

HATCHING ASYNCHRONY OCCURS AS A BYPRODUCT OF MAINTAINING EGG
VIABILITY IN THE FLORIDA SCRUB-JAY

by

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ABSTRACT

For many organisms, embryonic development begins directly after an egg (ovum) has been fertilized by sperm; however, some organisms delay the onset of embryonic development until conditions are favorable for raising young. This delayed onset of development could occur by delaying implantation of fertilized ovum on the uterine wall, as seen in many mammals. Birds delay embryonic development by laying a set of fertilized ova over a period of consecutive days. These fertilized ova are protected from the ambient environment by an exterior shell, and it is in this shell outside of the female's body that embryonic development occurs, but only when females initiate incubation. The number of fertilized ova (eggs) that can be laid by a single female in a single clutch varies among and within bird species, and understanding this variation remains a vital, unanswered question in ornithology. A latitudinal gradient in clutch size is widely recognized, but the reason for this pattern is unclear. Some birds lay relatively large clutches over many days, thus we should expect that eggs could withstand fairly long exposure to ambient temperature and remain viable. However, recent evidence suggests that egg viability declines with increased exposure to ambient temperatures. The egg viability hypothesis predicts that eggs will fail to hatch if exposed to warm ambient temperatures for prolonged periods.

I conducted a natural experiment to determine whether egg viability can explain site-specific variation in hatching failure. Hatching failure is higher in a suburban population of Florida Scrub-Jays than it is in a wildland population, possibly because suburban scrub-jays lay larger clutches. Scrub-jays, like many bird species, lay one egg per day and begin incubation with the last-laid egg, thus first-laid eggs in the larger suburban clutches should be exposed to the warm ambient temperatures of sub-tropical Florida longer than first-laid eggs in the smaller

clutches typical of the wildland population. As predicted, I found hatching failure is higher in first-laid eggs in the suburbs, and these eggs experience increased exposure to warm ambient temperatures. At both sites, females appear to begin incubation earlier in the laying period as ambient temperatures increase seasonally, possibly to minimize exposure to warm ambient temperatures and minimize hatching failure in first-laid eggs. However, early onset of incubation causes eggs to hatch asynchronously (> 24 hours between the first and last-hatched egg), and hatching asynchrony increases within-brood size-asymmetries, which leads to an increased frequency of brood reduction (the nonrandom loss of last-hatched young because of starvation). Thus, a tradeoff may exist between beginning incubation earlier in the laying period to minimize hatching failure in first-laid eggs and delaying the onset of incubation to minimize hatching asynchrony and brood reduction. This tradeoff can have profound effects on avian clutch sizes, and may potentially explain the widely known negative relationship between latitude and clutch size.

I dedicate my thesis to Dr. Glen Everett Woolfenden (1930-2007), a wonderful mentor who is responsible for most of the extensive research on Florida Scrub-Jays over the last 40 years. It was Glen's knowledge of the scientific literature and his interest in avian natural history that allowed him to recognize the unique breeding system of the Florida Scrub-Jay when he visited Archbold Biological Station in the 1960s. These visits sparked the beginning of a long-term research project that helped introduce cooperative breeding to the ornithological world and has provided countless papers on the demographics of the cooperatively breeding Florida Scrub-Jay. Glen was passionate about ornithology, but his contribution to ornithology extended well beyond his work on the Florida Scrub-Jay. Glen's greatest passion was teaching young biologists; he always was willing to drop anything he was doing to mentor a student, namely me and my wife. Glen taught me how to be a professional ornithologist, and, more importantly, how to be a professional human being. I only hope that I will remember how important it was for me, as a student, to have a mentor like Glen, so that someday I might be at least half the teacher that Glen was for me.

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INTRODUCTION

Evolution should shape reproductive ‘decisions’ so that behaviors that increase the likelihood that offspring will survive to breeding age and successfully reproduce are favored and eventually propagate through populations and/or species. ‘Decisions’ refers to selected reproductive responses to specific environmental factors or cues that indicate the suitability of conditions for breeding. For example, an increase in photoperiod may provide a reliable cue that soon conditions will be favorable for breeding (i.e., warmer temperatures, more food). Individuals that respond to this cue by breeding at the appropriate time produce more offspring, and eventually the physiological response to that particular environmental change increases in frequency within a population. Similarly, animals can respond to environmental cues such as rainfall or temperature, which may be more variable than photoperiod. However, evolution will favor only those ‘behavioral decisions’ that more accurately predict optimal future conditions for raising young. Reproductive ‘decisions’ do not include behaviors that change based on prior experience, even though plasticity in behavioral responses might constitute an evolutionary ‘decision’. Possibly the most important reproductive decisions made by all organisms are 1) when to begin breeding and 2) how much effort should go into each reproductive bout.

Birds lay a set of fertilized ova (eggs) over consecutive days and delay the onset of embryonic development until they begin incubation, which usually occurs with the ultimate (last-laid) egg (Hebert 2002). Because birds typically lay one egg per day, beginning incubation prior to clutch completion initiates embryonic development in first-laid eggs before last-laid eggs are present. Because development time typically is stable for all eggs within a clutch, first-laid eggs will hatch before last-laid eggs. Incubation onset appears to influence variations in hatching synchrony (time between first and last-hatched egg). Females beginning incubation at or before

the penultimate (second to last-laid) egg should have nests that hatch asynchronously (> 24 hours between first and last-hatched egg). Hatching asynchrony produces competitive size asymmetries between nestlings within a nest because nestlings from early-laid eggs are fed before their younger siblings hatch. These competitive size asymmetries result in brood reduction or the nonrandom death of late-hatched young because of starvation when food is limiting (Lack 1966). Many hypotheses have been proposed to explain patterns of hatching asynchrony and brood reduction (Stoleson and Beissinger 1997). The brood reduction hypothesis suggests that females begin incubation early in the laying period as an adaptive strategy to promote rapid reduction of last-hatched young when nestling food is unpredictable (Lack 1966). Thus, female birds should produce the maximum number of offspring (eggs) they can raise in optimal conditions, begin incubation prior to the last-laid egg to create competitive size asymmetries within nests, and reduce the brood to a manageable size when food is limiting. Therefore, females may begin incubation earlier in the laying period as an evolutionary ‘decision’ to match brood sizes to local food resources.

