Upper lethal temperatures of Northern Bobwhite embryos and the thermal properties of their eggs

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ABSTRACT Northern Bobwhite eggs in the southern United States are often exposed to ambient temperatures in excess of their normal incubation temperature when unattended during their typical extended preincubation period. In drought years, typified by high ambient temperatures, Bobwhite eggs are often exposed to temperatures >45°C, well-above the upper lethal temperature of most other birds. Because the upper lethal temperature of Bobwhite embryos is not currently known, simulated clutches of eggs were exposed to preincubation temperatures ranging from 39 to 52°C for exposure times of 1, 3, or 6 h. The upper lethal temperatures and the temperatures resulting in ≥50% death of Northern Bobwhite embryos were recorded in addition to the time to thermal equilibrium of Bobwhite eggs. The upper lethal temperature for 1, 3, and 6 h of preincubation exposure was 51, 49, and 46°C, respectively. The temperatures resulting in ≥50% death were 46, 44, and 40°C for eggs exposed to elevated temperatures for 1, 3, and 6 h, respectively. The mean time for the inner-egg temperature to reach the ambient temperature was 38 ± 1 min (± SE). The thermal tolerances of Northern Bobwhite embryos were much higher than expected, and among the highest reported for birds, indicating an adaptation to the naturally occurring temperature extremes that often occur in the Bobwhite's semi-arid southern range. However, as the temperature increased above the incubation temperature, hatching success declined, showing that increased thermal tolerance has a cost. Although Bobwhite producers, managers, and researchers will find this information useful, it seems most interesting that high temperatures could plausibly have contributed to the population decline observed in the Bobwhite's semi-arid range.

Key words: upper lethal temperature, Northern Bobwhite, *Colinus virginianus*, avian development, incubation

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INTRODUCTION

One of the most important physicochemical forces ubiquitously acting on living organisms is temperature. Known to drive development, temperature plays a vital role in the ontogeny of avian species (Romanoff, 1960). Embryonic development begins, or resumes, above a temperature defined as physiological zero (Funk and Biellier, 1944; Miller and Wilson, 1975). For most avian species, physiological zero occurs from 24 to 26°C (see Kosin, 1964; Landauer, 1967; Wilson, 1991 for review). Above physiological zero, avian development increases as a function of increasing temperature, until a thermal optimum is reached; that is, the range of temperatures producing the highest survivorship of embryos (Romanoff, 1960). The thermal optimum for domestic poultry eggs, including chickens, Northern Bobwhites, turkeys (*Meleagris gallopavo*), and ducks (*Anas platyrhynchos*), falls within the range of 37 to 38°C (Romanoff, 1960; French, 1997). Although there are interspecific differences in incubation temperatures, even slight deviations (±1°C) from the optimum temperature within a species can disrupt embryonic development and affect hatchability of the eggs (Romanoff, 1960; Dawson, 1984; Wilson, 1991; Gill, 1999).

For all avian species, an upper lethal temperature (**ULT**) exists, beyond which life is extinguished (Gill, 1999). For instance, the ULT for chicken embryos (*Gallus gallus*) was reported to be 43°C and 44 to 45°C by Dareste (1891) and Dumas (1824), respectively, as reported by Romanoff (1960), although no age or exposure time was reported. However, Romanoff (1972) later showed that older chicken embryos were more susceptible to temperatures higher than that of incubation (>37.5°C), and younger chicken embryos were more susceptible to temperatures lower than that of incubation. Thus, thermal tolerance differs according to age. In addition, thermal tolerance seems quite similar across avian species. Lundy (1969) recorded a ULT for chicken embryos in fresh eggs as 42.4°C for 1 h. Heerman gull embryos (*Laris heermanni*) experienced a

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ULT of 43°C for 1 h (Bennett and Dawson, 1979), and Adélie penguin embryos (*Pygoscelis adeliae*) expired after exposure to 42°C for 96 min.

