

THE SHELF LIFE OF BIRD EGGS: TESTING EGG VIABILITY USING A TROPICAL CLIMATE GRADIENT

STEVEN R. BEISSINGER,^{1,3} MARK I. COOK,^{1,4} AND WAYNE J. ARENDT²

¹*Ecosystem Sciences Division, Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720-3114 USA*

²*International Institute of Tropical Forestry, USDA Forest Service, Catalina Service Center, Palmer, Puerto Rico 00721 USA*

Abstract. Avian parents influence the onset of development, hatching synchrony, and likelihood of brood reduction through the onset of incubation. Studies testing adaptive functions of brood reduction assume that eggs are protected by their shells and waiting for parents to initiate incubation in a manner that creates optimal hatching patterns, but the viability of early laid eggs may diminish over time due to putative effects of ambient temperature. We tested effects of exposure to ambient temperature on the viability of unincubated bird eggs using an altitudinal climate gradient in Puerto Rico: daily maximum temperatures nearly always exceed developmental zero in the lowland forest, whereas temperatures rarely exceed developmental zero in the cloud forest and at intermediate altitudes. We removed 382 freshly laid Pearly-eyed Thrasher (*Margarops fuscatus*) eggs, placed them in holding boxes at three sites for 1–7 days, and returned them to nests to be incubated. Additional control eggs ($n = 39$) tested effects of both handling and movement on hatching success, and unmanipulated eggs ($n = 139$) provided a measure of natural hatching success. Hatching success of control eggs (82.0%) and unmanipulated eggs (84.9%) did not differ, indicating no impacts of handling on hatchability. Hatching success of experimental eggs exposed for one day (78.6%) was high but declined very strongly after exposure for three days (41.9%), five days (11.5%) and seven days (2.1%). Hatchability of eggs held at the cloud forest did not differ from eggs held at the lowland site but was lower for eggs exposed at mid-elevation. Hatching success was positively related to minimum temperature and marginally related to mean temperature and proportion of time above developmental zero. Exposure duration, treatment site, and maximum temperature were the only significant effects identified by stepwise logistic regression. Eggs held at the lowland site had shorter developmental periods than eggs held at other sites. Most embryo mortality (80.8%) occurred at very early stages. Our results demonstrate that viability of unincubated eggs exposed to moist tropical conditions declines strongly but suggest that ambient temperature is not the sole cause. We compare rates of egg viability decline among species, examine alternative mechanisms for the loss of viability, and discuss the significance of egg viability on avian life histories.

Key words: altitudinal gradient; birds; developmental constraint; hatching asynchrony; climatic gradient; egg viability; incubation; parental care; Pearly-eyed Thrasher.

INTRODUCTION

The behavior of vertebrate parents often has little effect on the time between emergence of their first and last young, which are usually born within a short time of each other compared to the time required for their development. Birds, however, have an unusual life history because parents influence the onset of development, and resultant synchrony of hatching, by determining when to initiate incubation (White and Kinney

1974, Drent 1975, Carey 1980). Birds lay no more than one egg daily, so if incubation starts before the last egg is laid, eggs in a clutch typically hatch over 1–3 days and up to 17 days apart (Beissinger and Waltman 1991, Stoleson and Beissinger 1995). Asynchronous hatching often leads to the mortality of last-hatched young and occurs in avian families nearly as frequently as synchronous hatching (Clark and Wilson 1981, Ricklefs 1993, Stoleson and Beissinger 1995). Studies of avian hatching patterns have focused on identifying adaptive functions for the nestling size disparities that result in brood reduction with equivocal success (Amundsen and Slagsvold 1991, Stoleson and Beissinger 1995, 1997). Such studies implicitly assume eggs are protected by their shells and waiting for parents to initiate incubation in a manner that creates optimal

Manuscript received 26 October 2004; revised 25 January 2005; accepted 25 January 2005. Corresponding Editor: R. Greenberg.

³ E-mail: beis@nature.berkeley.edu

⁴ Present address: South Florida Water Management District, 3301 Gun Club Road, P.O. Box 24680, West Palm Beach, Florida 33416 USA.

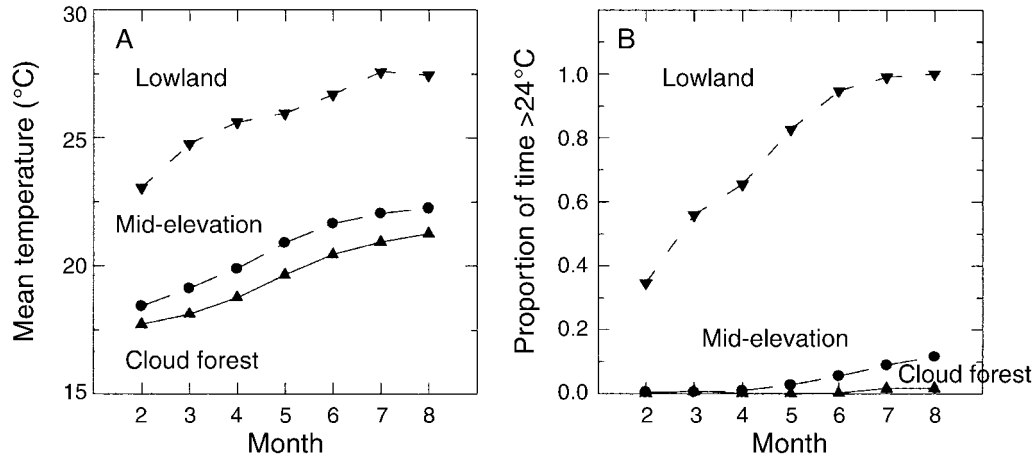


FIG. 1. Monthly ambient temperature characteristics calculated from measures made at 5-min intervals at the lowland, mid-elevation, and cloud forest sites in Puerto Rico during the three years of study: (A) mean ambient temperatures, and (B) the proportion of time that temperatures were greater than developmental zero (24°C). See Appendix for the entire time series.

hatching patterns. Alternatively, initiating incubation before a clutch is completed could serve to maintain the viability of early laid eggs, which diminishes over time due to putative effects of ambient temperature (Hussell 1985, Arnold et al. 1987, Veiga 1992).

Temperature is posited to be the most critical mechanism affecting hatchability of unincubated eggs, although humidity, gaseous environment, egg orientation, and egg turning are also important (Wilson 1991, Fasnko et al. 1992, Meijerhof 1992, Walsberg and Schmidt 1992). Avian embryos do not develop below physiological or developmental zero ($24^{\circ}\text{--}27^{\circ}\text{C}$), and viability of unincubated eggs maintained in cold torpor declines very slowly (Decuyper and Michels 1992, Ewert 1992). When birds in temperate springtime climates delay incubation until the last egg, cold torpor suspends development of earlier eggs and allows hatching to be synchronous (Drent 1975). In warmer conditions, however, extended exposure of unincubated eggs to temperatures above developmental zero, but below optimal incubation temperature ($36^{\circ}\text{--}38^{\circ}\text{C}$), results in abnormal development and embryo mortality either early or late in development due to unsynchronized growth when some, but not all, embryonic tissues develop in the absence of incubation (Webb 1987, Deeming and Ferguson 1992, Meijerhof 1992, Stoleson 1999). Development of neurological and brain tissues in very young embryos is particularly sensitive to prolonged exposure to temperatures in this range (Romanoff and Romanoff 1972, Webb 1987). Thus, once development begins, parent birds might be obliged to initiate incubation early to maintain the viability of first-laid eggs, which affects not only hatching asynchrony but perhaps also the evolution of clutch size (Stoleson and Beissinger 1995, 1999). Latitudinal and seasonal declines in clutch size, which are widespread in birds (Lack 1968, Klomp 1970, Skutch 1985, Stutch-

bury and Robertson 1988, Perrins and McCleery 1989), may occur partly because the benefits of laying additional eggs are offset by the decline in hatchability with increasing temperatures (Beissinger 1999, Stoleson and Beissinger 1999).