An alternative explanation recently has been proposed, which suggests that declining egg viability, and not nestling food availability, may explain why females begin incubation prior to the last-laid egg. According to the egg viability hypothesis, hatching asynchrony may occur when females begin incubation early in the laying sequence to minimize the negative effect of prolonged exposure of eggs to deleterious temperatures. The egg viability hypothesis states that prolonged exposure to temperatures between physiological zero (24° to 27° C) and normal incubation temperatures (34° to 36° C) increases hatching failure (Arnold 1987; Stoleson and Beissinger 1997). Physiological zero is the temperature above which development is initiated in the embryo (Cook et al. 2003). Therefore, prolonged exposure to temperatures high enough to

promote development but below temperatures necessary for normal development should lead to abnormal development or mortality (Lundy 1969; Hebert 2002). If hatching failure increases with increased egg exposure to ambient temperatures, adults may be able to minimize hatching failure in first-laid eggs by beginning incubation earlier in the laying period when laying large clutches. However, early incubation onset incurs a cost of increased hatching asynchrony and brood reduction. According to the egg viability hypothesis, the timing of the onset of incubation may be an evolutionary ‘decision’ that balances the number of eggs that hatch successfully against the number of nestlings lost to brood reduction as a result of hatching asynchrony.

I studied *a priori* predictions of the egg viability hypothesis to determine whether Florida Scrub-Jays vary incubation onset to 1) minimize hatching failure in first-laid eggs or 2) minimize hatching asynchrony and brood reduction or both. I examined site-specific differences in incubation onset between a suburban and wildland population of Florida Scrub-Jays. Suburban scrub-jays begin breeding earlier and lay larger clutches than wildland jays. Scrub-jays lay one egg per day and begin incubation at clutch completion, thus hatching failure should be higher in first-laid eggs in the suburbs because of increased exposure in larger clutch sizes. Because scrub-jays breed from late February to early June, a substantial warming occurs from the beginning to the end of the breeding season. If incubation onset is regulated to reduce exposure of eggs to warm ambient temperatures, scrub-jays in both sites should begin incubation earlier in the laying sequence as ambient temperatures increase seasonally to minimize hatching failure in first-laid eggs. This early incubation onset with progressing season should translate into a seasonal increase in hatching asynchrony and brood reduction in both sites. Therefore, I predicted: 1) hatching failure will be higher in the suburbs than in the wildlands, 2) scrub-jays in both sites will begin incubation earlier in the laying sequence as ambient temperatures increase seasonally

to reduce exposure of clutches and minimize hatching failure in first-laid eggs, 3) earlier onset of incubation will result in increased hatching asynchrony and increased brood reduction, and 4) hatching asynchrony and brood reduction will increase as the season progresses because of seasonal changes in incubation behavior.

CHAPTER ONE: SITE-SPECIFIC DIFFERENCES IN HATCHING FAILURE BETWEEN A WILDLAND AND SUBURBAN POPULATION OF FLORIDA SCRUB-JAYS¹

Abstract

Hatching failure occurs in approximately 10% of all avian eggs. This reduction in viable offspring can have significant fitness consequences for breeding parents. Therefore, it is important to understand what factors influence hatching failure in natural populations. We analyzed differences in the rate of hatching failure between a suburban and wildland population of Florida Scrub-Jays using reproductive data collected from 1993-2007. We found that hatching failure was higher in the suburbs than in the wildlands. From 2003-2007, we performed two alternative experiments to examine whether increased hatching failure in the suburbs resulted from 1) increased length of off-bouts during incubation (predation risk hypothesis, 2003-2004) or 2) increased exposure to ambient temperature during laying (egg viability hypothesis, 2005-2007). Although suburban jays appeared to alter nest attentiveness strategies with increased perception of predation risk, nest attentiveness during the incubation period did not explain site-specific differences in hatching failure. Alternatively, hatching failure increased with increasing exposure of eggs to ambient conditions prior to incubation. First-laid eggs in the suburbs had the greatest pre-incubation exposure and the greatest rate of hatching failure, consistent with the egg viability hypothesis. Urbanization influences hatching failure through a series of complex interactions. Access to predictable food sources advances mean laying date in suburban jays,

¹ This chapter will be submitted as a co-authored manuscript. I will be the primary author and I will include the following co-authors, respectively; Sonya C. LeClair, Glen E. Woolfenden, and Reed Bowman

leading to larger median clutch sizes. Because scrub-jays begin incubation with the ultimate egg, first-laid eggs in the suburbs are exposed to ambient temperatures for longer periods, thus reducing their viability.

Introduction

Hatching failure is defined as an egg that is still in the nest at the end of the incubation period but fails to hatch. Hatching failure may be influenced by a variety of ecological, geographical and social factors (Koenig 1982). Among species, hatching failure tends to be slightly higher for cooperative breeders with shared incubation and slightly lower for uniparental incubators, monogamous pairs, birds with all-purpose territories and monogamous pairs with non-breeding helpers (Koenig 1982; Woolfenden and Fitzpatrick 1984; Innes and Johnston 1996). Hatching failure also tends to be higher for cavity nesting species than for open-cup nesters (Koenig 1982). Scrub-jays appear to conform to these among species patterns of hatching failure because hatching failure explains a small proportion of nest failures in the Florida Scrub-Jay (Bowman and Woolfenden 2001), a cooperative breeder consisting of a monogamous breeding pair with non-breeding helpers that lays its eggs in open-cup nests. Within species, a variety of factors may contribute to variation in hatching failure among populations, including inbreeding depression (van Noordwijk and Scharloo 1981; Bensch et al. 1994), ambient and nest temperatures (Taylor 1949; Lundy 1969), increased population density (Koenig 1982), clutch size (Reid et al. 2000; Wiebe and Martin 2000; Erikstad and Tveraa 1995), timing of breeding (Harmeson 1974; Hipfner et al. 2004), female condition (White 1991; Saino et al. 2002), and predation risk (Conway and Martin 2000).

