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Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds

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ABSTRACT

Incubation is a vital component of reproduction and parental care in birds. Maintaining temperatures within a narrow range is necessary for embryonic development and hatching of young, and exposure to both high and low temperatures can be lethal to embryos. Although it is widely recognized that temperature is important for hatching success, little is known about how variation in incubation temperature influences the post-hatching phenotypes of avian offspring. However, among reptiles it is well known that incubation temperature affects many phenotypic traits of offspring with implications for their future survival and reproduction. Although most birds, unlike reptiles, physically incubate their eggs, and thus behaviourally control nest temperatures, variation in temperature that influences embryonic development still occurs among nests within a population. Recent research in birds has primarily been limited to populations of megapodes and waterfowl; in each group, incubation temperature has substantial effects on hatchling phenotypic traits important for future development, survival, and reproduction. Such observations suggest that incubation temperature (and incubation behaviours of parents) is an important but underappreciated parental effect in birds and may represent a selective force instrumental in shaping avian reproductive ecology and life-history traits. However, much more research is needed to understand how pervasive phenotypic effects of incubation temperature are among birds, the sources of variation in incubation temperature, and how effects on phenotype arise. Such insights will not only provide foundational information regarding avian evolution and ecology, but also contribute to avian conservation.

Key words: incubation temperature, life history evolution, maternal effects, phenotypic variation.

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I. INTRODUCTION

Parents often have tremendous influence over the developmental environment of their young which has many implications for their offspring's phenotype. In birds, incubation largely determines the developmental environment of the embryo, influencing, for instance, the temperature at which embryos develop. Little is known, however, about how naturally occurring variation in incubation temperature shapes avian phenotype. By contrast, a wealth of literature exists on the phenotypic effects of incubation temperature in reptiles, and this work provides tremendous insight for avian biologists exploring the relationship between incubation temperature and avian phenotypic development. Herein we summarise the effects of incubation temperature on reptilian offspring phenotype and its influence on reptile reproductive ecology and life history. We then explore the existing literature on incubation temperature and its effects on offspring phenotype in birds, what factors influence the temperatures experienced by avian embryos during development, and the ecological, evolutionary and conservation implications of temperature-induced phenotypes in birds. Finally, we conclude with priorities for future research.

II. INCUBATION TEMPERATURE AND PHENOTYPIC DEVELOPMENT IN REPTILES

The discovery of temperature-dependent sex determination in reptiles over 50 years ago (Charnier, 1966) spawned decades of intensive molecular, physiological, and ecological research on the influence of temperature on early reptilian development (Bull, 1983; Valenzuela & Lance, 2004). It is now well established that incubation temperature is the predominant factor determining sex in some turtles and squamates, and all crocodilians (Valenzuela, 2004*a*, *b*). In recent years research has begun to reveal possible molecular mechanisms by which temperature influences sex ratios of reptiles (Rhen & Schroeder, 2010), as well as the evolutionary and ecological consequences of environmental sex determination (Shine, 2004; Valenzuela, 2004*a*, *b*; Warner & Shine, 2008, 2011; Robert & Thompson, 2010). In addition, recent theoretical and empirical research has been directed at determining the consequences of global climate change on reptile development and population dynamics (Booth, 2006; Mitchell & Janzen, 2010).

In addition to determining sex, incubation temperature can have a wide array of other more subtle effects on phenotypic characteristics and life-history traits of reptiles (Table 1). For example, body size, shape, and body condition (i.e. mass relative to structural size) of hatchlings are influenced by incubation temperature in lizards, crocodilians, and turtles. Likewise, early growth, locomotor performance, and physiological performance are influenced by nest temperatures in a wide variety of species and in some cases these effects may be long-lasting. Most importantly, a few studies have demonstrated that incubation temperatures influence the survival and lifetime reproductive success of reptilian offspring in the wild. For example, Parker & Andrews (2006) artificially incubated lizard (*Sceloporu sundulatus*) eggs at 22, 24, and 27°C and released them in the field. They found significant reductions in early survival in hatchlings from eggs incubated at the coolest temperature that were also correlated with differences in locomotor performance. In a seminal study by Warner & Shine (2008), eggs of the lizard *Amphibolurus muricatus* were incubated across a range of temperatures, and hatchlings were then released into field enclosures where their lifetime reproductive success was monitored. Incubation temperature influenced fitness of lizards, but affected male and female offspring differently. These findings demonstrate that early developmental experiences can influence the fitness of reptiles, and that temperature-dependent sex determination may control sex ratios in a manner that is adaptive given particular environmental conditions that preferentially favour one sex over the other (Warner & Shine, 2008). Although the importance of incubation temperature in determining reptilian phenotypes is clear, surprisingly little is known about how temperature influences the phenotypic characteristics of birds despite their similarly structured amniotic egg. As one researcher stated, 'rather belatedly it has been realized that incubation temperature can influence more than hatchling morphology in poultry' (Deeming, 2004).

