size in Laughing Gulls (*Larus atricilla*) and Wood Ducks (*Aix sponsa*) (Ricklefs et al. 1978, Hepp et al. 1987). This wide range of variation between proportional composition of fresh eggs and hatchlings suggests that caution is ad-

visable when speculating about the possible adaptive function of allometric variation in egg composition.

Protein and lipid content of newly-hatched chicks were inversely correlated with incubation time (controlling for variation in fresh egg mass), presumably because embryos metabolized more of these nutrients if hatching was delayed. Although variation in egg composition is usually interpreted in terms of its influence on energy reserves of hatchlings, nutrient rich eggs may also be beneficial for the energy they provide to embryos before and during hatching. Tarsus length of hatchlings was equally influenced by incubation time and egg mass, suggesting that embryos in larger eggs had faster growth rates, at least up until hatching (see also Hassan and Nordskog 1971; cf. Ricklefs 1984). This does not appear to be caused by arrested development arising from space constraints in small eggs, because growth rate of Japanese Quail embryos during the last six days of incubation is constant (Spiers and Baummer 1990).

An unlooked for result in our study was the slightly longer incubation period for eggs immersed in distilled water on incubation day 14, relative to eggs receiving similar treatment on incubation day 0 or 4 (day 14 eggs required 3.3 additional hr to hatch; hatchability was unaffected by this treatment). Presumably, this hatching delay was a result of embryo cooling. Webb (1987) summarized evidence from other studies that have demonstrated correlations between low incubation temperatures (often resulting from egg neglect) and increased incubation time (see also Evans 1990). Our data suggest a relatively high cost in terms of additional incubation time (3.3 hr) for a relatively short period of embryo cooling (30 sec emersion, <30 min interruption of incubation), and may help explain why birds are reluctant to take incubation breaks during inclement weather (e.g., Afton 1980, Zerba and Morton 1983). In addition, these data suggest that investigators that use the egg immersion technique to estimate future hatching dates (e.g., Nol and Blokpoel 1983) may be affecting, as well as estimating, their results.

CONCLUSION

We observed very weak correlations among fresh egg mass, incubation time, and daily water loss in a large sample of Japanese Quail eggs artifi-cially incubated under controlled conditions. These results were inconsistent with interspecific studies of these relationships (Rahn and Ar 1974, Ar and Rahn 1985). We view the constancy of incubation conditions among eggs as a benefit in our study, although perhaps in wild conditions large eggs have advantages not expressed in our artificial environment (e.g., slower cooling rates of large eggs during incubation recesses). But in the wild, the frequent nest visits required to accurately determine hatch time would likely also affect incubation constancy of the parents (Forbes and Ankney 1988). We used Japanese Quail eggs for our experiment because they were readily available, but we recognize that the importance of synchronous hatching among many precocial nidifugous birds (e.g., Vince 1966) may have resulted in strong stabilizing selection on incubation time (Table 1; CV's). Ideally, our experiment would have been conducted using a bird with asynchronous hatching and intense intersibling rivalry. Such is the case among larids, where field studies have generated equivocal support for relationships among incubation time and egg size (see discussion). Alternatively, the narrow range of egg mass in our study may have contributed to the weakness of the relationships among egg mass, incubation time, and water loss; however, the coefficient of variation for egg mass of Japanese Quail (CV = 7.0%) is typical of that found in other birds (mean CV = 7.3 ± 1.8 % [1 SD] for 48 populations, range = 4.0 to 10.9 %; based on references in our Literature Cited), indicating that these relationships are likely to be weak within other species of birds. Other comparative studies have also found that allometric relationships derived across a broad taxonomic array (i.e., the mouse to elephant, or hummingbird to ostrich approach) are inappropriate when applied to lower taxonomic levels (Pagel and Harvey 1988). We recognize the enormous advances that studies of interspecific egg-size variation have made to our understanding of egg physiology, but we believe that it would be erroneous to assume that these same functional relationships apply to variation in egg size within a species.

tion, and D. Ankney of the University of Western Ontario. K. Risi assisted with incubator maintenance and hatchling care, and provided us with a much needed break from hatch-checks. J. Amery conducted the lipid extractions. The reviewers made many valuable suggestions for improving the manuscript.

LITERATURE CITED

AFTON, A. D. 1980. Factors affecting incubation rhythms of Northern Shovelers. Condor 82:132–137.

ALISAUSKAS, R. T. 1986. Variation in the composition of the eggs and chicks of American Coots. Condor 88:84–90.

ANKNEY, C. D. 1980. Egg weight, survival, and growth of Lesser Snow Goose goslings. J. Wildl. Manage. 44:174–182.

AR, A., AND H. RAHN. 1980. Water in the avian egg: overall budget of incubation. Am. Zool. 20:373–384.

AR, A., AND H. RAHN. 1985. Pores in avian eggshells: gas conductance, gas exchange and embryonic growth rate. Respir. Physiol. 61:1–20.

ARNOLD, T. W., F. C. ROHWER, AND T. ARMSTRONG. 1987. Egg viability, nest predation, and the adaptive significance of clutch size in prairie ducks. Am. Nat. 130:643–653.