Inbreeding tends to promote homozygosity, and it can increase the incidence of recessive lethal alleles (Koenig 1982; van Noordwijk and Scharloo 1981). In a population of the Great Tit (*Parus major*), the number of eggs that failed to hatch and the proportion of clutches that experienced hatching failure increased with the degree of inbreeding (van Noordwijk and Scharloo 1981), possibly because of an increase in embryo death because of an increase in homozygous alleles that are recessive and deleterious. Furthermore, lifetime reproductive success was lower in inbred female Song Sparrows (*Melospiza melodia*), largely as a result of increased hatching failure (Keller 1998). Hatching failure also was positively correlated with the degree of genetic similarity among mates in the Great Reed Warbler (*Acrocephalus arundinaceus*) (Bensch et al. 1994).

Ambient temperature can affect embryo development and potentially hatching failure. Normal embryonic development occurs between 34° and 36° C, and eggs are sensitive to temperature fluctuations outside of this range, especially after incubation has been initiated. Contact with the female's brood patch, as well as her behavior at the nest relative to both ambient and nest temperatures, helps regulate egg temperature (Drent 1975; Wilson and Verbeek 1995). Consequently, long periods of egg neglect may reduce the regulation of egg temperature relative to ambient temperature, thus increasing hatching failure. Embryos are particularly sensitive to overheating (Lundy 1969), and exposure to temperatures greater than 38° C while the female is off the nest can increase embryo mortality. In urban and suburban areas, where temperatures typically are warmer than nearby rural areas (Fan and Sailor 2005), periods of egg neglect may increase the potential for hatching failure because of this increased chance of exposure to higher ambient temperatures.

The presence of non-breeding helpers in cooperative breeding species may contribute to lower rates of hatching failure by decreasing the amount of time a female must spend defending the territory, thereby allowing her to remain on the nest (Woolfenden and Fitzpatrick 1984). This hypothesis parallels a prediction that increased population density contributes to greater egg neglect through increased territorial disputes (Koenig 1982). Suburban jays tend to have fewer non-breeding helpers than wildland jays (Shawkey et al. 2004), and suburban scrub-jay territories are smaller with higher densities of territories, possibly leading to more territorial interactions between breeding adults in the suburbs than in the wildlands (Fleischer et al. 2003). Periods of neglect may leave eggs exposed to temperatures outside the range of ideal temperatures for development (e.g., normal incubation temperatures; 34° to 36° C), leading to developmental abnormalities and embryo death (Lundy 1969).

Hatching failure also may be related to clutch size. First, a female may be limited in her ability to produce several high-quality eggs (Potti and Merino 1996). In large clutches, last-laid eggs may be smaller, and small eggs may contain insufficient reserves for normal embryonic development, or they may be more vulnerable to fluctuations in nest temperature (Lundy 1969; Potti and Merino 1996). Therefore, smaller, last-laid eggs may be more likely to experience hatching failure. Second, female passerines may not be able to incubate large clutches effectively. Large clutches may become warmer or lose more water if females cannot adequately regulate the nest microclimate, and these changes may increase the potential for hatching failure (Reid et al. 2000). Finally, many species begin incubation on the day the penultimate or ultimate egg is laid. First-laid eggs in large clutches may be exposed to ambient temperatures for a longer period of time prior to the onset of incubation. According to the egg viability hypothesis, prolonged exposure to warm ambient temperatures can lead to developmental abnormalities or

embryo death, resulting in increased hatching failure (Lundy 1969; Hebert 2002; Arnold et al. 1987; Stoleson and Beissinger 1997).

Timing of breeding is another important factor in successful reproduction. First, females nesting early in the season may encounter a food shortage because food availability often is most scarce at the beginning of the breeding season (Perrins 1970). Therefore, early-nesting females may increase the duration of their off-bouts to forage (Harmeson 1974). Conversely, female condition at the end of the breeding season may be poor relative to early nesters, thus late-nesting females may need to increase the frequency or duration of off-bouts to meet their foraging needs (Hipfner et al. 2004). In each case, longer off-bouts increase the time eggs may be exposed to temperatures outside normal incubation temperatures, leading to increased embryo mortality (Lundy 1969; Drent 1975).

Predation risk also influences incubation behavior, which may increase the possibility of hatching failure. Increased activity of adults at the nest can lead to an increased risk of nest predation (Skutch 1949). Thus, birds should alter their incubation behavior to decrease activity at the nest in areas of high nest predation. Previous studies have shown that males may decrease incubation feedings and females may take fewer, but longer off-bouts from the nest where they perceive an increased risk of nest predation (Conway and Martin 2000; Fontaine and Martin 2006). Although this strategy decreases the risk of nest predation, it may increase the frequency of partial or whole clutch loss through hatching failure since unattended eggs may be exposed to temperatures outside the range of normal incubation temperatures. As a result, a tradeoff may exist in areas with high nest predation between increasing nest activity to maintain endogenous reserves for the incubating parent(s) and minimizing nest activity to decrease the risk of nest predation.

Long-term research on the demography of the Florida Scrub-Jay (*Aphelocoma coerulescens*) in both wildland (Woolfenden and Fitzpatrick 1984, 1996) and suburban habitats (Bowman 1998; Bowman and Woolfenden 2001) suggest that rates of whole hatching failure are significantly higher in the suburban population than in the nearby wildland population, but the reason for this pattern is unclear (Bowman and Woolfenden 2001). Inbreeding is rare, thus it appears that inbreeding should not explain hatching failure differences between the two sites (Woolfenden and Fitzpatrick 1996). Female condition also appears to be similar between sites (Schoech and Bowman 2003), thus female condition likely does not explain differences in hatching failure between sites. However, other differences exist that could influence hatching failure. Scrub-jays in the suburbs tend to have fewer non-breeding helpers, if they have any helpers at all (Shawkey et al. 2004). This absence of non-breeding helpers could lead to higher hatching failure in the suburbs because females need to leave the nest to help defend the territory against intruding jays. Suburban scrub-jays also lay larger clutches than wildland jays (Bowman et al. 1998); the median clutch size is 4 eggs in the suburbs and 3 eggs in the wildlands. Larger clutch size in the suburbs may increase hatching failure by increasing exposure of eggs to ambient temperatures. Suburban jays also initiate breeding earlier and the breeding season lasts longer in the suburban site; therefore, suburban jays invest more in reproduction annually (Bowman and Woolfenden 2001). This site difference in breeding season length could lead to suburban jays either breeding before food supplies are adequate or breeding later and in poorer condition.