III. AVIAN INCUBATION TEMPERATURE AND ITS INFLUENCE ON PHENOTYPE

Incubation is a necessary component of avian reproduction that is costly to parents, but critical to the development and hatching of eggs. Metabolic rates of parents incubating eggs can exceed metabolic rates of birds thermoregulating at cool temperatures by 40–50% (reviewed in Tinbergen & Williams, 2002), and prolonged incubation periods are associated with reduced adult survival, immunity, and future reproductive success (Hanssen *et al.*, 2005; de Heij, van den Hout & Tinbergen, 2006). However, spending more time on the nest can prove beneficial to the parents by increasing hatching success of eggs, and increasing temperatures that embryos experience. Higher incubation temperatures are known to speed embryonic development and reduce incubation period, thus reducing the risk of nest predation (Tombre & Erikstad, 1996; Neuchterlein & Buitron, 2002; Reid, Monaghan & Nager, 2002; Martin *et al.*, 2007). To increase reproductive success it is in the parent's best interest to provide an optimal incubation environment, but variation in intrinsic factors (e.g. body condition, lipid stores, etc.) and/or extrinsic factors (e.g. weather, predation) can limit the ability of the parents to achieve such optima.

In most birds, providing an optimal incubation environment is often achieved by spending more time actively incubating the eggs. Thus, for most birds embryos

Table 1. Examples of phenotypic traits and life-history characteristics of reptilian offspring affected by incubation temperature

Phenotypic trait/ life-history characteristic	Reptile taxa[a]
Sex	All crocodilians, tuatara, some turtles and lizards
Hatchling body size	Turtles, lizards, crocodilians
Hatchling body shape/ scalation	Lizards, snakes, crocodilians
Hatchling body condition/ composition	Turtles, lizards, crocodilians
Colour	Turtles, lizards, crocodilians
Locomotor performance	Turtles, lizards, snakes
Behaviour	Turtles, lizards, snakes
Metabolism	Turtles
Thermoregulation	Turtles, lizards, snakes, crocodilians
Growth	Turtles, lizards, tuatara, crocodilians
Immune function	Turtles
Endocrinology	Turtles, lizards
Survival	Turtles, lizards, crocodilians
Lifetime reproductive success	Lizards

Compiled from: Booth & Astill (2001), Andrews (2004, 2008), Birchard (2004), Deeming (2004), Rhen & Lang (2004), Shine (2004), Valenzuela (2004*a*, *b*), Valenzuela & Lance (2004), Booth (2006), Parker & Andrews (2006), Freedberg *et al*. (2008), Warner & Shine (2008), and Paitz *et al*. (2010*a*, *b*).

[a]Failure to include a taxonomic group usually indicates that studies have not evaluated that phenotypic trait in that group.

Taxa included represent those in which the phenotypic trait is known to be affected in at least one species.

experience temperatures conducive to rapid development while the parent is incubating, but considerable reductions in temperature, often below physiological zero (temperatures at which development is suspended; Webb, 1987), occur when a parent leaves the nest (e.g. Greeney, 2009). This is in stark contrast to most reptiles and megapode birds in which nest-site selection and nesting materials/substratum have a stronger influence on the temperatures experienced by developing embryos. This type of nesting can result in long periods of time in which embryos are incubated at temperatures above or below the 'ideal' developmental temperature. Distinguishing between these two forms of incubation is important because many avian embryos are not very tolerant of incubation for long periods (i.e. days to weeks) at sub-optimal temperatures, but are very tolerant of exposure to the short periods of cool temperatures below physiological zero that occur when parents leave the nest (Webb, 1987). By contrast, reptile and megapode embryos are tolerant of exposure for long periods of time (days) at sub-optimal incubation temperatures (Eiby & Booth, 2008). Regardless of incubation strategy, the average temperature and the variability of temperatures that embryos experience have different implications for phenotype (e.g. Du & Shine, 2010) and need to be explored in birds.