Because avian embryos reside within a cleidoic egg, the ambient temperature outside of the egg is not always equal to the inner-egg temperature. When the ambient temperature changes, the inner-egg temperature lags behind the ambient temperature until a thermal equilibrium is reached. As a result, the ULT for avian embryos of like ages is often listed as a combination of ambient temperature and duration of egg exposure (Lundy, 1969). For instance, the ULT for embryos in fresh chicken eggs is frequently reported as 43°C at thermal equalization, which takes approximately 2 h to reach when starting from room temperature (22–25°C; Kaplan et al., 1978). Thus, the ULT would be reported as 43°C for 2 h. If the fertile chicken eggs were exposed to 43°C for <2 h, the embryos within may survive because the inner-egg temperature, or embryonic temperature, did not reach 43°C.

The thermal properties of chicken eggs and lethal temperatures of avian embryos are well-characterized (Alsop, 1919; Romanoff et al., 1938; Romanoff, 1960; Wilson et al., 1979; Gill, 1999). Unfortunately, such data are lacking for Northern Bobwhites (*Colinus virginianus*), a near-threatened game bird (IUCN, 2008) whose population has declined by 82% since 1983 (Sauer et al., 2008). Bobwhite populations have declined concurrently with global warming (Guthery et al., 2000) and, in their semi-arid range, population fluctuations are known to correlate with extreme heat loads and drought conditions (Bridges et al., 2001; Guthery et al., 2001, 2004; Reyna, 2008). Indeed, their populations seem to be, at least partially, driven by temperature.

Determining the ULT for Northern Bobwhites is of interest because of their potential exposure to extremely high, diurnally-fluctuating temperatures during an extended preincubation period. The Northern Bobwhite is a ground-nesting gallinaceous game bird that lays 1 egg/d for approximately 12 to 14 d (Stoddard, 1931; Lehmann, 1984; Roseberry and Klimstra, 1984). The eggs remain in the nest (typically a scrape nest under a clump of grass) largely unattended, until the hen begins incubation ≤7 d after laying is complete (Stoddard, 1931); meaning that the clutch is unattended and exposed to ambient temperatures for 15 to 21 d without the thermal buffer of the hen. During this time, Bobwhite eggs are potentially exposed to temperatures that exceed their normal incubation temperature (i.e., >37.5°C) in their southern climes. In nondrought years, internal temperatures of nonincubated Bobwhite eggs followed diurnal ambient fluctuations and often exceeded 40°C during peak thermal intensity (Guthery et al., 2004). Bobwhite nest temperatures in simulated drought conditions regularly peaked at approximately 45°C and were recorded to be as high as 60°C (Guthery et al., 2000) during drought conditions in a 1-yr-old nest. Thus, in their southern range, it is plausible that extreme temperatures experienced during preincubation severely affect production, resulting in decreased population numbers in drought years.

Yet, the effect of these temperature extremes on the viability of Bobwhite eggs (specifically the ULT) is not known. Therefore, the objectives of this study were to determine the ULT of Northern Bobwhite embryos, the hatching success rates of sublethal temperature doses, and the thermal equalization times of freshly laid Northern Bobwhite eggs. We hypothesized that Bobwhite eggs must exhibit a higher thermal tolerance than other avian species (e.g., chickens) because they are often subjected to temperatures exceeding 42.4°C (lethal temperature for chickens) in nature. We expected reduced hatching rates in response to extreme temperatures that would mimic age ratios observed in nature during drought years. We also hypothesized that Bobwhite eggs would reach thermal equilibrium with high heat loads faster than chicken eggs because of their smaller size.

MATERIALS AND METHODS

Fertilized Northern Bobwhite eggs were collected from flying-type breeding pairs (1–2 yr of age) at Lake Cumberland Game Bird Farm (Mill Springs, KY,), a source approved for egg production by the US Department of Agriculture and certified by the US Department of Agriculture National Poultry Improvement Plan. Eggs were packaged and shipped to the University of North Texas (Denton) on the day of collection. Eggs arrived on-site within 2 to 3 business days with a written record of the date and time of egg collection. This research was approved by the University of North Texas Institutional Animal Use Care Committee, protocol No. 0808.

Upon arrival, Bobwhite eggs were weighed and randomly divided into groups of 15 eggs. Each group was placed on plastic egg trays with the blunt end up, and they were labeled with an indelible marker as either a control (no preincubation exposure to high temperatures) or treatment (exposed to high temperatures during preincubation) egg and assigned an identifying number according to group, egg number, preincubation temperature, and exposure time (e.g., T1–41–6 = Treatment group egg No. 1, 41°C preincubation temperature, 6 h exposure). All eggs were acclimated at 22 to 25°C (room temperature) for ≥3 h before the experiment to ensure a uniform temperature throughout the egg and a common starting temperature for all of the treatments.