A decline in the viability of eggs resulting from prolonged exposure to ambient temperatures has long been known in synchronously hatching domestic fowl (Drent 1973, 1975), but was only recently demonstrated for wild birds. Declines in viability of waterfowl, House Sparrow (*Passer domesticus*), and Green-rumped Parrotlet (*Forpus passerinus*) eggs occurred with 3–5 days of exposure to ambient conditions and thereafter declines accelerated rapidly (Arnold et al. 1987, Veiga 1992, Arnold 1993, Stoleson and Beissinger 1999). Although these experiments demonstrate loss of egg viability with increased duration of preincubation exposure, none exposed eggs to a critical set of fluctuating temperatures above or below developmental zero thought to either erode or preserve the viability of unincubated eggs. Instead, they relied on incubators run at constant temperatures (Arnold et al. 1987), or were conducted in the wild at single locations where they were unable to subject eggs to ambient temperatures thought to conserve or erode viability (Veiga 1992, Arnold 1993, Stoleson and Beissinger 1999). Thus, the role of ambient temperature on egg viability has not received strong tests with wild bird eggs.

Here we report on a three-year study to test the effects of exposure to ambient temperatures on unincubated bird eggs using an altitudinal climate gradient along the slope of a tropical mountain that permits eggs to be exposed to a critical set of thermal environments in situ. At the base of the mountain in lowland forests, daily maximum temperatures nearly *always* exceed developmental zero (Fig. 1 and Appendix), whereas temperatures *rarely* exceed developmental zero at the top

of the mountain in the cloud forest. Along the slope at intermediate altitudes, temperatures mostly remain cool but increasingly exceed developmental zero as summer advances. We removed eggs of the Pearly-eyed Thrasher (*Margarops fuscatus*), a species that nests in cavities throughout this gradient, on the morning they were laid, placed them in holding boxes at each of the three altitudes for 1–7 days to expose them to ambient temperatures, and then returned eggs to nests to be incubated by females. Other eggs served as controls for the effect of movement on hatching success.

Hatching success, development times, and developmental stage at death of experimental and control eggs were compared among the three sites to test the predicted effects of ambient temperatures: (1) Hatching success should decline with increasing exposure period and the relationship should differ by treatment site because of the effects of ambient temperatures above developmental zero. Cloud forest eggs should show a slight decline in hatching success with duration of exposure time, whereas hatching success of eggs held at the lowland should be strongly negatively related to duration of exposure, and hatching success of mid-elevation eggs should be negatively but less strongly related to exposure time. Thus, the interaction of exposure period with ambient temperatures (mean, minimum, maximum, and proportion of time above developmental zero) should be negatively related to hatching success. (2) Exposure of newly laid eggs to ambient temperatures above developmental zero should induce some embryological development in the absence of incubation, and as a result those eggs should require slightly shorter incubation periods than non-exposed eggs. Thus, incubation periods for lowland eggs should be shorter than eggs held at the cloud forest or mid-elevation sites. (3) If prolonged exposure to temperatures above developmental zero but below optimal incubation levels causes abnormal development, a greater proportion of embryos should die at moderate to advanced developmental stages in eggs exposed at the lowland than at the other two sites.

METHODS

Study species

Pearly-eyed Thrashers are medium-sized, omnivorous passerines that nest in cavities in forests from the lowlands to the cloud forest throughout Puerto Rico (Raffaele 1989). They reach highest densities at mid-elevations (Snyder et al. 1987). We erected 134 wooden nest boxes 2–15 m above the ground in trees ~0.1 km apart at the cloud forest, mid-elevation, and lowland sites. Thrashers lay one egg daily or every second day until a typical clutch of 3–4 eggs is completed over 3–6 days; and only females incubate (Arendt 1993). Young typically hatch over a 1–2 day interval. The relatively synchronous hatching pattern and observations of diurnal nest attendance behavior suggested that thrashers began incubation on the penultimate egg

(Arendt 1993), and that early laid eggs might be exposed for 2–4 days prior to the onset of full incubation. Placing temperature loggers in thrasher nests, we discovered that the onset of incubation is variable; partial diurnal incubation (<20% of daylight hours) began on the first through third eggs, full (>98%) nocturnal incubation began on the first or second egg, and full diurnal incubation (~50% of daylight hours) occurred at clutch completion (M. I. Cook, S. R. Beissinger, and W. J. Arendt, *unpublished manuscript*). Partial incubation prior to clutch completion appears widespread in birds and has been documented in most species that have been monitored intensively during laying: geese (Poussart et al. 2000, Hanssen et al. 2002), raptors (Bortolotti and Wiebe 1993, Wiebe et al. 1998), pheasants (Persson and Goransson 1999), swallows (Banbura and Zielinski 1995), parids (Haftorn 1981), sparrows (Anderson 1997), and warblers (Hebert and Sealy 1992). Even waterfowl, which hatch their eggs completely synchronously and were thought to initiate incubation at the termination of laying, partially incubate after laying the second egg of their large clutches (Loos and Rohwer 2004). Thus, the partial incubation exhibited by Pearly-eyed Thrashers is similar to many birds that hatch their eggs relatively synchronously.

Holding sites

Three sites along an altitudinal gradient in or near the Luquillo Experimental Forest (LEF) within the Caribbean National Forest in Puerto Rico (18.3° N, 65.3° W) were chosen to place holding boxes to expose thrasher eggs: (1) a cloud forest at East Peak near the top of the LEF (810 m above sea level); (2) a mid-elevation site in palo colorado forest in the Icacos Valley at 600 m above sea level along the slope of the mountain where thrashers nest in large numbers, and (3) a lowland forest at the base of the mountain at Roosevelt Roads Naval Station (6 m above sea level) in 2000, and in 2001 and 2002 at Las Paulinas (20 m above sea level). The three sites differed dramatically in ambient temperature during the experiment (two-way ANOVA: Site $F_2 = 27$, 719.2, $P < 0.001$; Year $F_2 = 20.7$, $P < 0.001$; and Site \times Year $F_4 = 40.6$, $P < 0.001$) and also in relative humidity (two-way ANOVA: Site $F_2 = 75$, 500.8, $P < 0.001$; Year $F_2 = 3$, 938.4, $P < 0.001$; Site \times Year $F_4 = 781.7$, $P < 0.001$; Fig. 1 and Appendix), based on hourly means calculated from measurements recorded in holding boxes every five minutes throughout the study using thermistors and data loggers (Onset Computer, Pocasset, Massachusetts, USA). During the months that experiments were implemented (Fig. 1 and Appendix), the lowland site had a mean \pm SE daily temperature of $25.8^\circ\text{C} \pm 0.1^\circ\text{C}$ (range: $16^\circ\text{--}38^\circ\text{C}$) and a mean relative humidity of $79.0\% \pm 0.1\%$ (range: 33–98%), while the cloud forest site experienced daily mean temperatures of $19.4 \pm 0.2^\circ\text{C}$ (range: $12^\circ\text{--}25^\circ\text{C}$) and high relative humidity averaging $99.2\% \pm 0.1\%$ (range: 77–100%).

The mid-elevation site resembled the cloud forest in both temperature (mean = $20.4^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$, range = $14^{\circ}\text{--}28^{\circ}\text{C}$) and relative humidity (mean = 97.4 ± 0.1 , range = 53–100%). All sites experienced a warming effect as summer advanced (Fig. 1 and Appendix). Most importantly, ambient temperatures exceeded developmental zero (conservatively designated as 24°C should development begin at the lower end of the range) from 35–99% of the time during months at the lowland site, from 0.4–12% at the mid-elevation site, and from 0.1–1.7% at the cloud forest site (Fig. 1 and Appendix).