In passerine birds, embryos develop optimally between 36 and 40°C, temperatures above 40.5°C are lethal, and development is suspended below 24–26°C (i.e. physiological zero; Arnold, Rohwer & Armstrong, 1987; Webb, 1987; Stoleson & Beissinger, 1995; Cooper *et al*., 2005). If eggs are exposed for extended periods to incubation temperatures between physiological zero and the optimum, embryos experience unsynchronized tissue growth, abnormal development and mortality (Deeming & Ferguson, 1992). After the onset of incubation, 3 days of exposure to ambient conditions due to egg neglect by the parent can cause embryonic mortality (Stoleson & Beissinger, 1999; Beissinger, Cook & Arendt, 2005), but sensitivity of embryos to egg neglect varies among species, stages of development and environments(Webb, 1987). Incubation not only maintains eggs in a proper thermal environment, but it also reduces trans-shell infection by microbes (Cook *et al*., 2005; Shawkey *et al*., 2009 but see Walls, Hepp & Eckhardt, 2011), which can also be an important and independent source of mortality to unincubated eggs (Cook *et al*., 2005). Partial incubation during egg-laying may be an important response to these threats (Wang & Beissinger, 2011). Despite our long-held understanding that maintenance of egg temperatures by physical incubation is a necessity for birds to hatch eggs successfully, we know very little about how variations in temperature influence phenotypes of offspring that do hatch. If fluctuations in egg temperatures are sufficient to influence variation in phenotypes of avian offspring, important implications for the fitness of both young and parents can arise.

Perhaps the relationship between temperatures experienced during development and phenotypes of avian offspring has been considered unlikely in birds because parental incubation of eggs was perceived to reduce substantially variation in nest temperatures compared to most reptiles that provide no parental care. Even megapodes, whose eggs are laid in soil mounds full of decomposing materials and warmed using chemical heat, typically maintain nest temperatures by adding organic material to their nest or changing the shape of the mound (average temperature: 34°C; range within a nest: 28–40°C; Booth, 1987; Booth & Jones, 2002; Göth & Booth, 2005; Eiby & Booth, 2008). However, temperatures within megapode nests do vary and nest temperatures can sometimes drop to approximately 25°C and increase to 40°C for extended periods of time (Booth, 1987; Eiby & Booth, 2008). Nest temperatures of birds that brood their eggs also vary. Nest temperatures (which are often used as a proxy for egg temperatures)of most incubating birds typically range between 32 and 38°C (Huggins, 1941), and temperatures among nests of a single species can vary within a population (Webb, 1987). For example, within-population mean temperatures (averaged over the entire course of incubation) of wood duck (*Aix sponsa*) nests in Alabama, USA, ranged between 34.9 and 37.8°C (Hepp, Folk & Manlove, 2005), mean nest temperatures of American robin (*Turdus migratorius*) in Ohio, USA, ranged between 31.6 and 39.9°C (Huggins, 1941), and mean nest temperatures of willow tit (*Parus montanus*) in Norway ranged between 35.3

and 36.8°C (Haftorn, 1979; also see Haftorn, 1988). Mean nest temperatures of a population of yellow-eyed juncos in Arizona, USA, ranged between 33.3 and 36.8°C (Weathers & Sullivan, 1989). The average nest temperature of six western bluebird (*Sialia mexicana*) nests on the first day of incubation ranged from approximately 30.0–37.0°C (Wang & Weathers, 2009). Despite behavioural regulation of nest temperatures, the observed variation in temperatures among naturally incubated nests within a species is enough to produce variation in offspring phenotype. For example, recent work demonstrates that differences of only 1–1.5°C can affect a wide variety of phenotypic traits in avian offspring (Hepp, Kennamer & Johnson, 2006; DuRant *et al*., 2010, 2011, 2012*a*, *b*; Hopkins *et al*., 2011; Nord & Nilsson, 2011).

The majority of work investigating the effects of incubation temperature on hatchling characteristics in birds comes from studies of domesticated species. In turkeys and chickens incubation temperature, which was typically manipulated during a portion of the incubation period, influenced thermoregulation, post-hatching growth, metabolism, food-conversion efficiency, and hatchling morphology of offspring (e.g. Lourens & van Middelkoop, 2000; Hill, 2001; Lourens, 2001; Nichelmann & Tzchentke, 2002; Hulet *et al*., 2007), and these effects have subsequent implications for the individual's ability to survive and reproduce. Here we explore the effects of incubation temperature on avian phenotype in wild birds and the implications of these phenotypic effects for avian ecology and evolution.