Control Incubation

Control eggs (15 eggs/group; 3 replications) received no preincubation exposure to a high temperature. Once labeled and acclimated, control eggs were placed into an incubator with circulating air (model 1502, G.Q.F.

Manufacturing Co., Savannah, GA) to undergo normal development. The temperature of the control incubator was 37.5 ± 0.5°C with an RH of 60% (common commercial settings; Romanoff, 1960). Eggs were turned automatically every 4 h for the first 19 d of the 23-d incubation period (Romanoff, 1960). On d 20, the control group was placed in a separate hatching chamber within a Hatchrite incubator (Hatchrite Inc., Kirkwood, MO) and the eggs were no longer turned (Romanoff, 1960). The Hatchrite incubator was an emu incubator with 6 large metal chambers with rubber lining, which was ideal for hatching Northern Bobwhite eggs of different treatment groups. Hatching was determined when the eggs were star-pipped, defined as an externally pipped egg where the embryo created a small hole (approximately 3 mm^2) in the shell to initiate hatching (MacCluskie et al., 1997). This definition of hatching was used to compensate for any artificial hatching difficulties associated with any microclimates within the incubator. Upon star-pipping, the percentage of eggs hatched was recorded for further comparison with the treatment groups.

ULT

Treatment egg groups were assigned elevated temperature treatments ranging from 39 to 52°C (control eggs were assigned a temperature of 37.5°C) to determine a ULT. For each temperature, 3 egg groups (15 eggs/group) were assigned to different durations of exposure (1, 3, and 6 h, respectively) to determine the range of temperatures that may cause death.

Once labeled and acclimated, treatment groups were placed in a thermal chamber (G.Q.F. 1583, G. Q. F. Manufacturing Co.; with circulated air; RH = 60%) at their assigned temperature. A class T thermocouple that was connected to a digital converter (model bat-12; Physitemp Instruments, Clifton, NJ) was placed approximately 1 mm above the eggs to record the chamber temperature. Group exposure time began when the chamber temperature reached the assigned temperature.

After the assigned exposure time, eggs were promptly removed from the thermal chamber and immediately placed into the control incubator (37.5°C; RH = 60%) to ensure they received the same quality of incubation as the control eggs. Treatment eggs were turned automatically every 4 h for the first 19 d of the 23-d incubation period (Romanoff, 1960). On d 20, each treatment group was placed in a separate hatching chamber within a Hatchrite incubator and the eggs were no longer turned. Upon star-pipping, hatching success was recorded as the percentage of eggs hatched. Each treatment was replicated 3 times.

Thermal Properties of Fertile Eggs

To determine the amount of time until the inner-egg, or embryo, reached equilibrium with the chamber temperature, an additional fresh Bobwhite egg was placed into the incubator with the treatment eggs at 37.5, 40, 43, 45, and 50°C. Prior to thermal exposure, a class T thermocouple that was connected to a digital converter was inserted into a 1-mm^2 hole in the blunt end of the additional egg until it reached the inner-shell membrane. The thermocouple was then affixed to the eggshell (hole sealed) with modeling clay. The egg and inserted thermocouple were held at 22 to 25°C (room temperature) for ≥3 h before the experiment to ensure a uniform temperature throughout the egg and a common starting temperature for all of the treatments. Inner-egg temperature and chamber temperature were recorded every 1 to 3 min to determine the time to thermal equilibrium.

Statistical Analyses

All data were tested with a Shapiro-Wilks normality test (Zar, 1999) and Hartley's F_{max} test (Zar, 1999) before specific statistical analyses were performed. An ANOVA (Zar, 1999) was used to identify differences in mean values among treatment groups. A Holm-Sidak pairwise multiple comparison procedure was subsequently used to test for significance between control and treatment groups (Zar, 1999). All statistical tests were conducted using SigmaStat 3.5 software (Systat Software Inc., San Jose, CA) with statistical decisions made with a 0.05 level of probability.