Historical Data

We examined the effects of site (suburban versus wildland), year, presence of non-breeding helpers, clutch size and clutch initiation date (timing of breeding) on hatching failure rates within and between these two populations of Florida Scrub-Jays using data collected from 1993-2007. As expected hatching failure was higher in the suburbs than in the wildlands. Nest attentiveness may be an important factor explaining site-specific differences in hatching failure. Avian eggs are particularly sensitive to fluctuations in ambient temperature (Lundy 1969), and females regulate exposure of eggs to ambient temperature by altering nest attentiveness strategies, which may differ by period of the nesting cycle (laying or incubation). During the laying period nest attentiveness is low because females are away from the nest accumulating endogenous reserves for the upcoming incubation period. Thus, egg temperatures fluctuate with variation in ambient temperatures. Alternatively, nest attentiveness is high during the incubation period because eggs need to be maintained constantly within a narrow temperature range (34°-36° C). Thus, egg temperatures vary only for short intervals as females leave the nest to forage. Although nest attentiveness strategies may affect egg exposure differently based on period of the nesting cycle, low nest attentiveness (high egg neglect) in either period can cause increased hatching failure by increasing egg exposure to deleterious temperature ranges (e.g., long exposure periods, large temperature fluctuations, high ambient temperatures, etc.). Therefore, we tested the predictions of two alternative hypotheses, 1) the predation risk hypothesis and 2) the egg viability hypothesis, to determine whether period-specific differences in nest attentiveness could explain site-specific differences in hatching failure.

Predation Risk Hypothesis

Nest predation may vary across an urban-rural gradient (Haskell et al. 2001). Overall rates of nest predation are similar between our suburban and wildland sites, but both sites differ according to when nest predation is most prevalent (Bowman and Woolfenden 2001). Nests in the wildlands are more likely to fail during the incubation period, and nests in the suburbs are more likely to fail in the nestling period. This difference in nest failure may occur because of differences in the abundances or types of nest predators in each site, or it may occur because of period-specific differences in nest activity. Within North America, populations of American Crows typically increase with increasing urbanization (Haskell et al. 2001). Previous research suggests that birds are the main nest predators in our suburban site (Thorington and Bowman 2003). Because many bird species are visual predators, suburban scrub-jays may perceive an increased risk of nest predation, which should cause females to take fewer, longer off-bouts to decrease nest activity and minimize the number of nests lost to avian nest predators.

Although predation risk can be affected by a number of interacting factors, human activity may be perceived by birds in a manner analogous to increased predation risk (Frid and Dill 2002). Many animals change their behavior in response to humans, and this change can lead to lower reproductive success if eggs or young are neglected for long periods of time (Frid and Dill 2002). If perception of predation risk increases in urbanized areas as a result of human activity, females may alter their incubation behavior by taking fewer, longer off-bouts to minimize nest activity in suburban areas (Conway and Martin 2000). Longer off-bouts during incubation may increase the potential for hatching failure by increasing the amount of time eggs are exposed to temperatures outside the range of normal incubation temperatures.

In 2003 and 2004, we tested predictions of the predation risk hypothesis by performing an experiment to determine whether female scrub-jays altered their nest attentiveness strategies to increased human activity, which was used as a proxy for increased perception of predation risk, and whether differences in nest attentiveness during the incubation period explained site-specific differences in hatching failure. We predicted that 1) suburban females would take fewer, longer off-bouts and males would have lower rates of incubation feedings because of the increased perception of predation risk, and 2) these longer off-bouts would cause increased hatching failure rates in the suburbs.

Egg Viability Hypothesis

Alternatively, we tested predictions of the egg viability hypothesis from 2005 to 2007 to determine whether nest attentiveness strategies during the laying period could explain site-specific differences in hatching failure. According to the egg viability hypothesis, longer exposure to warmer ambient temperatures increases hatching failure (Arnold et al. 1987; Stoleson and Beissinger 1997). Previous research shows that urban and suburban environments experience warmer ambient temperatures than nearby rural and wildland areas because of the urban heat island effect (Fan and Sailor 2005). We also know that suburban jays tend to lay larger clutches than wildland jays (Bowman et al. 1998). Scrub-jays typically lay one egg per day and begin incubation at clutch completion (Woolfenden and Fitzpatrick 1996). Since suburban clutches are larger and exposed to a warmer environment, first-laid eggs in the suburbs should experience longer exposure to warmer ambient temperatures than first-laid eggs in the wildlands.

Beissinger et al. (2005) showed that the minimum exposure to ambient conditions necessary for eggs to experience decreased viability is three days in other passerines. Because scrub-jays lay one egg per day, and suburban jays lay 4-egg clutches, the first-laid egg from most suburban nests is at or above this 3-day threshold; in wildlands, where median clutch size is three, most first-laid eggs fall below this threshold. Thus, site-specific differences in clutch size and ambient temperature are consistent with *a priori* predictions of increased hatching failure in the suburbs. Therefore, we predicted that hatching failure will be higher in first-laid eggs in the suburbs because of constraints imposed on egg viability. We also predicted that hatching failure will increase as the season advances because ambient temperatures increase seasonally, and the temperature difference between the beginning and end of the long scrub-jay breeding season (late February to early June) is much greater than the 1-2° C temperature difference between sites.