(1) Temperature effects on phenotype in wild birds

The effects of natural variations in incubation temperature on offspring phenotypes in non-domesticated species primarily have been studied in wood ducks and megapodes. Incubation temperature affected hatchling body size and composition of Australian brush turkey (*Alectura lathami*) hatchlings (Eiby & Booth, 2009). In addition, incubation temperature influenced sex ratios of brush turkeys *via* temperature-dependent embryonic mortality (Göth & Booth, 2005; Eiby, Wilmer & Booth, 2008; also see Batt & Cornwell, 1972). In wood ducks that hatched from eggs artificially incubated at different temperatures within the range of naturally incubated nest temperatures, incubation temperature differentially influenced an array of phenotypic traits important for early survival, including locomotor performance, metabolism, thermoregulation, stress physiology, immune responses, body size and composition, and growth (Hepp *et al*., 2006; DuRant *et al*., 2010, 2011, 2012*a*, *b*; Hopkins *et al*., 2011). Results from wood ducks suggested that most measures of physiological performance of ducklings were poorest at the lowest incubation temperature and maximized at an intermediate temperature, while performance at the highest incubation temperature was either similar to performance at the intermediate temperatures or slightly worse (Fig. 1). The results suggest that parents may experience trade-offs between the benefits of incubating eggs at higher temperatures to reduce risks of nest predation by shortening the incubation period, *versus* incubating eggs at intermediate temperatures (extending the incubation period) and producing hatchlings of higher quality. A study that examined the effects of incubation temperature on offspring phenotype in an altricial species, the cavity-nesting blue tit, *Cyanistes caeruleus* (Nord & Nilsson, 2011) found that temperature influenced both nestling size and metabolic rate. Taken together, results from studies of precocial and altricial species are similar and suggest that the effects of incubation temperature on offspring phenotypes may be widespread.

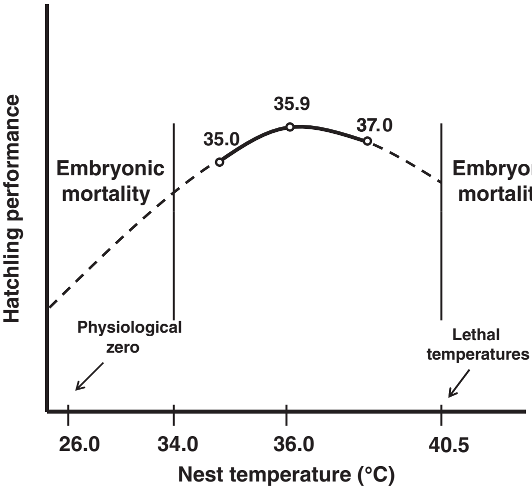

Fig. 1. The relationship between average temperature experienced during incubation and hatchling performance in wood ducks (*Aix sponsa*).Individual points on the graph represent physiological performance (e.g. immune responses) of wood duck embryos incubated at either 35.0, 35.9 or 37.0°C. Based on studies conducted in wood ducks, duckling performance is optimized at approximately 36°C (DuRant *et al*., 2010, 2011, 2012*a*, *b*; Hopkins *et al*., 2011). Dotted lines represent predicted performance at incubation temperatures that have yet to be tested. Natural nest temperatures in wood ducks are typically not higher than 38.5°C (Manlove & Hepp, 2000; Folk & Hepp, 2003; Hepp *et al*., 2006) and research suggests that even short exposure to temperatures exceeding 40.5°C is lethal to most avian embryos (Webb, 1987). A temperature below 24–26°C is considered physiological zero for most avian embryos. Although not tested, we propose that a similar curve is probably representative of the relationship between nest temperature and hatchling performance for most avian species.

Additional studies that did not directly measure egg temperatures, or account for other parental effects or other aspects of developmental microclimate provide indirect, but valuable evidence that incubation temperatures influence offspring phenotypes in birds. For example, female European starlings (*Sturnis vulgaris*) were exposed to 30 min stressors four times a day for 8 days while they were incubating eggs (Cyr & Romero, 2007). Sixteen days after hatching, nestlings from disturbed mothers had altered stress hormone profiles relative to those from unstressed mothers. Stressed mothers spent more time away from their nest than unstressed mothers suggesting that incubation temperature could have been a factor that influenced offspring phenotype. Subsequent work in wood ducks demonstrated that slight changes in incubation temperature can in fact influence stress hormone profiles of offspring (DuRant *et al*., 2010). In other studies, tree swallow (*Tachyci netabicolour*) nestlings that developed in experimentally cooled nest boxes had lower innate immune responses than nestlings from unmanipulated nest boxes (Ardia, Perez & Clotfelter, 2010; also see Nilsson, Stjemman & Nilsson, 2008; Martin, Arriero & Majewska, 2011). However, the cause of compromised immune response due to cool incubation temperatures is potentially confounded with the influence of female behaviour or physiology during incubation [e.g. duration of on- and off-bouts; heart rate (Walker, Boersma & Wingfield, 2005)] and other aspects of nest microclimate (e.g. humidity).

Historically, the importance of incubation in birds has been evaluated primarily in terms of hatching success, but recent research on the effects of incubation temperature on offspring phenotypes indicate that the influence of incubation temperatures extends beyond egg viability (Cooper *et al*., 2005; Olsen *et al*., 2008) to substantial influences on phenotype during the juvenile period with subsequent fitness implications. Thus, the additional selection pressure for maintaining incubation temperatures that enhance hatchling phenotype has implications for many aspects of avian reproductive biology and life history. In addition to providing important insights into the reproductive ecology, life history, and evolution of birds, a better understanding of how incubation temperature influences avian development will also have practical implications for conservation. Factors that directly influence the nest environment such as climate change or nest-site quality could affect egg incubation temperatures and exert additional selective pressures on adults to maintain optimal incubation conditions (Matthysen, Adriaensen & Dhondt, 2011). Additionally, anthropogenic influences such as environmental pollutants, reduced habitat quality, and nest-site disturbance that reduce nest attendance by adults could cause deviations from optimal incubation conditions. The relationships among environmental variables, incubation conditions, offspring phenotypes, and the subsequent life-history traits of adults remain unexplored in birds.