RESULTS

ULT

Upon arrival, mean fresh egg mass (± SE) was 9.82 g ± 0.82. Control eggs, receiving no elevated preincubation temperature, had a mean hatch success rate (± SE) of 84.6% ± 2.1. Treatment eggs exposed to elevated preincubation temperatures for 1 h showed a decline in viability at temperatures >45°C (Figure 1A). The lethal temperature at which 50% of the sample perished (**LT-50**) for groups exposed for 1 h occurred at temperatures ≥46°C. The ULT for 1 h of exposure occurred at 51°C (Figure 1A). Eggs exposed to elevated preincubation temperatures for 3 h showed declining hatchability at ≥40°C, had an LT-50 of ≥44°C, and exhibited a ULT of 50°C (Figure 1B). Finally, eggs exposed to the most extreme conditions (6 h of elevated preincubation temperatures) displayed a decline in hatching success at >38°C, exhibited an LT-50 of ≥40°C, and incurred a ULT of 47°C (Figure 1C).

Mean hatch rates were significantly different between groups exposed to 1, 3, and 6 h of elevated preincubation temperatures of 44 to 47°C ($P = 0.03$; Figure 2). No difference between groups was observed from 38 to 42°C ($P > 0.05$) or ≥48°C ($P > 0.05$). Temperatures leading to 30, 50, 70, 90, and 100% mortality are illustrated in Figure 3.

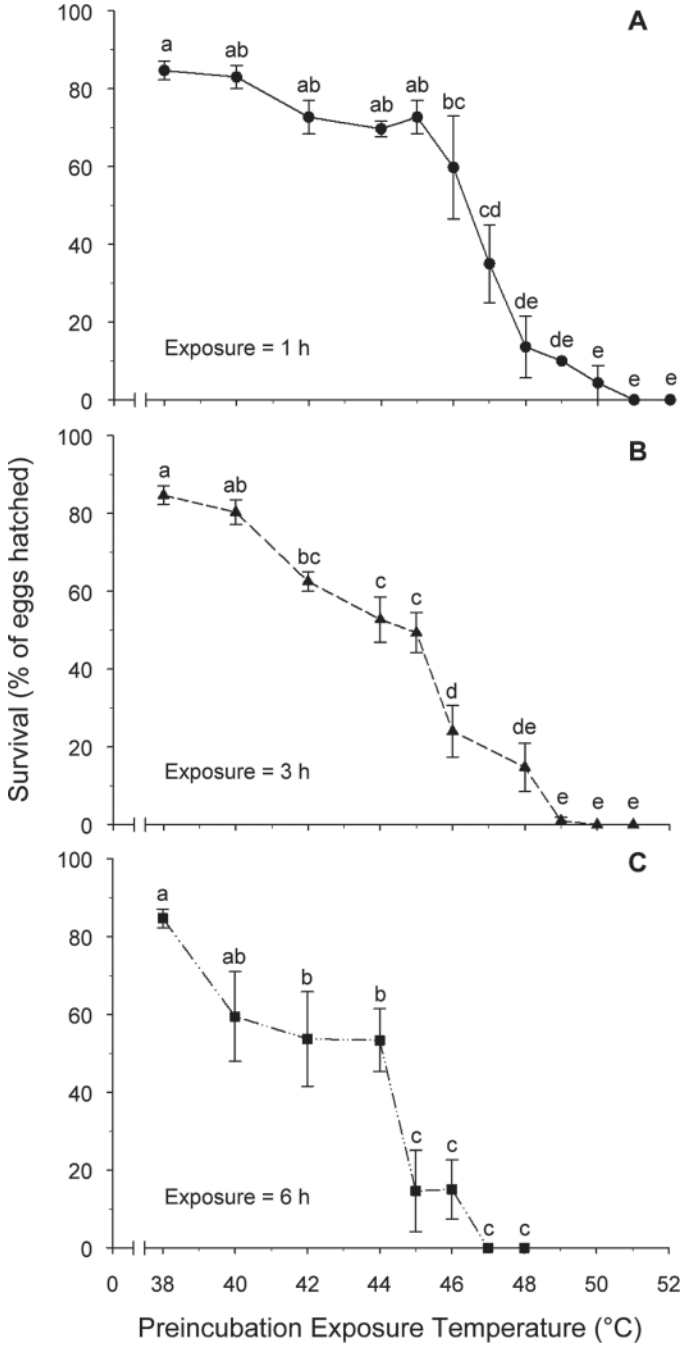

Figure 1. Mean hatching success rate (%) of Bobwhite eggs exposed for 1 h to temperature treatments ranging from 38 to 52°C before incubation. The upper lethal temperature (ULT) for 1, 3, and 6 h of preincubation exposure was 51, 49, and 46°C, respectively. Letters indicate statistical groupings; error bars = ±1 SE.