Methods

Study Organism and Study Sites

The Florida Scrub-Jay is a cooperative breeder, consisting of one monogamous breeding pair and zero to six non-breeding helpers (Woolfenden and Fitzpatrick 1996). Scrub-jays are long-lived species that set up and defend permanent, year-round territories. Florida Scrub-Jays are dependent on the fire-dominated xeric oak scrub habitat that occurs mainly on the Lake Wales Ridge in central Florida. Over 85% of Florida's original oak scrub habitat has been destroyed through conversion to urban or suburban areas and agriculture (Peroni and Abrahamson 1985). This habitat destruction has been instrumental in the loss of over 80% of the Florida Scrub-Jay population (Stith et al. 1996).

We examined differences in hatching failure rates between two populations of Florida Scrub-Jays near Lake Placid, Highlands County, Florida. One of the populations occurs in Placid Lakes Estates (27°15'N, 81°25'W), a 1,500 ha residential housing subdivision that has undergone substantial development since the mid-1960s. Within this residential development, scrub-jay habitat occurs as fragmented patches of sub-optimal (overgrown) oak scrub because of a lack of periodic fires. This suburban population has been studied extensively over the past 16 years (Bowman 1998; Bowman and Woolfenden 2001). The second population occurs at Archbold Biological Station, a 2,000 ha natural preserve that is 8 km south of the suburban site. Here, the scrub is fire-maintained and occurs in large contiguous blocks. This wildland population has been studied extensively over the past 39 years (Woolfenden and Fitzpatrick 1984).

Historical Data

Extensive demographic data were available from each population as a result of long-term on-going studies at each site. From these demographic data we constructed an historical database to examine differences and potential causes of hatching failure between these two populations (suburban vs. wildland). Although studies began in the wildland site in 1969, the first year for which complete data were available from the suburban site was 1993. Therefore, we only used years in which concurrent data were available for both sites (1993-2007).

We used territory-specific data collected between 1993 and 2007 to assess the effects of site (suburban versus wildland), year, presence of helpers, clutch size, and clutch initiation date (timing of breeding) on the occurrence of hatching failure. We defined hatching failure in two ways, whole hatching failure and partial hatching failure. Whole hatching failure (WHF) was defined as all eggs in a clutch surviving the incubation period (~18 days) but subsequently

failing to hatch. Partial hatching failure (PHF) was defined as an egg that survived incubation but failed to hatch in a nest where at least one egg hatched. We included partial hatching failure as a binary variable for entire clutches because (1) it is difficult to compare proportions of different-sized clutches (e.g., one egg lost from a 2-egg clutch (0.5) vs. a 5-egg clutch (0.2)) and (2) individual eggs within a clutch cannot be considered independent samples.

We considered an egg to have failed to hatch if the egg survived incubation and was observed in the nest at least two days after the first egg hatched. Eggs seen on the day the first egg hatched, but missing prior to hatch completion (e.g., two days post-hatching) may have hatched then disappeared. Therefore, these eggs were assumed to have hatched but then failed because of partial brood loss rather than hatching failure, which provided a conservative estimate of hatching failure and reduced the likelihood of biasing comparisons between sites.

Predation Risk Hypothesis

In 2003 and 2004, we performed an experiment to determine whether females altered nest attentiveness strategies during the incubation period because of human activity near the nest (perceived predation risk), and whether those changes in incubation behavior (i.e. less frequent, but longer off-bouts) were consistent with site-specific differences in hatching failure. Within the suburban site, we assigned nests to one of two treatments, normal visitation (“control suburban”) or increased visitation (“experimental suburban”). Within the wildland site, we assigned nests only to normal visitation (“control wildland”). Normal visitation consisted of following protocols of the on-going long-term demography project. We visited these “control” nests once during the laying period and once at clutch completion. Since scrub-jays lay a maximum of five eggs, our clutch completion visit occurred on the day the fifth egg should be laid. We also visited all nests

at the middle of the incubation period (day 9), and the day before the first egg was supposed to hatch (day 18) and then each day until all eggs had hatched. We visited experimental nests on the same schedule; however, these nests also were visited twice daily (between 1000-1200 and 1300-1600) on each of three consecutive days (days 4, 5 and 6) before we measured nest microclimate and female incubation behavior.

We placed a thermocouple (HOBO H8 Pro Series, Onset Computer Corporation, Bourne, Massachusetts, USA) to record nest microclimate and a time-lapse remote video camera (Sandpiper Technologies Basic Sentinel System, Sandpiper Technologies, Inc., Manteca, California, USA) to record parental activity at each nest seven days after clutch completion. Both were placed at the nests between 1000 and 1400, when diurnal predator activity is at its lowest. The thermocouples have two temperature sensors, a thermocouple unit plus one wire sensor. The wire sensor was woven into the nest lining as close to the eggs as possible to record nest temperature, and the thermocouple unit was placed at least one meter below the nest, protected by shade to record ambient temperature. The thermocouple unit and wires were camouflaged to minimize detection by the jays. Thermocouples recorded temperature data for 24 hours. The video cameras were placed from within 1 to 3m of the nest, depending on the thickness of the vegetation. The camera recorded monochrome images during the day and night. Female activity was detected at some nests at night via infrared lighting when the camera was placed within 1m of the nest. Each camera recorded nest attentiveness for 24 hours. Each camera was placed at a nest with a thermocouple, but camera equipment was limited, thus not all nests that had thermocouples had cameras.

We began recording nest attentiveness approximately 15 minutes after the videotape began to ensure the female had resumed her normal activities. We recorded off-bouts in minutes

and we recorded feedings as the number of feedings between sunrise and sunset. We considered a female “on” the nest if she was settled on the eggs or shading them. We considered a female “off” the nest if she was on the rim of the nest and clearly not attempting to incubate or shade the eggs, or off the nest and out of sight. Incubation feeding occurred any time the male arrived at the nest with food and offered it to the female.