IV. FACTORS INFLUENCING AVIAN INCUBATION TEMPERATURE

Understanding the factors that create within-population variability in avian incubation temperatures is critical to illuminating the role that incubation temperature plays in avian ecology, evolution, and conservation. Factors that can contribute to variations in egg temperature within a population include nest-site microclimate, nest structure, clutch size, parental condition, differences in parental physiology (e.g. size or vascularization of the brood patch), nest initiation date, and of course incubation behaviour (e.g. duration of on/off bouts, onset of incubation). Studies are needed that examine these factors in relation to embryonic developmental temperatures and offspring phenotype.

The most obvious and best-studied factor contributing to egg-temperature variation is incubation behaviour (Deeming & Ferguson, 1992; Zicus, Hennes & Riggs, 1995; Martin *et al*., 2007). When eggs are in direct sunlight, parents often shield them from lethally high temperatures (Ar & Sidis, 2002). However, under cooler conditions when parents spend time away from the nest, decreased nest temperatures result in longer incubation periods because temperature and developmental rate co-vary (Deeming & Ferguson, 1992; Martin *et al*., 2007), and small differences in incubation behaviour can have important implications for egg temperatures and variation in incubation period (Boersma & Wheelwright, 1979; Lill, 1979; but see Wang & Beissinger, 2009). For example, in common goldeneye (*Bucepha laclangula*) an incubation period of 30 days instead of 29 days could be attributed to 13 min less parental nest attendance per day (Zicus *et al*., 1995).

Many factors, including disturbance, predation risk, climate, and nest initiation date can influence incubation behaviour of parents and the resulting variation in temperature among nests within a population. For instance, work in passerines suggests that high levels of nest predation are associated with parents adopting tactics that minimize activity around the nest by increasing the length of on- and off-bouts (Conway & Martin, 2000). Incubating parents also modify incubation behaviour as ambient temperatures fluctuate to keep their nest at relatively similar average temperatures in both cold and hot conditions (Yerkes, 1998; Deeming, 2002; Hepp & Kennamer, 2011). Parents take longer nest breaks when it is warm and shorter breaks during cold weather when the decline in nest temperature is more rapid (Caldwell & Cornwell, 1975; Afton & Paulus, 1992; Hepp & Kennamer, 2011). In two subspecies of swamp sparrow (*Melospiza georgiana*) apparent incubation length (clutch completion to hatching) was negatively related to ambient temperature (Olsen *et al*., 2008). In temperate environments, nest-initiation date also affects the temperatures that eggs experience as eggs laid earlier in the season experience greater reductions in temperature when parents leave the nest to forage (Bentzen *et al*., 2010), whereas later in the season embryos experience smaller decreases in temperature when parents are off the nest (Hepp & Kennamer, 2011). Research in reptiles suggests that fluctuations in temperature within nests can be instrumental in determining reptilian offspring phenotype (Ashmore & Janzen, 2003; Birchard, 2004; Du & Shine, 2010; Paitz *et al*., 2010*b*), and the same could be true for birds as well. Surprisingly, few studies have specifically discussed how subtle variations in average, minimum, and maximum nest temperatures among nests within a population correlate with the parental behaviour of the adults. Presumably under similar environmental conditions, avian parents that take longer incubation breaks could produce lower mean incubation temperatures. Alternatively, parents taking longer breaks may incubate their eggs at slightly higher temperatures than parents that take shorter or less frequent breaks resulting in similar mean incubation temperatures among nests within a population (Reneerkens *et al*., 2011).

Nest location, one of the primary factors affecting egg temperatures in many reptiles (Congdon & Gibbons, 1990; Wilson, 1998; Weisrock & Janzen, 1999), also can affect egg temperatures within bird populations. Birds tend to select sites that offer shelter from direct sunlight and wind, and that provide some measure of insulation (Gloutney & Clark, 1997; Hansell & Deeming, 2002; D'Alba, Monaghan & Nager, 2009);both variation in nest location and nest construction influence nest microclimates (Wachob, 1996; Weibe, 2001; Hansell & Deeming, 2002). In megapodes, parents select areas to construct their nests that are protected from direct exposure to sunlight and with leaves from plant species that rapidly decompose (Booth & Jones, 2002). A study on northern flickers (*Colaptu sauratus*) revealed that cavity temperatures positively correlated with clutch size (Weibe, 2001), suggesting that higher quality female flickers procure better nest sites. A study of yellow warblers (*Dendroi capetechia*) demonstrated that individuals nesting in colder locations constructed nests that were less porous and retained heat better than those nesting in warmer locations (Rohwer & Law, 2010).