Thermal Properties of Fertile Eggs

Egg temperature lagged behind chamber air temperature by 30 to 50 min (Table 1) with a mean equilibration time (± SE) of 38 ± 1 min for chamber temperatures between 37.5 and 50°C and an initial egg temperature of 22 to 25°C. Thus, embryos were fully exposed to the ambient temperature for 10 to 30 min of the nominal 1 h exposure treatment, 2.2 to 2.5 h for the 3 h exposure treatment, and 5.2 to 5.5 h for the 6 h exposure treatment of eggs to chamber temperature regimens.

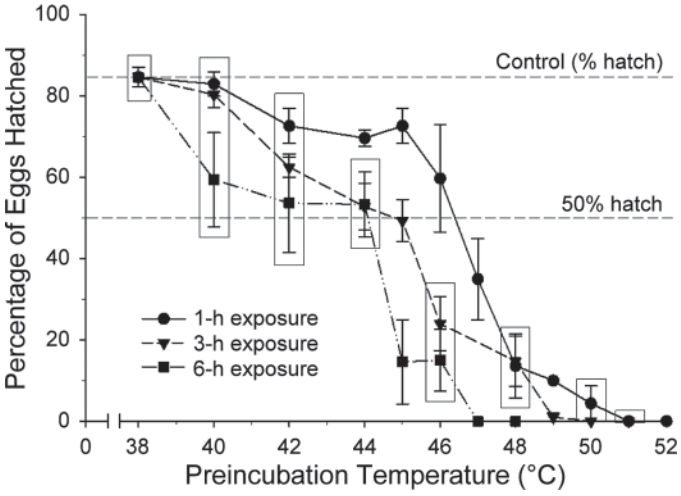

Figure 2. Mean hatching success rate (%) of Bobwhite eggs exposed to temperature treatments of 38 to 52°C for 1, 3, and 6 h before incubation; n = 45 eggs per data point (1,350 eggs total). Boxes indicate no statistical difference; error bars = ±1 SE.

DISCUSSION

ULT of Northern Bobwhite

Webb (1987) reviewed thermotolerance of 15 avian species and reported that significant hyperthermic mortality occurred interspecifically at 38 to 39°C for ≥24 h in Adélie penguins, anatids, galliforms, kestrels, and gulls. However, most avian species can withstand the temperature of 41°C for an exposure period of ≤10 h. In contrast to this long-standing finding, the present study reports that preincubated Bobwhite embryos exhibit a much higher and rather remarkable tolerance to brief hyperthermic exposures, surviving temperatures of up to 50°C for 1 h, 49°C for 3 h, and 46°C for 6 h. The ability of very early Bobwhite embryos to persist

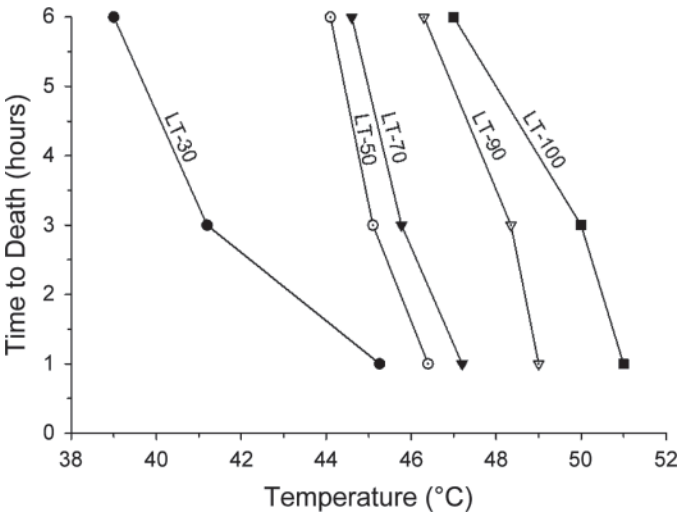

Figure 3. Estimated lethal temperatures at exposure times ranging from 1 to 6 h, for Northern Bobwhite eggs leading to 30, 50, 70, 90, and 100% mortality (LT-30, LT-50, LT-70, LT-90, and LT-100, respectively) from data illustrated in Figure 2.