BATT, B.D.J. 1969. The effects of cold on Mallard embryos. M.S.thesis, Univ. of Florida, Gainesville.

BOAG, P. T., AND A. J. VAN NOORDWIJK. 1987. Quantitative genetics, p. 45–78. In F. Cooke and P. A. Buckley [eds.], Avian genetics. Academic Press, London.

BOHREN, B. B., L. B. CRITTENDEN, AND R. T. KING. 1961. Hatching time and hatchability in the fowl. Poult. Sci. 40:620–633.

BROCKELMAN, W. Y. 1975. Competition, the fitness of offspring, and optimal clutch size. Am. Nat. 109:677–699.

BURTON, F. G., AND S. G. TULLETT. 1985. The effects of egg weight and shell porosity on the growth and water balance of the chicken embryo. Comp. Biochem. Physiol. 81A:377–385.

CAREY, C. 1979. Increase in conductance to water vapor during incubation in eggs of two avian species. J. Exp. Zool. 209:181–186.

CAREY, C. 1986. Tolerance of variation in eggshell conductance, water loss, and water content by Redwinged Blackbird embryos. Physiol. Zool. 59:109–122.

CAREY, C., S. D. GARBER, E. L. THOMPSON, AND F. C. JAMES. 1983. Avian reproduction over an altitudinal gradient. II. Physical characteristics and water loss of eggs. Physiol. Zool. 56:340–352.

DAVIS, J.W.F. 1975. Age, egg-size and breeding success in the Herring Gull Larus argentatus. Ibis 117:460–473.

DOBUSH, G. R., C. D. ANKNEY, AND D. G. KREMENTZ. 1985. The effect of apparatus, extraction time, and solvent type on lipid extractions of Snow Geese. Can. J. Zool. 63:1917–1920.

EVANS, R. M. 1990. Terminal-egg chilling and hatching intervals in the American White Pelican. Auk 107:431–434.

FALCONER, D. S. 1981. Introduction to quantitative genetics. Longman, London.

FORBES, M. R. L., AND C. D. ANKNEY. 1988. Nest attendance by adult Pied-billed Grebes, Podilymbus podiceps (L.). Can. J. Zool. 66:2019–2023.

FURNESS, R. W. 1983. Variations in size and growth of Great Skua Catharacta skua chicks in relation to adult age, hatching date, egg volume, brood size and hatching sequence. J. Zool. 199:101–116.

HASSAN, G. M., AND A. W. NORDSKOG. 1971. Effects of egg size and heterozygosis on embryonic growth and hatching speed. Genetics 67:279–285.

HEPP, G. R., D. J. STANGOHR, L. A. BAKER, AND R. A. KENNAMER. 1987. Factors affecting variation in the egg and duckling components of Wood Ducks. Auk 104:435–443.

HOCHACHKA, W. M. 1988. The effect of food supply on the composition of Black-billed Magpie eggs. Can. J. Zool. 66:692–695.

KERR, D. C., C. D. ANKNEY, AND J. S. MILLAR. 1982. The effect of drying temperature on extraction of petroleum ether soluble fats of small birds and mammals. Can. J. Zool. 60:470–472.

KRAPU, G. L. 1979. Nutrition of female dabbling ducks during reproduction, p. 57–70. In T. A. Bookhout [ed.], Waterfowl and wetlands—an integrated review. North Central Section—The Wildlife Society, Madison, WI.

LACK, D. 1948. The significance of clutch-size. Part III. Some interspecific comparisons. Ibis 90:25–45.

LACK, D. 1967. The significance of clutch-size in waterfowl. Wildfowl 18:125–128.

LESSELLS, C. M., F. COOKE, AND R. F. ROCKWELL. 1989. Is there a trade-off between egg weight and clutch size in wild Lesser Snow Geese (Anser c. caerulescens)? J. Evol. Biol. 2:457–472.

LOMHOLT, J. P. 1976. Relationship of weight loss to ambient humidity of birds eggs during incubation. J. Comp. Physiol. 105:189–196.

LUNDBERG, C.-A., AND R. A. VÄISÄNEN. 1979. Selective correlation of egg size with chick mortality in the Black-headed Gull (Larus ridibundus). Condor 81:146–156.

MARTIN, P. A. 1990. Effects of carbofuran, chlorpyrifos, and deltamethrin on hatchability, deformity, chick size, and incubation time of Japanese Quail (Coturnix japonica) eggs. Environ. Toxicol. Chem. 9:529–534.

MATHER, C. M., AND K. F. LAUGHLIN. 1976. Storage of hatching eggs: the effect on total incubation period. Br. Poult. Sci. 17:471–479.

MCARDLE, B. H. 1988. The structural relationship: regression in biology. Can. J. Zool. 66:2329–2339.

MCLAURY, D. W., AND W. M. INSKO, JR. 1968. Relation of pre-incubation factors and post-hatching performance to length of incubation period. 1. Effects of egg weight and storage time on length of incubation period. Poult. Sci. 47:305–311.