Differences in the number of eggs within a nest and location of an egg within a clutch can produce variability in temperatures experienced by individual embryos during incubation. Incubating adults may not be able to maintain high incubation temperatures for all the eggs in a large clutch *versus* a small clutch resulting in substantial differences in temperatures experienced by eggs in the centre *versus* the periphery of the nest (Huggins, 1941; Caldwell & Cornwell, 1975). Spatial differences in incubation temperature within a nest are most noticeable early in incubation and disappear as incubation progresses due to metabolic heat production of later-staged embryos (Caldwell & Cornwell, 1975). Parents can reduce some of these temperature differences by shifting eggs around, but in very large clutches it may be difficult to keep all eggs at optimal temperatures for a significant proportion of the incubation period. A study in northern lapwings (*Vanellus vanellus*) found that experimentally enlarged clutches had longer incubation periods and produced young of smaller size than smaller clutches (Larsen, Lislevand & Byrkjedal, 2003), findings that are consistent with the effects of lower average incubation temperatures on offspring phenotype (Hepp *et al*., 2006; DuRant *et al*., 2010, 2012*a*, *b*; Hopkins *et al*., 2011; Nord & Nilsson, 2011). Thus, maintaining optimal incubation temperatures for all eggs within a large clutch may be difficult for incubating parents, a problem that could be exacerbated by brood parasitism. For example, in wood ducks the average clutch size is approximately 12 eggs, but can exceed 30 eggs when nests are parasitized by other females (Bellrose & Holm, 1979) and larger clutches experience longer incubation periods in this species (Hepp, Kennamer & Harvey, 1990; Hepp *et al*., 2005). Currently, it is unclear how much variation in incubation temperature exists within clutches in most bird species.

Finally, variation in incubation temperature within a population can be due to differences in the ability of individual birds to incubate eggs at optimal temperatures. For example, adults in poorer condition might be less effective incubators than those in good body condition. Parents in poor body condition should have greater foraging demands particularly in colder weather when it can be metabolically costly to provide heat for their embryos. Incubating birds have been known to increase rates of breathing, oxygen consumption, heartbeat, and onset of shivering, all energetically expensive processes, to generate heat in response to artificial cooling of their eggs (Lea & Klandorf, 2002, and citations therein). Regulating incubation temperatures also requires the ability of the parent to sense the temperature of their eggs *via* thermoreceptors (Tøein, 1993) which could also vary among birds in a population. Variation in size, vascularization, and vasodilatation in the brood patch (Midtgård, Sejrsen & Johansen, 1985; Massaro, Davis & Davidson, 2006) can all contribute to variation in the temperatures experienced by embryos during incubation as well.

V. ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS OF INCUBATION TEMPERATURE-INDUCED PHENOTYPES

In some cases incubation temperatures have been shown to be an important source of variation in avian offspring phenotypes upon which natural selection can act. If such effects are widespread among avian taxa, incubation temperature could be an important determinant of many aspects of avian ecology and evolution *via* its effects on offspring quality. Here we explore several aspects of avian life history that may be influenced by incubation temperature.

Selection for offspring quality might be the basis for evolution of nest-site selection and more elaborate nest construction. Most work on effects of incubation temperatures on offspring quality has been done on cavity-nesting species, and it is clear that differences in the quality of nest sites exist within these species (Stoleson & Beissinger, 1999; Weibe, 2001; Hepp *et al*., 2006). However, differences in nest-site quality also exist in species that build platform and cup nests, as well as in ground-nesting birds (e.g. Walsberg, 1983). The literature on nest-site selection related to microclimate has primarily focused on the energetics of incubating adults rather than effects on embryo development. Within species, individuals construct nests of differing quality in relation to the climate of the region in which they are nesting; in colder areas nests generally have higher heat-retaining characteristics than do those constructed in warmer areas (Rohwer & Law, 2010; Crossman, Rohwer & Martin, 2011). Traditionally nest-site selection and nest type were thought to be influenced primarily by predation risks (e.g.

Nilsson, 1984). However, nest microclimate may play an equal or larger role through the influence of incubation temperature on offspring quality.