We exposed eggs to a “controlled set” of natural thermal regimes rather than use incubators because: (1) the temperature fluctuations that cause embryo mortality and mechanisms of developmental failure are so poorly understood that choosing the proper incubator regime is difficult, and (2) large temperature fluctuations in incubators can be accompanied by humidity changes that may be difficult to control and greater than humidity fluctuations in situ.

Experimental procedures

Unincubated thrasher eggs were exposed in a wooden holding box identical in design to the box in which they were laid ($24 \times 22 \times 73$ cm with a 16 cm diameter hole located 6 cm from the top) for periods of 1, 3, 5, or 7 d, and then returned to natal nests to be incubated by their parents. Eggs were removed from nests on the morning of laying, marked, weighed, and replaced with plaster dummy eggs. Each egg from a clutch was randomly assigned to a holding site, held there in a secure, shaded holding box, and turned twice daily. Holding box temperature and humidity were recorded every 5 min using Hobo thermistors and data loggers. Thus, all eggs in a clutch had the same exposure period, which was randomly determined, and ensured the proportion of eggs from each female was similar for each treatment.

We intended to use thrasher eggs laid at all three sites in a balanced design, but few thrashers nested in boxes at the top and bottom of the altitudinal transect. Most eggs came from females nesting at the mid-elevation site (90.5%) with 7.5% from females nesting in the cloud forest and 2% from lowland females. It is unlikely that eggs produced at different sites along the altitudinal gradient would create variation that could confound the results. Eggshell porosity, but not thickness or initial water content, may vary along an altitudinal gradient of much greater magnitude, which may affect oxygen exchange and water vapor loss (Rahn et al. 1977, Carey et al. 1983, Carey 1994). However, changes in oxygen tension along the gradient we used are unlikely to affect egg viability, because O_2 requirements of newly developing embryos are very low and embryos apparently receive sufficient O_2 to support normal metabolism up to 3600 m above sea level (Carey et al. 1982, Carey 1994, Vleck and Vleck 1996),

three times the altitude of the cloud forest site. Finally, early developmental patterns and incubation temperatures vary little among avian species and there is no evidence of avian embryo adaptation to local environmental conditions (Webb 1987).

After exposure was complete, experimental eggs were weighed, returned to their natal nest, and parentally incubated until hatching. Increasing the pre-incubation exposure period increased the period that females incubated eggs by up to 7 d (i.e., up to 21 d instead of the usual 14). Only eight treatment clutches (18 eggs) were abandoned by females prior to the end of incubation. Twelve of these eggs did not hatch but were included in analyses because candling indicated the embryos had died prior to desertion. Four deserted eggs were excluded from analysis because the timing and cause of mortality could not be ascertained. The remaining two eggs hatched and were included in analyses.

Two types of eggs were designated to test for potential detrimental effects of handling eggs during experimentation. “Control” eggs tested the effects of both handling and movement on hatching success by transporting them to and from a randomly assigned holding box on the day of laying. “Unmanipulated” eggs were not removed from nests and provide a measure of natural hatching success. Hatching success of control eggs (82.0%, $n = 39$) and unmanipulated eggs (84.9%, $n = 139$) did not differ ($\chi^2 = 0.185$, $df = 1$, $P > 0.7$). Given the apparent lack of handling effects on hatchability, control and unmanipulated eggs were pooled for all further comparisons with treatment eggs. It is conceivable, however, that the duration of transportation from the nest to the holding box and back again, rather than transportation per se, influenced hatching success. Transportation time did not differ significantly ($t = 0.74$, $df = 33$, $P = 0.46$) between control eggs that hatched (120.1 ± 13.6 min [mean \pm SE]) and those that did not hatch (111.8 ± 44.5 min), nor did it differ significantly ($t = 0.23$, $df = 365$, $P = 0.82$) between experimental eggs that hatched (154.7 ± 9.9 min) and those that did not hatch (147.9 ± 6.7 min). Nevertheless, due to the logistics of site locations, transportation time did differ significantly among experimental eggs held at different sites ($F_2 = 162.1$, $P < 0.001$). Eggs held at the slope site spent the least amount of time in transit (70.0 ± 5.0 min, $n = 130$) compared to eggs held at cloud forest (131.4 ± 6.2 min, $n = 124$) and lowland sites (263.6 ± 8.3 min, $n = 113$). To ensure that transportation did not influence results, we included transportation time along with other factors in logistic regression analyses of hatching success of experimental eggs (see *Methods*, *Data analysis*).

Eggs from all nests were candled using a portable video candler at both the mid-stage (day 4–6) and late-stage (day 9–12) of parental incubation to determine embryo developmental status and viability. After day

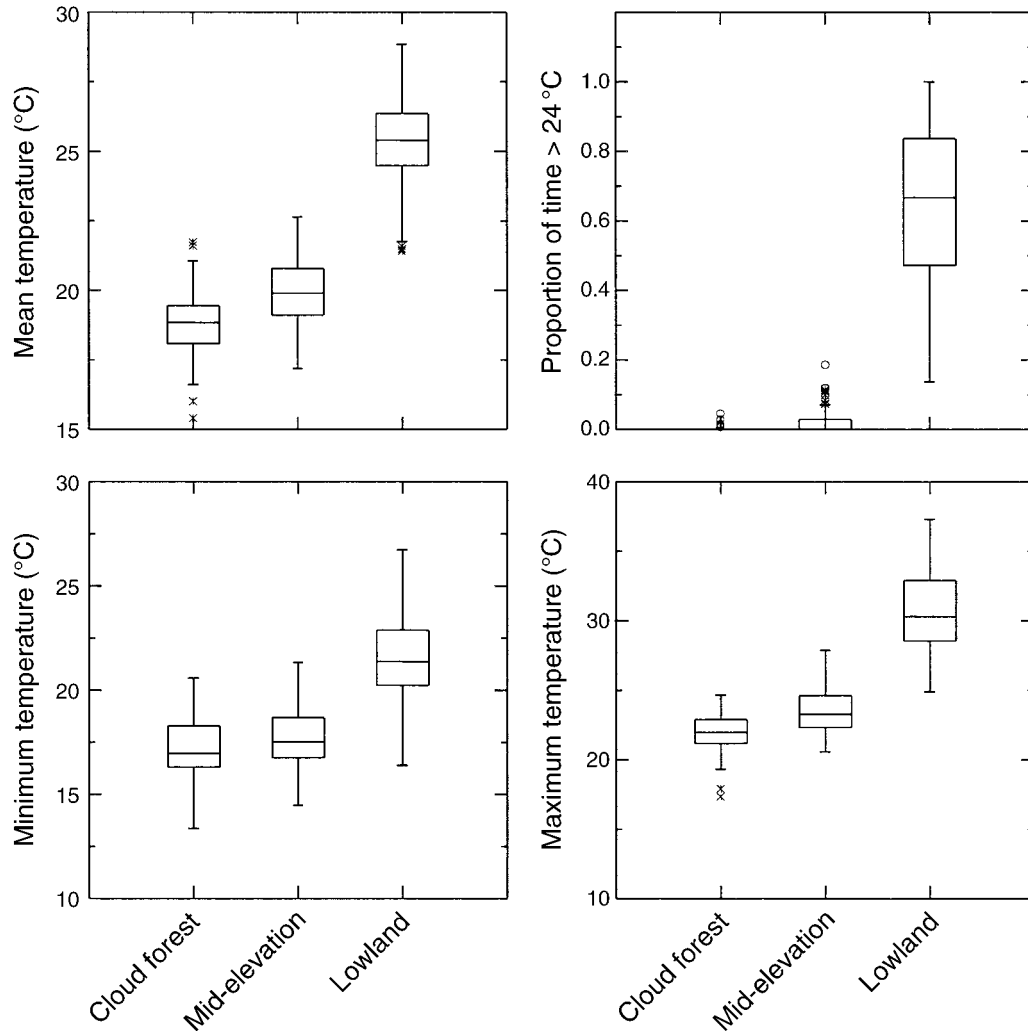


FIG. 2. Mean, minimum, and maximum temperatures and the proportion of time that temperatures exceeded developmental zero (24°C) for eggs exposed in holding boxes at cloud forest, mid-elevation, and lowland sites.