Egg Viability Hypothesis

From 2005 to 2007, we performed a natural experiment to determine whether egg exposure during the laying period explained site-specific differences in hatching failure. All nests were found prior to egg-laying and followed until either the nest failed or some young successfully fledged. Each egg was weighed within 24 hours of being laid to determine initial egg weight, and laying order was identified by marking eggs with an indelible marker. All eggs within a clutch were candled three days after clutch completion to determine the amount of embryonic development (Lokemoen and Koford 1996). We chose three days after clutch completion because we found that three days after the last-laid egg is the last day all eggs in a clutch showed no embryonic development at the beginning of the season when scrub-jays begin incubation at clutch completion (Aldredge, unpub. data). We photographed each clutch to determine the size and volume of each egg (Bridge et al. 2007) and weighed all eggs. Sixteen days after clutch completion we again weighed all eggs to determine total egg water loss. We recorded hatching by visiting nests at least once daily from seventeen days after clutch completion until all eggs were hatched. Non-viable eggs were recorded as eggs that failed to hatch a minimum of 48 hours after the last-hatched egg. Eggs that failed to hatch were opened

and egg contents were examined to determine whether development had occurred during the incubation period, thus indicating the egg had been fertilized.

In 2006 and 2007, we placed thermoprobes (HOBO H12-001, Onset Computer Corporation, Bourne, Massachusetts, USA) in a subset of nests on the day the first egg was laid. Thermoprobes were gently pushed through the nest lining at the bottom of the nest until the sensor was just exposed at the interface of the eggs and lining. Thermoprobes collected temperature data every minute from laying of the first egg until three days after clutch completion. The onset of incubation was apparent because nest temperature closely tracked ambient temperature until the onset of incubation when nest temperature became stable at night between 31° and 36°C (Fig. 1). Artificial eggs, which could give a more accurate estimate of incubation temperature, were not used because scrub-jays reject artificial eggs (Fleischer and Woolfenden 2004).

HOBO thermocouples (HOBO H8 Pro Series, Onset Computer Corporation, Bourne, Massachusetts, USA) were placed within 2m of sites known to have been selected by jays for nesting (e.g., abandoned, failed and successful nests for each year) to quantify site-specific differences in ambient temperature at nest sites. Unlike thermocouples that were used at specific nests while studying the predation risk hypothesis, these thermocouples were placed simultaneously in each site throughout the entire season to quantify site-specific differences in ambient temperature. Thus, only the thermocouple unit was used. Thermocouple units were placed in three-sided housings approximately 2m above the ground to avoid ground irradiance from the white sand and each housing faced north to avoid direct sunlight. Ambient temperature was recorded every fifteen seconds and each thermocouple was moved to a new location every six days. Two thermocouples were used simultaneously in each site and their data were pooled to

avoid the confounding effect of variation in ambient temperature because of individual housing placement. Each year data collection began prior to initiation of the first clutch in late February and lasted until cessation of the laying period of the final clutch of the year in early June.

Statistical Analysis

Data were analyzed using SPSS 13.0. Parametric tests were used only when data fit basic statistical assumptions. All values are reported as means (± 1 SEM).

Historical Data

Scrub-jays rarely lay clutches smaller than two eggs or larger than five eggs, thus only clutches of two to five were included in our analyses. We included data for all nests where clutch initiation and hatch dates were known, and for nests where clutch initiation dates were unknown but hatch dates were known. Scrub-jays have an 18 day incubation period, so clutch initiation dates were back-calculated using the date the first egg hatched. We excluded data from nests that were found during the nestling period and nests that failed prior to hatching.

We modeled whole and partial hatching failure as a function of the main and additive effects of our five variables (site, year, presence of helpers, clutch size, and clutch initiation date) using multiple binary logistic regression. We used the maximum log-likelihood estimate from each of our 29 models to determine which models provided the most parsimonious explanation of the variation in whole and partial hatching failure using Akaike's Information Criterion (AIC) (Burnham and Anderson 2002). Models with a ΔAIC_i greater than 10 were excluded from further analysis because these models have essentially no support that the fitted model is the best model. Models with ΔAIC_i values between 2 and 7 have some support, and those with ΔAIC_i values below 2 have substantial support. Thus, only models with a ΔAIC_i less than 7 are reported. As

expected, site was highly significant in both analyses. Therefore, we split the data by site and reran a forward stepwise binary logistic regression using the best-fit model to determine which variables significantly explained the variation in hatching failure in each site.

Predation Risk Hypothesis

We recorded temperature continuously over 24 hours and calculated the mean, minimum and maximum ambient and nest temperatures. We used ANOVA to test for differences in temperature between sites and treatments. We used ANOVA to test for differences in nest attentiveness between treatments. We used binary logistic regression to examine the effects of temperature and nest attentiveness on hatching failure. Since whole hatching failure is rare in Florida Scrub-Jays we examined the effects of temperature and nest attentiveness only on partial hatching failure.

Egg Viability Hypothesis

A year effect existed when examining ambient temperature differences between sites. Mean ambient temperatures were higher in the suburbs than in the wildlands in 2005 and 2006, but mean temperatures were similar between sites in 2007. No year effect existed for minimum or maximum ambient temperatures. Minimum ambient temperature appears to be the most biologically relevant temperature parameter for testing egg viability hypothesis predictions because higher minimum ambient temperatures may increase exposure to temperatures above physiological zero. Because minimum ambient temperatures did not differ between years and site-specific differences in minimum ambient temperatures were below physiological zero, we pooled ambient temperature by year for further analysis.

Although clutch size in Florida Scrub-Jays ranges from 2-5 eggs, the median clutch sizes are four eggs in the suburbs and three eggs in the wildlands, thus only three and 4-egg clutches

were used in subsequent analyses. Forward stepwise binary logistic regression was used to determine which factors influence hatching failure. We examined site, clutch initiation date, number of laying days, clutch size, number of apparent incubation days (days between last laid egg and first hatched egg), pre-incubation and total egg water loss, and pre-incubation embryonic development as potential factors affecting egg viability.