Incubation temperature may also play an important role in the evolution of clutch size and egg size. More recent theory on the evolution of clutch size has begun to consider incubation temperature (Arnold *et al.*, 1987; Stoleson & Beissinger, 1999; Cooper *et al.*, 2005; Wang, Firestone & Beissinger, 2011) in relation to thermal properties of clutches of varying size (e.g. large clutches are more difficult to heat uniformly, but they have higher thermal inertia) and its implications for hatching success of embryos. The importance of incubation temperature may be greater than previously suspected if adults must maintain temperature conditions not only for successful hatching of eggs, but also to produce young with the most adaptive phenotypes. Likewise, recent theory suggests that the evolution of egg size may be related to incubation temperature. Martin (2008) proposed that because incubation temperature correlates negatively with incubation period, eggs incubated at lower temperatures must be larger in order to cope with the energetic demands of an extended incubation period. Recent work on the influence of incubation temperature on embryonic bioenergetics supports Martin's (2008) hypothesis because eggs incubated at lower temperatures indeed use more energy during development (DuRant *et al.*, 2011). However, the relationship between egg size and incubation temperatures may be more complex than previously proposed because temperature also affects many aspects of hatchling phenotype important for survival and reproduction (Eiby *et al.*, 2008; DuRant *et al.*, 2010, 2012*a*, *b*; Hopkins *et al.*, 2011; Nord & Nilsson, 2011) that may not be mediated by energetic constraints.

The influence of incubation temperature on offspring phenotype could have implications for many other aspects of avian life history and ecology, including adult incubation patterns (e.g. incubation constancy, duration and frequency of off/on bouts), mating systems, the evolution of parental care, and latitudinal and altitudinal gradients in avian life-history traits. For example, the effects of temperature on offspring phenotype may favour biparental incubation in some species, allowing parents to forage without compromising the temperatures their eggs experience. Just as studies on incubation temperature revolutionized our understanding of reptilian reproductive ecology, exploring the various attributes of birds from the perspective of incubation temperature could yield novel insights into their ecology and evolution.

VI. CONSERVATION IMPLICATIONS OF AVIAN INCUBATION TEMPERATURE

A number of environmental factors, many of which can be attributed to human activities, could influence the temperatures that eggs experience during incubation. For instance, environmental contaminants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are ubiquitous and known to have an array of adverse effects on the reproductive ecology of birds. Many of these and other contaminants disrupt the avian endocrine system, including reproductive hormones (e.g. androgens and estrogens), thyroid hormones, and prolactin (Fernie *et al.*, 2003; Verreault *et al.*, 2008; Marteinson *et al.*, 2010). As a result, these compounds have the potential to alter parental behaviours including those that influence the temperature of the nest. For example, American kestrels (*Falco sparverius*) fed PCBs exhibited reduced nest attendance and showed poor coordination of incubation duties, largely due to aberrant attendance by males (Fisher *et al.*, 2006). Although egg temperatures were not measured, the result of reduced nest attentiveness were consistent with the predicted relationship between cooler nest temperatures and longer incubation duration; kestrel eggs from less-attentive adults fed PCBs took significantly longer to develop and were less likely to hatch than those from control parents. Moreover, exposure of male kestrel embryos to PBDEs affected their subsequent fertility and reproductive behaviours as adults, suggesting that maternal transfer of these contaminants may have lifelong and multigenerational consequences for incubation behaviours in birds (Marteinson *et al.*, 2010).

We know of only one study that has explored the relationship between contaminants and nest temperatures. Verboven *et al.* (2009) found negative relationships between plasma levels of organic pollutants and nest temperatures in glaucousgulls (*Larus hyperboreus*) and postulated that these reductions in temperature were caused by physiological aberrations in incubating adults (e.g. brood patch development). To the best of our knowledge, however, no ecotoxicological studies have explicitly linked changes in adult nest attendance or physiology to incubation temperatures and resulting phenotypic consequences to offspring. Similarly, no studies have examined the interactive consequences of embryonic exposure to maternally transferred contaminants and suboptimal incubation conditions, which could act synergistically or additively to produce effects on the embryo/hatchling that neither would produce alone.

Nest disturbances such as those caused by military activities, urbanization, introduced predators, and ecotourism could influence nest attendance by forcing incubating adults to leave their eggs unattended more frequently or for longer periods of time ultimately affecting incubation temperatures and offspring phenotype. Numerous studies have demonstrated that improperly managed ecotourism and outdoor recreation can influence the physiology, behaviour, and survival of birds (reviewed in Carney & Sydeman, 1999; Buckley, 2004; Müllner, Linsenmair & Wikelski, 2004; Ellenberg *et al.*, 2007). Many of these studies indicate that adults are flushed from the nest when they perceive the threat of approaching humans, but the consequences for egg incubation temperatures have not been adequately explored.