12 of parental incubation, nests were checked daily to determine hatching date.

Data analysis

The completed experiment was comprised of a total of 560 eggs (382 treatment, 39 control, and 139 unmanipulated eggs) from 213 clutches. All analyses were conducted with SYSTAT 10 (SPSS 2002).

We quantified the thermal environment that each experimental egg experienced while it was exposed from temperatures recorded in holding boxes (Fig. 2), except for 34 eggs that were exposed during a data logger malfunction. For each egg, we calculated the proportion of time that it was exposed to temperatures above developmental zero (conservatively designated as >24°C), and the mean, minimum, and maximum temperatures it experienced during exposure. A two-way multivariate analysis of variance (MANOVA) indicated that: (1) all four temperature measures differed signifi-

cantly among holding sites (Wilks' lambda = 0.001, $F_{24, 1267} = 494.4$, $P < 0.001$), and (2) duration of exposure significantly affected some temperature measures (Wilks' lambda = 0.781, $F_{12, 944} = 7.72$, $P < 0.001$). Mean temperature and proportion of time above developmental zero did not differ significantly among exposure periods ($F_{3, 360} < 0.41$, $P > 0.75$ for both), whereas minimum and maximum temperatures differed significantly among exposure durations ($F_{3, 360} > 9.1$, $P < 0.001$ for both). The latter result is to be expected, since the range (extremes) of a distribution often increases with increasing sample size; and, (3) the interaction of holding site and exposure time was not significant (Wilks' lambda = 0.948, $F_{24, 1246} = 0.808$, $P = 0.730$). To determine if eggs lost mass during exposure due to water loss, we conducted a two-way ANOVA with holding site and exposure time as factors.

We compared hatching success between control and experimental eggs exposed for varying durations using

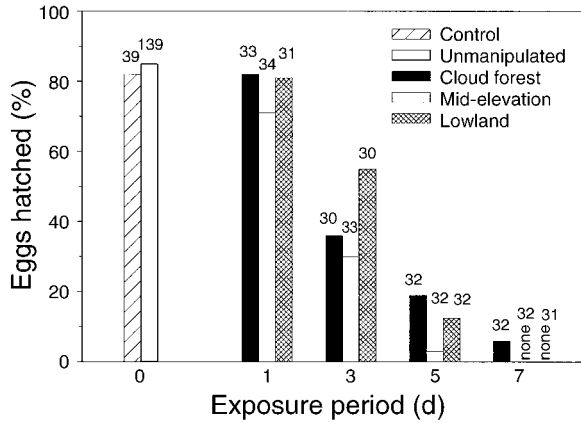


FIG. 3. Percentage of Pearly-eyed Thrasher eggs that hatched in relation to exposure period and holding site. Sample sizes are above bars.

Chi-squared (χ^2) tests. Logistic regression was used to examine effects of treatment site, exposure, and ambient temperature on hatching success of experimental eggs. We included proportion of time above developmental zero and mean, minimum, and maximum temperatures, as well as the interaction of exposure with the latter two temperatures. We also included transportation time to test for confounding results from movement of eggs, and egg mass, and change in egg mass during exposure to test for egg size effects and effect of water vapor loss, respectively. Results from individual models for each effect and from a mixed stepwise model with only significant effects ($P \leq 0.15$ to enter and remain in the model) are presented. Following the approach of Arnold et al. (1987) and Stoleson and Beissinger (1999), we modeled the decline in hatchability (H) of thrasher eggs with increasing exposure for each site separately using nonlinear regression and forcing the intercept through hatching success (84.3%) for control eggs [$H = 0.843/(1 + a(\text{Exposure})^b)$].

To examine the effect of treatment site and exposure period on the rate of egg development, we calculated incubation periods for experimental eggs as the number of days from returning the egg to the nest until hatching and for control eggs as the number of days from laying to hatching. We combined five- and seven-day exposures, as few eggs exposed for these durations hatched, and used a two-way ANOVA with holding site and exposure period as factors. As the onset of incubation varies among females (M. I. Cook, S. R. Beissinger, and W. J. Arendt, *unpublished manuscript*), we restricted analysis of unmanipulated eggs to last-laid eggs. Incubation period was determined for 127 experimental eggs that hatched and 34 last-laid unmanipulated eggs that hatched. Finally, logistic regression was used to examine differences in the timing of embryo mortality among sites and among durations of pre-incubation exposure. Time of embryo death was cat-

egorized as little (1–2 days) or no development vs. moderate to advanced development (4–12 days). Cochran's test was used to test for a linear trend of mortality with increasing periods of pre-incubation exposure.

RESULTS

Does egg viability decline with exposure to warm ambient temperatures and differ among sites?

Hatching success of experimental eggs declined markedly with increasing exposure period (Fig. 3). Hatchability of control eggs (84.3%, $n = 178$) did not differ significantly ($\chi^2_{1,276} = 1.4$, $P = 0.24$) from experimental eggs exposed for one day (78.6%), but was significantly higher than eggs exposed for three days (41.9% hatched, $\chi^2_{1,271} = 51.9$, $P < 0.001$), five days (11.5% hatched, $\chi^2_{1,274} = 136.4$, $P < 0.001$) and seven days (2.1% hatched, $\chi^2_{1,273} = 169.5$, $P < 0.001$).

Duration of exposure had by far the greatest single impact on hatching success (Table 1). Hatching success differed significantly among treatment sites. However, contrary to expectation, hatching success was lowest for eggs held at the mid-elevation site and tended to be highest at the lowland site, while eggs held at the cool cloud forest did not differ significantly from either site (Figs. 3 and 4). Among the temperature variables, only minimum temperature significantly affected hatching success, while mean temperature and the proportion of time above developmental zero had marginally significant effects. However, hatchability was positively, rather than negatively, related to all temperature

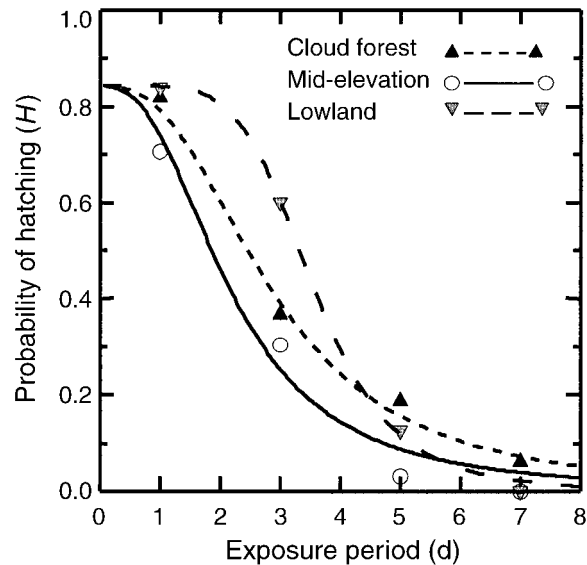


FIG. 4. Decline in hatchability (H) of thrasher eggs with exposure, modeled using nonlinear regression forcing the intercept through hatching success (84.3%) for control eggs: $H = 0.843/[1 + a(\text{Exposure})^b]$ for cloud forest ($a = 0.065$, $b = 2.615$, $r^2 = 0.59$), lowland ($a = 0.001$, $b = 5.352$, $r^2 = 0.689$), and mid-elevation ($a = 0.141$, $b = 2.556$, $r^2 = 0.564$) sites.

TABLE 1. Individual and mixed stepwise logistic regression models for the effects of pre-incubation exposure duration (days), treatment site (referenced against the mid-elevation site), temperature during exposure, travel time, and egg mass on the viability of Pearly-eyed Thrasher eggs.