Results

Historical Data

We examined hatching failure in 1908 nests in both sites; 1102 nests from the suburbs and 806 nests from the wildlands. Hatching failure appeared to be consistent with previous findings by Koenig (1982); hatching failure occurred in 14.0% of suburban eggs and 9.1% of wildland eggs. Four different models were chosen that best explained patterns of whole hatching failure, and all of these models contained more than 3 factors (Table 1). The cumulative weight of each factor suggests that clutch size (0.987) was the most important factor in explaining whole hatching failure, followed by site (0.914), year (0.756), clutch initiation date (0.738) and helpers (0.619). According to the best-fit model, whole hatching failure was significantly ($p < 0.05$) affected by site and clutch size; however, an interaction existed between site and clutch size. Whole hatching failure was higher in the suburbs (0.026) than in the wildlands (0.010) (Fisher's Exact, $p = 0.011$). Whole hatching failure also decreased with increasing clutch size in the suburbs, but whole hatching failure did not change relative to clutch size in the wildlands.

Partial hatching failure also appeared to be most affected by site and clutch size (Table 2). Site was included in all of the top-ranked models and appeared to best explain patterns of partial hatching failure, followed by clutch size, which occurred in seven of the top-ten ranked

models. Partial hatching failure was higher in the suburbs (0.338) than in the wildlands (0.240) (Fisher's Exact, $p < 0.001$; Fig. 2). Additionally, rates of partial hatching failure were much higher than rates of whole hatching failure in both sites. Partial hatching failure also increased with increasing clutch size in both sites ($X^2 = 13.349$, $df = 3$, $p = 0.004$; Fig. 2).

Predation Risk Hypothesis

We followed 35 nests during the predation risk experiment; 13 nests in the wildland treatment, 10 nests in the control suburban treatment, and 12 nests in the experimental suburban treatment. Mean ambient temperature was higher in the suburbs (for both control and experimental sites) than in the wildlands. This site-specific difference in ambient temperature appeared to be driven by higher minimum ambient temperature in the suburban site; minimum ambient temperature was approximately 5°C warmer in the suburbs than in the wildlands (Fig. 3). Maximum ambient temperature did not differ between treatments or years. Nest and ambient temperatures were highly correlated (Pearson, $p=0.001$); therefore, we used only ambient temperature data to test the effect of temperature on hatching failure (*see Discussion*). None of the temperature variables (mean, minimum or maximum ambient temperature) explained partial hatching failure patterns (Table 3).

Incubating females always remained on the nest throughout the night, except when disturbed by a potential predator. When examining the influence of human activity on the frequency of off-bouts, an interaction existed between treatment and year because of year differences in control suburban and wildland females (Fig. 4). In 2003, control suburban females took more off-bouts than wildland females, but in 2004, wildland females took more off-bouts than control suburban females. Overall, experimental suburban females took significantly fewer

off-bouts than either control suburban or wildland females (Duncan's post-hoc test) Therefore, increased human activity significantly decreased the frequency of off-bouts by incubating females.

Mean duration of off-bouts did not differ by treatment, but year was significant because the duration of off-bouts in experimental suburban and wildland treatments tended to be longer in 2003 than in 2004 (Fig. 4). Because the frequency of off-bouts differed with treatment, but the duration of those off-bouts did not differ, experimental suburban females spent a smaller proportion of their time off their nests than females in any other treatment. This difference was significant only between experimental and control suburban females (Duncan's post-hoc test). An interaction existed between treatment and year when examining minimum duration of off-bouts. Again, this interaction existed because of year differences in control suburban and wildland treatments. In 2003, the minimum duration of off-bouts was much greater in the wildland treatment than in the control suburban treatment, but in 2004, minimum duration of off-bouts was greater in control suburban than wildland treatments. Maximum duration of off-bouts did not differ between treatments or years.

Although females at experimental nests took fewer off-bouts and were off their nests for a shorter proportion of the day, experimental suburban nests did not experience less hatching failure than control suburban or wildland nests. Rates of hatching failure were highest in control (0.1270) and experimental suburban (0.0996) treatments and lowest in the wildland treatment (0.0537). Only the frequency of off-bouts was marginally significant in explaining partial hatching failure patterns (Table 3); nesting females that experienced partial hatching failure took fewer off-bouts than nesting females where all eggs within the nest hatched.

Experimental suburban males tended to have slightly higher rates of incubation feedings than control suburban or wildland males, but these differences were not significant. Feeding rates were negatively correlated with mean duration of off-bouts (Pearson, $p=0.011$), but feeding rates were not correlated with any other female behavior. No differences existed among incubation feeding rates between years, and feeding rates were not significant in explaining partial hatching failure patterns.

Egg Viability Hypothesis

Ambient temperatures were higher in the suburbs than in the wildlands ($F = 1.805$, $df = 23$, $p = 0.011$; Fig. 5) in the egg viability experiment (2005-2007), which parallels results of the predation risk experiment (2003-2004). These differences occurred in both the mean ($F = 4.608$, $df = 1$, $p = 0.032$) and minimum ($F = 13.368$, $df = 1$, $p < 0.001$) ambient temperatures, but maximum temperature did not differ between sites ($F = 1.385$, $df = 1$, $p = 0.240$). Consistent with previous findings, the site difference in mean temperature appeared to be driven by higher minimum ambient temperatures in the suburbs. Ambient temperature increased significantly with season ($F = 138.575$, $df = 4$, $p < 0.001$), with mean ambient temperature warming by more than 6°C over the five month breeding season. This seasonal change in ambient temperature was greater than the $1\text{-}2^{\circ}\text{C}$ temperature difference between sites.

We determined the frequency of hatching failure in 183 nests between 2005 and 2007; 82 nests in the suburbs and 101 nests in the wildlands. Hatching failure was affected by site and the number of apparent incubation days (Table 4). Hatching failure was higher in the suburbs than in the wildlands, and hatching failure increased as apparent incubation exceeded 18 days, the average length of the scrub-jay incubation period ($\chi^2 = 11.055$, $df = 2$, $p = 0.004$; Fig. 6). Within

clutches, hatching failure was greater in first-laid eggs in the suburbs than in the wildlands (Fisher's Exact $(1-\alpha)$; $p = 0.045$). No site differences existed between hatching failure in eggs 2, 3 and 4 in the laying sequence.