Global environmental issues like climate change and invasive species also have the potential to alter incubation behaviour of parents. Shifts in temperature and rain patterns can create asynchony in avian nesting and the emergence of invertebrate prey important for feeding nestlings (Both *et al*., 2006), and changes in food resources during incubation also could reduce the amount of time parents spend incubating. Introduced avian predators could affect parental care of incubating birds as well (Massaro *et al*., 2008), which could subsequently affect nest temperatures or alter a parent's incubation tactics (Conway & Martin, 2000). Examining threats of changing environmental temperatures and introduced species in relation to nest temperatures and resultant phenotypic quality of young will provide more comprehensive insights into their importance than examining hatching success alone.

Finally, habitat attributes that influence resource availability could force adults to take more frequent or longer recesses from the nest to forage. For example, depleted fisheries and invertebrate prey are known to influence the foraging behaviour and success of some seabirds (Kitaysky *et al*., 2000; Furness, 2007) and increased competition for fish prey can force birds to travel longer distances to forage (Lewis *et al*., 2001). However, most studies consider parental foraging in relation to provisioning young, and not incubation patterns. Future studies designed explicitly to examine habitat conditions in relation to incubation behaviour, particularly in uniparental incubators where adults must balance the competing demands of attending to their eggs and maintaining their own body condition, will prove useful to land managers.

VII. FUTURE RESEARCH PRIORITIES

As we begin to explore the role incubation temperatures play in shaping avian offspring phenotype, the diversity of research opportunities requires prioritization. Perhaps most pertinent is to determine how widespread are the effects of incubation temperature on offspring phenotype in birds and whether embryos of some species are more sensitive to changes in incubation temperature than others. Comparisons among cavity- and open-nesting species may be particularly revealing in this regard. Similarly, we know nothing about the long-term or latent effects of incubation temperatures on avian phenotypes. In wood ducks, some performance deficits remain and can even be exacerbated 20 days post hatching (DuRant *et al*., 2012*a*), but we do not know whether these or other effects persist and ultimately affect fitness. Likewise, it is possible that the influence of incubation on certain traits may not be evident until later in ontogeny. For example, age at sexual maturity, clutch sizes, sperm viability, plumage colouration, flight performance, song, and incubation behaviour could all be traits influenced by early developmental experiences. However, before investigating temperature effects in any species, a better understanding of the natural variation in incubation temperature for that species is required to ensure that temperature manipulations are ecologically relevant.

Researchers should also identify the mechanisms by which temperature effects are mediated in birds. Several possible mechanistic pathways warrant attention. Does temperature alter gene expression, having lasting impacts on development? For example, there is evidence that gene expression in reptiles may be the underlying mechanism for temperature-dependent sex determination (Lance, 2008). Does temperature affect embryonic developmental rates directly or through the production of hormones important in orchestrating developmental processes? Several studies have demonstrated that incubation temperature can influence both developmental rates and embryonic bioenergetics (Booth, 1987; Olson, Vleck & Vleck, 2006; DuRant *et al*., 2011). Longer incubation periods result in embryos expending more energy during development and converting more yolk to hatchling tissue (Eiby & Booth, 2009) leaving fewer remaining energy reserves in hatchlings; these effects could have implications for post-hatching phenotypic traits of young birds.

Lastly, birds produce offspring that span the altricial-precocial spectrum, and the effect of incubation temperature on offspring phenotype is expected to differ for birds at opposing ends of this spectrum. Precocial hatchlings undergo most of their development in ovo whereas altricial birds continue to develop rapidly after hatching. As a result, nest temperatures experienced during the post-hatching period may also shape the phenotype of altricial young. Understanding how incubation conditions influence early avian development across the altricial-precocial spectrum will ultimately provide novel insights into the evolution of diverse avian life histories and reproductive ecologies.

VIII. CONCLUSIONS

(1) Discovering the importance of incubation temperature on phenotypic development in wild birds has many exciting implications for avian biology. Current research demonstrates that incubation temperature can influence traits ranging from hatchling size and composition to juvenile locomotor performance weeks post hatching.

(2) Many factors contribute to nest temperatures, but how these factors relate to changes in avian phenotypic expression is unknown. Yet this natural variation in incubation temperature in wild populations of birds has many implications for avian development, phenotype, and ultimately fitness.

(3) Given the wide array of environmental variables that can influence the temperatures avian embryos experience, the effects of incubation temperature on avian phenotype certainly have conservation implications. Understanding how disturbance, contaminants, and changing climates affect the phenotype of avian offspring and not just hatching success will allow more successful mitigation of these perturbations.

(4) Much more research is needed before we can understand the relative roles of incubation temperature as a selective pressure on avian reproduction and its

importance in avian life histories, ecology and evolution, and conservation. Several key areas to investigate include: (*i*) the prevalence of incubation temperature-induced phenotypes across bird species; (*ii*) the persistence and latent emergence of temperature-induced phenotypes; and (*iii*) the underlying mechanisms behind the effects of incubation temperature on avian phenotype.

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