Table 1. Time to reach thermal equilibrium of Northern Bobwhite eggs transferred from 22°C to thermal chambers at 37.5 to 50°C

Chamber temperature (°C)	Mean equilibration time[1] (min ± SE)
37.5	29.7 ± 0.8
40	31.7 ± 0.3
43	37.3 ± 0.9
45	42.7 ± 0.6
50	49.0 ± 0.6

[1]n = 3 eggs per treatment.

in a wide range of temperatures is likely an adaptation to the naturally occurring temperature extremes that can occur in their semi-arid climate while awaiting incubation (Guthery et al., 2001, 2004; Hernandez and Peterson, 2007)

The increased tolerance to high heat loads has costs, however. As the preincubation temperature increased, mortality increased as a function of exposure time at a given temperature (Figure 3). This phenomenon may partially explain why differences in Bobwhite productivity (as indicated by an age ratio of the number of juveniles per adult) are observed in populations of different latitudes where the average number of juveniles per adult is 4.0 in northern US populations (i.e., colder climates) and 2.3 in southern US latitudes, or warmer climates (Guthery, 2002). In the northern regions, temperatures are unlikely to reach detrimental values, even in drought years; whereas in the southern climates, high temperature extremes are more common. It is plausible that heat associated with drought, experienced during preincubation, contributes to the reduced age ratios and ultimately contributes to the population decline in the Bobwhite's southern range.

Embryos remain viable when exposed to temperatures of ≤45°C for ≤1 h and ≤40°C for ≤6 h (Reyna, 2010), demonstrating that embryos can be exposed to extreme temperatures with minimal disruption of development, albeit at the cost of reduced hatch rates. This finding helps explain how embryos survive exposure to thermal stress as a result of solar radiation during preincubation (mostly during hot drought years) and as a result of nest inattentiveness during incubation when incubating parents forage or leave the nest when they enter hyperthermia. Insight might also be gained from this experiment into how avian eggs and embryos respond to quickly changing weather patterns and how they may adapt to changing climates. Bobwhite producers, managers, and researchers should find this information useful given that eggs are exposed to extreme temperatures in the laboratory or in the wild. However, of greater interest is that high temperatures, experienced during drought, could plausibly have contributed to the quail decline observed in the Bobwhite's semi-arid range.

Future research may be directed toward understanding the physiological mechanisms involved in embryo quiescence when exposed to temperature extremes. Investigations into the time of death, protein composition (e.g., the presence, quantity, or type of heat-shock proteins) or genetic structure of surviving embryos compared with those of deceased embryos may provide a good start. Protein profiles and their thermal stabilities of all embryonic stages and adult Bobwhites may prove useful when examining the thermal limits imposed by climate change or drought conditions. Additionally, the humidity during this experiment was kept constant to single out the effects of high temperatures; however, it would be interesting to replicate this study with different levels of RH.

Thermal Properties of Fertile Eggs

The time required for inner-egg temperature and the resident embryo to equalize with the outer-egg or chamber temperature is important, although not always reported with ULT. Knowledge of the thermal properties of the egg as a physical entity is important when determining lethal heat loads or the effect of high temperatures on the embryo in ovo. For instance, a Northern Bobwhite embryo with a mean heating equilibrium time of 38 min exposed to high temperatures for ≥2 h will experience high temperatures for approximately 1.4 h longer than a chicken embryo whose egg has a mean heating response time of 2 h. Additionally, it is imperative to link ULT with the duration of exposure and heating equilibrium time to understand the severity of the embryonic thermal environment. Future understanding of how egg surface area and volume interacts with heating response time of eggs and the physiological responses to thermal stress may further our understanding of temperature effects on embryos.

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