Model and parameter	Estimate	SE	<i>t</i> ratio	<i>P</i>
Individual				
Exposure duration	-0.843	0.085	-9.515	0.001
Site: cloud forest	0.443	0.270	1.645	0.101
Site: lowland	0.549	0.270	2.034	0.042
Mean temperature	0.066	0.035	1.901	0.057
Minimum temperature	0.146	0.042	3.473	0.001
Maximum temperature	0.014	0.026	0.549	0.583
Proportion of time >24°C	0.598	0.323	1.853	0.064
Travel time	0.001	0.001	0.584	0.560
Egg mass	0.118	0.140	0.842	0.400
Egg mass change	0.334	1.004	0.333	0.739
Stepwise				
Constant	-3.796	1.845	-2.058	0.040
Exposure duration	-1.010	0.110	-9.195	0.001
Maximum temperature	0.247	0.083	2.969	0.003
Site: cloud forest	1.289	0.405	3.183	0.001
Site: lowland	-0.347	0.662	-0.524	0.600

variables. Travel time and egg mass did not significantly affect hatching success. Change in egg mass during exposure was also unrelated to hatching success (Table 1), perhaps because very little mass was lost in the humid tropical environment (mean = 0.04 g or 0.5% of the average egg mass: 8.65 g) and mass loss was unrelated to treatment site ($F_{2,266} = 0.484$, $P = 0.617$), duration of exposure ($F_{3,266} = 0.773$, $P = 0.510$), and their interaction ($F_{6,266} = 0.0138$, $P = 0.991$). Including all significant main effects in Table 1 and the interactions of temperature measures with exposure duration in a mixed stepwise logistic regression yielded a final model (Table 1) with exposure duration, treatment site, and maximum temperature as significant effects (log likelihood = -132.3, LRT = 186.3, df = 4, $P < 0.001$, McFadden's rho-squared = 0.413).

Does exposure to ambient temperature induce pre-incubation development and are there differences among sites?

Prolonged exposure to temperatures above developmental zero should induce development and should reduce the incubation period (14.5 ± 0.1 days [mean \pm SE] for 34 last-laid control eggs) of eggs held at the lowland site, but not at other sites. A two-way ANOVA revealed that holding site had a significant effect on incubation period ($F_{2,118} = 5.47$, $P = 0.005$). Eggs held at the warmer lowland site had shorter developmental periods than eggs held at the cooler cloud forest and mid-elevation sites (Fig. 5). There was no effect of exposure period ($F_{2,118} = 0.044$, $P = 0.957$), but the interaction of site \times exposure period approached significance ($F_{4,118} = 2.09$, $P = 0.080$). Development time

TABLE 2. Summary of results from field experiments on egg viability in relation to environment and life history.

Characteristic	Domestic fowl	Dabbling ducks
Environment		
Location	Puerto Rico	Manitoba
Climate	tropical	high temperate
Life history		
Mode of development	precocial	precocial
Modal clutch size	8-12	8-12
Preincubation delay (d)	7-11	7-11
Viability		
Hatchability (%) after exposure of:		
0 days	83	90
3 days
5 days	31	83
7-10 days	...	68
Daily rate of decline (%), days 0-5	-10.4	-1.4
Source	Cook et al. (2003)†	Arnold (1993)†

† Used incubators to incubate all eggs after exposure including controls.

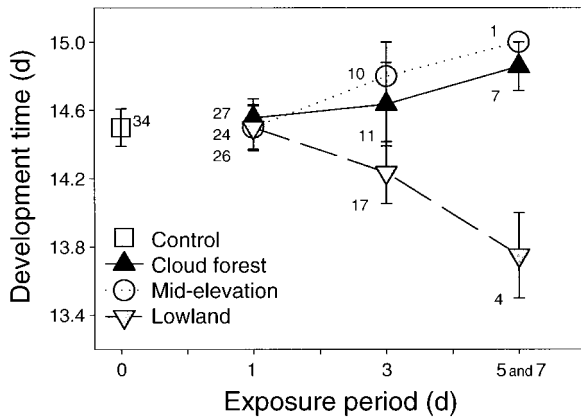


FIG. 5. Mean and standard errors of development periods for experimental and control eggs (last laid controls plus unmanipulated) that hatched, by holding site and exposure period. Numbers indicate sample sizes of eggs.

decreased with increasing exposure at the warm lowland site, whereas it tended to increase at the cooler sites.

Are there differences among sites in the timing of embryo mortality?

The majority of embryo mortality occurred at very early stages (Fig. 6), and most eggs (80.8%) showed little (1–2 days) or no development at death. Only 14.1% of eggs reached moderate development (4–8 days) at death and 5.1% died at advanced levels of development.

The prediction that a greater proportion of embryos should die at moderate to advanced developmental stages in eggs exposed at the lowland compared to the cloud forest or mid-elevation sites was not upheld. Logistic regression found no differences among sites (*t* ratio < 1.37, *P* > 0.17), but the timing of embryo mortality was strongly related to duration of pre-incubation exposure ($\chi^2_{4,276} = 34.9, P < 0.001$). The pro-

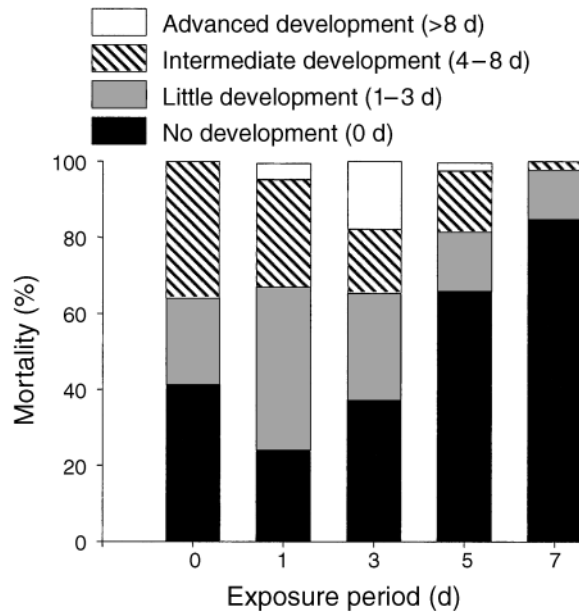


FIG. 6. Timing of embryo mortality in relation to length of preincubation exposure of thrasher eggs for 23 of 28 control (0 days exposure) and all 273 experimental eggs that failed to hatch.

portion of eggs dying with little or no development increased significantly (Cochran's test for linear trend = 30.0, *df* = 1, *P* < 0.001) with increasing periods of pre-incubation exposure (Fig. 6). Over 90% of the eggs (*n* = 178) that were exposed for 5–7 days died with little or no development, compared to 65% of eggs (*n* = 98) exposed for 0–3 days.

DISCUSSION

Predictions of the egg viability hypothesis were only partly upheld. Rates of hatching success and development supported the hypothesis: (1) viability of unincubated, freshly laid eggs declined rapidly and

TABLE 2. Extended.

Green-rumped parrotlet	Pearly-eyed thrasher	House sparrow
Venezuela lowland tropics	Puerto Rico tropical	Spain Mediterranean
altricial	altricial	altricial
7	3	5
0	2	4
86	84	81
70	42	66
63	11	58
14	2	...
-4.6	-14.6	-4.6
Stoleson and Beissinger (1999)	This study	Veiga (1992)

strongly with exposure to ambient conditions (Figs. 3 and 4), and (2) prolonged exposure to temperatures above developmental zero induced development and reduced the incubation period of eggs exposed at the warm cloud forest site, but led to increased development time for eggs exposed at cooler sites (Fig. 4). Nevertheless, critical predictions about the effects of ambient temperatures on egg viability were not upheld. Eggs at the cool cloud forest site that never experienced temperatures above developmental zero (Figs. 1 and 2, and Appendix) survived less well than eggs exposed to warm temperatures at the lowland forest (Figs. 3 and 4), and hatching success was positively related to ambient temperatures (Table 1). We will discuss the implications of these results for understanding the magnitude of, mechanisms responsible for, and life history significance of egg viability declines.