NISBET, I.C.T. 1975. Asynchronous hatching in Common and Roseate terns, Sterna hirundo and S. dougallii. Ibis 117:374–379.

NISBET, I.C.T. 1978. Dependence of fledging success on egg-size, parental performance and egg composition among Common and Roseate terns, *Sterna hirundo* and *S. dougallii*. Ibis 120:207–215.

NOL, E., AND H. BLOKPOEL. 1983. Incubation period of Ring-billed Gulls and the egg immersion technique. Wilson Bull. 95:283–286.

PACKARD, G. C., AND T. J. BOARDMAN. 1988. The misuse of ratios, indices, and percentages in ecophysiological research. Physiol. Zool. 61:1–9.

PACKARD, G. C., P. R. SOTHERLAND, AND M. J. PACKARD. 1977. Adaptive reduction in permeability of avian eggshells to water vapour at high altitudes. Nature 266:255–256.

PAGEL, M. D., AND P. H. HARVEY. 1988. The taxon-level problem in the evolution of mammalian brain size: facts and artifacts. Am. Nat. 132:344–359.

PARSONS, J. 1970. Relationship between egg size and post-hatching chick mortality in the Herring Gull (*Larus argentatus*). Nature 228:1221–1222.

PARSONS, J. 1972. Egg size, laying date and incubation period in the Herring Gull. Ibis 114:536–541.

PARSONS, J. 1976. Factors determining the number and size of eggs laid by the Herring Gull. Condor 78:481–492.

RAHN, H., AND A. AR. 1974. The avian egg: incubation time and water loss. Condor 76:147–152.

RAHN, H., C. V. PAGANELLI, I.C.T. NISBET, AND G. C. WHITTOW. 1976. Regulation of incubation water loss in eggs of seven species of terns. Physiol. Zool. 49:245–259.

RHYMER, J. M. 1983. Effect of egg size and renesting on thermoregulatory ability and growth of Mallard ducklings. M.S. thesis, Univ. of Manitoba, Winnipeg.

RHYMER, J. M. 1988. The effect of egg size variability on thermoregulation of Mallard (*Anas platyrhynchos*) offspring and its implications for survival. Oecologia 75:20–24.

RICKLEFS, R. E. 1977. Composition of eggs of several bird species. Auk 94:350–356.

RICKLEFS, R. E. 1984. Components of variance in measurements of nestling European Starlings (*Sturnus vulgaris*) in southeastern Pennsylvania. Auk 101:319–333.

RICKLEFS, R. E., D. C. HAHN, AND W. A. MONTEVECCHI. 1978. The relationship between egg size and chick size in the Laughing Gull and Japanese Quail. Auk 94:135–144.

RICKLEFS, R. E., AND C. A. SMERASKI. 1983. Variation in incubation period within a population of the European Starling. Auk 100:926–931.

ROHWER, F. C. 1988. Inter- and intraspecific relationships between egg size and clutch size in waterfowl. Auk 105:161–176.

RUNDE, O. J., AND R. T. BARRETT. 1981. Variation in egg size and incubation period of the Kittiwake *Rissa tridactyla* in Norway. Ornis Scand. 12:80–86.

SAS INSTITUTE INC. 1985. SAS user's guide: statistics. Version 5 edition. SAS Institute Inc., Cary, NC.

SCHIFFERLI, L. 1973. The effect of egg weight on the subsequent growth of nestling Great Tits (*Parus major*). Ibis 115:549–558.

SMITH, C. C., AND S. D. FRETWELL. 1974. The optimal balance between the size and number of offspring. Am. Nat. 108:499–506.

SOTHERLAND, P. R., G. C. PACKARD, T. L. TAIGEN, AND T. J. BOARDMAN. 1980. An altitudinal cline in conductance of Cliff Swallow (*Petrochelidon pyrrhonota*) eggs to water vapor. Auk 97:177–185.

SPIERS, D. E., AND S. C. BAUMMER. 1990. Embryonic development of Japanese Quail (*Coturnix coturnix japonica*) as influenced by periodic cold exposure. Physiol. Zool. 63:516–535.

TAIGEN, T. L., G. C. PACKARD, P. R. SOTHERLAND, T. J. BOARDMAN, AND M. M. PACKARD. 1980. Water-vapor conductance of Black-billed Magpie (*Pica pica*) eggs collected along an altitudinal gradient. Physiol. Zool. 53:163–169.

VINCE, M. A. 1966. Artificial acceleration of hatching in quail embryos. Anim. Behav. 14:389–394.

WEBB, D. R. 1987. Thermal tolerance of avian embryos: a review. Condor 89:874–898.

WHITTOW, G. C., G. S. GRANT, AND E. N. FLINT. 1985. Egg water loss, shell water-vapor conductance, and the incubation period of the Gray-backed Tern (*Sterna lunata*). Condor 87:269–272.

WORTH, B. C. 1940. Egg volumes and incubation periods. Auk 57:44–60.

ZERBA, E., AND M. L. MORTON. 1983. The rhythm of incubation from egg laying to hatching in Mountain White-crowned Sparrows. Ornis Scand. 14:188–197.