Magnitude and comparison of egg viability declines

Viability of unincubated thrasher eggs declined rapidly and strongly (Figs. 3 and 4). Less than half the eggs exposed for three days hatched and success was very poor for eggs exposed for five or seven days (11% and 2%, respectively). Loss of egg viability in thrashers was clearly due to prolonged exposure, as hatching success of control eggs that incurred the effects of both handling and movement was very high (82%), as was hatching success of eggs exposed for one day (78.6%). Thrasher eggs experienced the lowest hatching success when exposed at the mid-elevation site, which was the origin of most eggs used in this experiment. Thus, local adaptation cannot account for declines in thrasher egg viability. Furthermore, there is little evidence of avian embryo adaptation to local environmental conditions and early developmental patterns vary little among avian species (Webb 1987, Deeming and Ferguson 1992).

Viability of unincubated thrasher eggs declined more strongly than has been reported for other species (Table 2). The daily loss of viability for thrasher eggs in Puerto Rican forests was 3–10 times greater than for eggs of parrotlets exposed in tropical savannas of Venezuela, sparrows exposed in savannas of southern Europe, and dabbling ducks (*Anas* spp.) exposed in wetlands in temperate North America (Table 2). After 3–5 days of preincubation exposure, thrasher eggs hatched about one-third to one-half less often than Green-rumped Parrotlet and House Sparrow eggs treated similarly. However, viability of synchronously hatching waterfowl eggs began to decline only after 5–10 days of exposure at a high latitude site (Arnold et al. 1987, Arnold 1993). Waterfowl eggs may have lost viability more slowly because they were exposed to ambient temperatures that remained well below developmental zero, and because embryos of precocial species may have a different tolerance to exposure than altricial species (Cook et al. 2005). In support of the latter cause, domestic fowl (*Gallus gallus domesticus*) eggs exposed in Puerto Rico for five days under conditions identical to those

used in this experiment hatched three times more frequently than did thrasher eggs (Cook et al. 2003). Nevertheless, fowl eggs exposed in Puerto Rico lost viability twice as rapidly as eggs of parrotlets and House Sparrows (Table 2). Thus, the warm, moist conditions of tropical rain forests create challenging environments for maintaining viability of unincubated bird eggs.

Causes of egg viability declines

Our experiment is the first to expose wild bird eggs in situ to a critical set of temperatures thought to erode or preserve their viability. While viability of thrasher eggs declined strongly with duration of exposure to ambient conditions, hatchability differed little among exposure sites (Figs. 3 and 4) chosen because their ambient temperatures nearly always or very rarely exceeded developmental zero (Figs. 1 and 2, Appendix). Thus, effects of ambient temperature alone appear to be an inadequate explanation for the viability declines we observed. Eggs exposed at the cloud forest were held at temperatures that are ideal for storage of commercial poultry eggs (Webb 1987, Meijerhof 1992). Under optimal storage conditions, hatchability of commercial poultry eggs is reduced by only 0.5% per day (Meijerhof 1992) and under a variety of storage conditions viability declines averaged 2.8% per day (Stoleson 1999), compared to 11.7% per day for thrasher eggs held at the cool cloud forest site.

Mechanisms in addition to exposure to ambient temperatures must have caused mortality of thrasher embryos. Cook et al. (2003, 2005) have shown convincingly that trans-shell invasion by pathogenic bacteria and fungi is another mechanism of mortality for both thrasher and domestic fowl eggs. At the cloud forest, microbes invaded 90% and 60% of eggs exposed for five days of thrashers and fowl, respectively, whereas the chance of infection for lowland eggs was lower, the rate was slower, and the number of microorganisms was fewer. Nevertheless, invasion occurred in a sizeable proportion of lowland eggs (28% and 25% after five days for thrashers and fowl, respectively). Moreover, hatching success was high for eggs exposed at the cloud forest whose shells were disinfected twice daily, but was very low for uncleaned cloud forest eggs and for both cleaned and uncleaned lowland eggs. Thus, there were clear negative consequences to embryo mortality of exposure to high ambient temperature, but they became apparent only when the effects of microbial infection were controlled. Without well-designed experiments, separating the effects of microbial infection and ambient temperature can be difficult because both factors may act independently to considerably reduce hatching success. Thus, differences in egg size, eggshell thickness, pore number and size, preincubation delay, and perhaps levels of antibiotic properties in the albumen of eggs (Saino et al. 2002) may influence the probability of trans-shell infection, sus-

ceptibility to ambient temperatures, and the rate of viability decline (Cook et al. 2005).

Could a decline in viability of unincubated eggs be a consequence of early incubation rather than its cause? Artificial selection experiments indicate that egg hatchability is a conservative character that apparently cannot be changed without adverse pleiotropic effects (Crittenden and Bohren 1961, Nordskog and Hassan 1971). Although domestic hens have been selected for number and hatchability of eggs, their eggs still exhibit a loss of viability after 2–3 days of pre-incubation storage (Decuyper and Michels 1992, Meijerhof 1992) and have low hatching success after five days of exposure to ambient, tropical conditions (Cook et al. 2003). Eggs of domestic fowl can be exposed for >1 week prior to the onset of full incubation and did lose viability more slowly than thrasher eggs, which typically experience only 1–3 days of pre-incubation exposure (Table 2). On the other hand, thrasher eggs lost viability much more rapidly than parrotlet eggs that experience no pre-incubation delay. Too few species have been studied to date to make useful generalizations about causes of decline based on interspecific variation in egg viability.

Consequences of egg viability for avian life histories

Understanding how environmental conditions can constrain embryo fitness prior to the onset of full incubation may greatly influence how we think about avian life histories. Clutch size, hatching asynchrony, parental care roles, and social systems may be influenced by the amount of incubation required to sustain embryos during laying. Females cannot simultaneously incubate early laid eggs and gather resources to produce more eggs. An earlier onset of incubation, a larger clutch size and higher offspring quality may be associated with biparental incubation in altricial species (Nilsson 1993, Monaghan and Nager 1997, Smith and Härdling 2000, Reid et al. 2002).

Our results provide strong evidence that bird eggs have a limited “shelf life” under natural conditions prior to the onset of incubation and that the “shelf life” can be very short in moist tropical environments. Less than half of the first-laid thrasher eggs in four-egg clutches would hatch if thrashers did not initiate incubation prior to clutch completion, and only 70% of second-laid eggs would hatch (Fig. 4). These rates of embryo mortality suggest that egg viability may constrain not only the onset of incubation but potentially also clutch size in this species. If the clutch size of thrashers was one egg larger (five eggs), then the first-laid egg would have about a 22% chance of hatching in the absence of incubation prior to clutch completion. Thrasher eggs lost viability on average by 14.6% per day (Table 2), which greatly exceeds the range of daily nest predation rates (1.6–8.3%) of tropical forest birds (Robinson et al. 2000). Thus, the warm, moist conditions of tropical rain forests create challenging envi-

ronments for maintaining viability of unincubated bird eggs, which may be a factor contributing to latitudinal declines in clutch size (Beissinger 1999, Stoleson and Beissinger 1999) and increases in asynchronous hatching (Viñuela and Carrascal 1999).

Hatching asynchrony is likely to occur in tropical species even if they do not incubate first-laid eggs, because prolonged exposure to warm ambient temperatures can promote embryo growth and reduce development time. Thrasher eggs that survived pre-incubation exposure developed more rapidly at the warm lowland site than eggs exposed to cool temperatures at higher elevation sites (Fig. 5). Development proceeded slowly at temperatures below optimal incubation levels, as pre-incubation exposure for 3–7 days only reduced incubation periods of thrasher eggs by ~0.5–1 day. A reduction in incubation period of similar magnitude was found for parrotlet eggs exposed for similar durations (Stoleson and Beissinger 1999). Although warm temperatures initiated some development of thrasher eggs, they primarily led to embryo mortality at early stages of development (Fig. 6) and did not result in the bimodal timing of embryo mortality predicted by the egg viability hypothesis.

Partial incubation may be an important consequence of the strong decline in the viability of unincubated eggs. Studies of hatching asynchrony typically characterize hatching patterns by the egg on which full incubation is initiated (Clark and Wilson 1981, Stoleson and Beissinger 1995). Yet a great variety of birds, including thrashers, exhibit low to moderate levels of intermittent or partial incubation prior to the onset of full incubation (Drent 1970, Afton 1979, Lessells and Avery 1989, Hebert and Sealy 1992, Banbura and Zielinski 1995, Anderson 1997, Wiebe et al. 1998, Persson and Goransson 1999, Poussart et al. 2000, Hanssen et al. 2002, Loos and Rohwer 2004). Partial incubation has the potential to maintain the viability of early laid eggs by reducing the impacts of ambient temperature and also the probability and magnitude of microbial infection. Partial incubation may function to raise egg temperatures to levels sufficient to initiate and catalyze antibacterial defence mechanisms (e.g., lysozymes) in the albumen (Williams et al. 1968, Cook et al. 2005). Perhaps this may explain why hatching success in thrashers was positively related to ambient temperature, especially maximum temperature (Table 1). When analyzed by site, this relationship rose to significance solely at the lowland forest ($P = 0.008$), the only site where temperatures regularly exceeded enzyme activation levels (equal to or greater than ~27°C). In domestic chicken and turkey eggs, several hours of full incubation performed prior to 14 days of storage increased hatchability to that of eggs stored for only four days (Fasenko et al. 2001a, b).

The strong effects of exposure to ambient conditions on embryo mortality may select for the early onset of incubation even though the resulting hatching asyn-

chrony may lead to mortality of chicks hatched later (Beissinger 1999). Needed are well-designed experiments that can elucidate the conditions causing egg viability declines, and can be used to parameterize fitness models incorporating brood reduction and nest failure to explore how these forces interact with rates of egg viability decline to affect the onset of incubation and the evolution of clutch size (Stoleson and Beissinger 1995, Beissinger 1999).

ACKNOWLEDGMENTS

We thank M. Anderson, M. Ford, A. Kong, K. Janaes, A. Toledo, and K. Wakelee for field assistance, and R. Greenberg and three anonymous reviewers for suggestions that improved this paper. We are especially grateful to D. J. Lodge and J. Wunderle for logistic support. This study was financially supported by National Science Foundation grant 99-04754 to S. R. Beissinger and by the USDA Forest Service.

LITERATURE CITED

- Afton, A. D. 1979. Incubation temperatures of the Northern Shoveler. *Canadian Journal of Zoology* **57**:1052–1056.
- Amundsen, T., and T. Slagsvold. 1991. Hatching asynchrony: facilitating adaptive or maladaptive brood reduction? *Acta XX Congressus Internationalis Ornithologici* **3**:1707–1719.
- Anderson, T. R. 1997. Intermittent incubation during egg laying in house sparrows. *Wilson Bulletin* **109**:324–328.
- Arendt, W. J. 1993. The Pearly-eyed Thrasher: distribution, ecology and life-history strategy of an avian supertramp. Dissertation. University of Wisconsin, Madison, Wisconsin, USA.
- Arnold, T. W. 1993. Factors affecting egg viability and incubation time in Prairie Dabbling Ducks. *Canadian Journal of Zoology* **71**:1146–1152.
- Arnold, T. W., F. C. Rohwer, and T. Armstrong. 1987. Egg viability, nest predation, and the adaptive significance of clutch size in Prairie Ducks. *American Naturalist* **130**:643–653.
- Banbura, J., and P. Zielinski. 1995. The onset of incubation and hatching asynchrony in the barn swallow *Hirundo rustica*. *Ornis Fennica* **72**:174–176.
- Beissinger, S. R. 1999. Interaction of egg viability, brood reduction and nest failure on the onset of incubation. Pages 638–646 in N. J. Adams and R. H. Slotow, editors. *Proceedings of the 22nd International Ornithological Congress. BirdLife South Africa, Johannesburg, South Africa*.
- Beissinger, S. R., and J. R. Waltman. 1991. Extraordinary clutch size and hatching asynchrony of a neotropical parrot. *Auk* **108**:863–871.
- Bortolotti, G. R., and K. L. Wiebe. 1993. Incubation behavior and hatching patterns in the American kestrel *Falco sparverius*. *Ornis Scandinavica* **24**:41–47.
- Carey, C. 1980. The ecology of avian incubation. *BioScience* **30**:819–824.
- Carey, C. 1994. Structural and physiological differences between montane and lowland avian eggs and embryos. *Journal of Bioscience* **19**:429–440.
- 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. *Physiological Zoologist* **56**:340–352.
- Carey, C., E. L. Thompson, C. M. Vleck, and F. C. James. 1982. Avian reproduction over an altitudinal gradient: incubation period, hatchling mass, and embryonic oxygen consumption. *Auk* **99**:710–718.
- Clark, A. B., and D. S. Wilson. 1981. Avian breeding adaptations: hatching asynchrony, brood reduction, and nest failure. *Quarterly Review of Biology* **56**:253–277.
- Cook, M. I., S. R. Beissinger, G. A. Toranzos, and W. J. Arendt. 2005. Microbial infection affects egg viability and incubation behavior in a tropical passerine. *Behavioral Ecology* **16**:30–36.
- Cook, M. I., S. R. Beissinger, G. A. Toranzos, R. A. Rodriguez, and W. J. Arendt. 2003. Trans-shell infection by pathogenic micro-organisms reduces the shelf life of non-incubated bird's eggs: a constraint on the onset of incubation? *Proceedings of the Royal Society of London, Series B* **270**:2233–2240.
- Crittenden, L. B., and B. B. Bohren. 1961. The genetic and environmental effects of hatching time, egg weight, and holding time on hatchability. *Poultry Science* **40**:1736–1750.
- Decuypere, E., and H. Michels. 1992. Incubation temperature as a management tool: a review. *World's Poultry Science Journal* **48**:28–38.
- Deeming, D. C., and M. W. J. Ferguson. 1992. Physiological effects of incubation temperature on embryonic development in reptiles and birds. Pages 147–173 in D. C. Deeming and M. W. J. Ferguson, editors. *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge University Press, Cambridge, UK.
- Drent, R. H. 1970. Functional aspects of incubation in the Herring Gull. *Behavior (Supplement)* **17**:1–132.
- Drent, R. H. 1973. The natural history of incubation. Pages 262–322 in D. S. Farner, editor. *Breeding biology of birds*. National Academy of Science, Washington, D.C., USA.
- Drent, R. H. 1975. Incubation. Pages 333–420 in D. S. Farner and J. R. King, editors. *Avian Biology*. Volume 5. Academic Press, New York, New York, USA.
- Ewert, M. A. 1992. Cold torpor, diapause, delayed hatching and aestivation in reptiles and birds. Pages 173–191 in D. C. Deeming and M. W. J. Ferguson, editors. *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge University Press, Cambridge, UK.
- Fasenko, G. M., V. L. Christensen, M. J. Wineland, and J. N. Petite. 2001a. Examining the effects of prestorage incubation of turkey breeder eggs on embryonic development and hatchability of eggs stored for four or fourteen days. *Poultry Science* **80**:132–138.
- Fasenko, G. M., R. T. Hardin, F. E. Robinson, and J. L. Wilson. 1992. Relationship of hen age and egg sequence position with fertility, hatchability, viability and preincubation embryonic development in broiler breeders. *Poultry Science* **71**:1374–1383.
- Fasenko, G. M., F. E. Robinson, A. O. Whelan, K. M. Kremeniuk, and J. A. Walker. 2001b. Prestorage incubation of long-term stored broiler breeder eggs: 1. Effects on hatchability. *Poultry Science* **80**:1406–1411.
- Hafton, S. 1981. Incubation during the egg-laying period in relation to clutch size and other aspects of reproduction in the great tit, *Parus major*. *Ornis Scandinavica* **12**:169–185.
- Hansen, S. A., H. Engebretsen, and K. E. Erikstad. 2002. Incubation start and egg size in relation to body reserves in the common eider. *Behavioral Ecology and Sociobiology* **52**:282–288.
- Hebert, P. N., and S. G. Sealy. 1992. Onset of incubation in yellow warblers: a test of the hormonal hypothesis. *Auk* **109**:249–255.
- Hussell, D. J. T. 1985. On the adaptive basis for hatching asynchrony: brood reduction, nest failure and asynchronous hatching in snow buntings. *Ornis Scandinavica* **16**:205–212.
- Klomp, H. 1970. The determination of clutch size in birds: a review. *Ardea* **58**:1–124.
- Lack, D. 1968. *Ecological adaptations for breeding in birds*. Methuen, London, UK.
- Lessells, C. M., and M. I. Avery. 1989. Hatching asynchrony in European Bee-eaters *Merops apiaster*. *Journal of Animal Ecology* **58**:815–835.

- Loos, E. R., and F. C. Rohwer. 2004. Laying-stage nest attendance and onset of incubation in prairie nesting ducks. *Auk* **121**:587–599.
- Meijerhof, R. 1992. Pre-incubation holding of hatching eggs. *World's Poultry Science Journal* **48**:57–81.
- Monaghan, P., and R. G. Nager. 1997. Why don't birds lay more eggs? *Trends in Ecology and Evolution* **12**:270–274.
- Nilsson, J.-A. 1993. Bisexual incubation facilitates hatching asynchrony. *American Naturalist* **142**:712–717.
- Nordskag, A. W., and G. M. Hassan. 1971. Direct and maternal effects of egg size genes on hatchability. *Genetics* **67**:267–278.
- Perrins, C. M., and R. H. McCleery. 1989. Laying dates and clutch size in the Great Tit. *Wilson Bulletin* **101**:236–253.
- Persson, I., and G. Goransson. 1999. Nest attendance during egg laying in pheasants. *Animal Behaviour* **58**:159–164.
- Poussart, C., J. Larochelle, and G. Gauthier. 2000. The thermal regime of eggs during laying and incubation in greater snow geese. *Condor* **102**:292–300.
- Raffaele, H. A. 1989. *A guide to the birds of Puerto Rico and the Virgin Islands*. Princeton University Press, Princeton, New Jersey, USA.
- Rahn, H., C. Carey, K. Balmas, B. Bhatia, and C. Paganelli. 1977. Reduction of pore area of the avian eggshell as an adaptation to altitude. *Proceedings of the National Academy of Sciences (USA)* **74**:3095–3098.
- Reid, J. M., P. Monaghan, G. D. Ruxton. 2002. Males matter: the occurrence and consequences of male incubation in starlings (*Sturnus vulgaris*). *Behavioral Ecology and Sociobiology* **51**:255–261.
- Ricklefs, R. E. 1993. Sibling competition, hatching asynchrony, incubation period, and lifespan in altricial birds. Pages 199–276 in D. M. Power, editor. *Current Ornithology*. Volume 11. Plenum Press, New York, New York, USA.
- Robinson, W. D., T. R. Robinson, S. K. Robinson, and J. D. Brawn. 2000. Nesting success of understory forest birds in central Panama. *Journal of Avian Biology* **31**:151–164.
- Romanoff, A. L., and A. J. Romanoff. 1972. *Pathogenesis of the avian embryo*. John Wiley and Sons, New York, New York, USA.
- Saino, N., P. Dall'ara, R. Martinelli, and A. P. Moller. 2002. Early maternal effects and antibacterial immune factors in the eggs, nestlings and adults of the barn swallow. *Journal of Evolutionary Biology* **15**:735–743.
- Skutch, A. F. 1985. Clutch size, nesting success, and predation on nests of Neotropical birds, reviewed. *Ornithological Monographs* **36**:575–594.
- Smith, H. G., and R. Härdling. 2000. Clutch size evolution under sexual conflict enhances the stability of mating systems. *Proceedings of the Royal Society of London, Series B* **267**:2163–2170.
- Snyder, N. F. R., J. W. Wiley, and C. B. Kepler. 1987. The parrots of Luquillo: natural history and conservation of the Puerto Rican Parrot. Western Foundation of Vertebrate Zoology, Los Angeles, California, USA.
- SPSS. 2002. SYSTAT. Version 10. SPSS, Chicago, Illinois, USA.
- Stoleson, S. H. 1999. The importance of the early onset of incubation for the maintenance of egg viability. Pages 600–613 in N. J. Adams and R. H. Slotow, editors. *Proceedings of the 22nd International Ornithological Congress*. BirdLife South Africa, Johannesburg, South Africa.
- Stoleson, S. H., and S. R. Beissinger. 1995. Hatching asynchrony and the onset of incubation in birds, revisited: when is the critical period? Pages 191–270 in D. M. Power, editor. *Current Ornithology*. Volume 12. Plenum Press, New York, New York, USA.
- Stoleson, S. H., and S. R. Beissinger. 1997. Extreme hatching asynchrony, brood reduction, and food limitation in a neotropical parrot: an experimental study. *Ecological Monographs* **67**:131–154.
- Stoleson, S. H., and S. R. Beissinger. 1999. Egg viability as a constraint on hatching asynchrony at high ambient temperatures. *Journal of Animal Ecology* **68**:951–962.
- Stutchbury, B. J., and R. J. Robertson. 1988. Within-season and age-related patterns of reproductive performance in female Tree Swallows (*Tachycineta bicolor*). *Canadian Journal of Zoology* **66**:827–834.
- Veiga, J. P. 1992. Hatching asynchrony in the House Sparrow: a test of the egg-viability hypothesis. *American Naturalist* **139**:669–675.
- Viñuela, J., and L. M. Carrascal. 1999. Hatching patterns in non-precocial birds: a preliminary comparative analysis. Pages 584–589 in N. J. Adams and R. H. Slotow, editors. *Proceedings of the 22nd International Ornithological Congress*. BirdLife South Africa, Johannesburg, South Africa.
- Vleck, C. M., and D. Vleck. 1996. Embryonic energetics. Pages 417–460 in C. Carey, editor. *Avian energetics and nutritional ecology*. Chapman and Hall, New York, New York, USA.
- Walsberg, G. E., and C. A. Schmidt. 1992. Effects of variable humidity on embryonic development and hatching success of Mourning Doves. *Auk* **109**:309–314.
- Webb, D. R. 1987. Thermal tolerance of avian embryos: a review. *Condor* **89**:874–898.
- White, F. N., and J. L. Kinney. 1974. Avian incubation. *Science* **186**:107–115.
- Wiebe, K. L., J. Wiehn, and E. Korpimäki. 1998. The onset of incubation in birds: can females control hatching patterns? *Animal Behaviour* **55**:1043–1052.
- Williams, J. R., L. H. Dilliard, and G. O. Hall. 1968. The penetration patterns of *Salmonella typhimurium* through the outer structures of chicken eggs. *Avian Diseases* **12**:69–97.
- Wilson, H. R. 1991. Physiological requirements of the developing embryo: temperature and turning. Pages 145–156 in S. G. Tullett, editor. *Poultry science symposium*. 22: Avian incubation. Butterworth-Heinemann, London, UK.

APPENDIX

A figure showing ambient temperatures recorded hourly at the lowland, mid-elevation, and cloud forest sites during the three years of study is available in ESA's Electronic Data Archive: *Ecological Archives* E086-115-A1.