The effect of laying order on hatching failure approached significance in both sites ($X^2 = 7.014$, $df = 3$, $p = 0.071$; Fig. 7); however this trend was driven by higher rates of hatching failure in first-laid eggs in the suburbs relative to eggs laid later in the sequence ($X^2 = 8.207$, $df = 3$, $p = 0.042$). Laying order had no effect on hatching failure in the wildlands ($X^2 = 2.142$, $df = 3$, $p = 0.544$). Contrary to egg viability hypothesis predictions, hatching failure did not increase as seasonal ambient temperatures increased ($X^2 = 0.236$, $df = 3$, $p = 0.972$).

Embryonic development at day 3 post-clutch completion did not differ between sites ($X^2 = 2.335$, $df = 3$, $p = 0.506$). Within clutches, first-laid eggs exhibited greater embryonic development than later-laid eggs in both sites ($X^2 = 178.047$, $df = 9$, $p < 0.001$), and embryonic development decreased with laying order (Fig. 8), suggesting some development occurred in early-laid eggs prior to clutch completion. Embryonic development increased in first-laid eggs in both sites with advancing season ($X^2 = 16.279$, $df = 3$, $p = 0.001$).

The onset of incubation was determined for 44 nests during the 2006 and 2007 breeding seasons; 35 nests in the suburbs and 9 nests in the wildlands. Incubation onset was positively correlated with number of apparent incubation days (Spearman's rho = 0.418, $n = 33$, $p = 0.016$; Fig. 9). Scrub-jays initiating incubation at clutch completion had an apparent incubation period of 17.89 days; scrub-jays that began incubation prior to clutch completion had a shorter apparent incubation period as we defined it (see Methods), but eggs still hatched approximately 18 days after incubation onset. Furthermore, apparent incubation days decreased with advancing season ($F = 3.153$, $df = 3$, $p < 0.026$); the apparent incubation period lasted approximately 17.66 (\pm

0.177) days at the beginning of the season and declined to 17.27 (\pm 0.117) days by the end of the season. Onset of incubation also was negatively correlated with the amount of embryonic development in first-laid eggs (Spearman's $\rho = -0.338$, $n = 44$, $p = 0.025$); an earlier onset of incubation resulted in more embryonic development in first-laid eggs three days after the clutch was completed.

We used the relationship between incubation onset and apparent incubation days to calculate egg exposure for entire clutches, hereafter referred to as clutch exposure. Clutch exposure was calculated as the number of days between the first-laid egg (clutch initiation date) and the day females began incubation (using the correlation between incubation onset and apparent incubation days). Clutch exposure is a more appropriate measure of exposure to ambient conditions because this single parameter incorporates all of the variables affecting exposure of clutches, including clutch size, days skipped during the laying period, and onset of incubation. Hatching failure increased significantly as clutch exposure reached and exceeded 3 days ($X^2 = 6.135$, $df = 2$, $p = 0.047$; Fig. 10). Additionally, increased exposure to ambient temperatures was marginally significant in explaining hatching failure only in first-laid eggs in the suburbs ($F = 3.667$, $df = 1$, $p = 0.059$; Fig. 11).

Discussion

We have shown that rates of both whole and partial hatching failure are higher in a suburban population of Florida Scrub-Jays than in a wildland population. The reasons for higher rates of whole hatching failure still remain unclear. We know that inbreeding is low and female condition is similar in both of these populations, thus neither of these parameters should explain site-specific differences in hatching failure. Avian eggs are exposed to ambient temperatures

during two periods of the nesting cycle, laying and incubation, and previous research shows that avian eggs are particularly sensitive to fluctuations in ambient temperature (Lundy 1969). Females regulate egg exposure to ambient temperature by altering nest attentiveness strategies during laying and incubation. Our results suggest that nest attentiveness during the incubation period does not explain patterns of hatching failure in the Florida Scrub-Jay. However, nest attentiveness during the laying period does appear to explain site-specific differences in hatching failure.

Approximately half of the eggs that failed to hatch in our suburban site were first-laid eggs, and hatching failure was significantly different between sites only in first-laid eggs. Hatching failure increased as clutches reached or exceeded the 3-day threshold for maintaining egg viability. Thus, egg viability appears to explain higher hatching failure in first-laid eggs in our suburban population of Florida Scrub-Jays.

Historical Data

Whole hatching failure was rare in both sites, occurring in approximately 2.6% of suburban nests and 1.0% of wildland nests. These results are consistent with previous research, which suggested that higher rates of whole hatching failure in the suburbs might occur because the breeding population in the suburbs has a greater proportion of inexperienced breeders than the wildland population (Bowman and Woolfenden 2001). The best-fit model was the global model and all of the top-ranked models contained at least four of the five tested factors, indicating none of these factors gain overwhelming support in explaining patterns of whole hatching failure. Whole hatching failure occurred most frequently in the smallest clutches, often at the end of the breeding season, and was higher in pairs without helpers. This is consistent with

inexperienced pairs because they often lay small clutches, often lay later in the season than experienced pairs, and rarely have helpers.

Partial hatching failure was an order of magnitude higher than whole hatching failure in both sites, occurring in 33.8% of suburban nests and 24.0% of wildland nests. Because site occurred in all of the top-ranked models, site-specific differences should exist that explain patterns of partial hatching failure. Therefore, we performed two experiments to examine whether period-specific nest attentiveness strategies explain the site-specific difference in partial hatching failure. Partial hatching failure was higher in the suburbs than in the wildlands, and partial hatching failure increased with increasing clutch size. Because ambient temperatures are warmer in the suburbs than in the wildlands, and larger suburban clutch sizes should experience prolonged exposure to ambient temperatures, higher hatching failure in the suburbs closely follows predictions of the egg viability hypothesis. For each clutch size, hatching failure was higher in the warmer suburban site than in the cooler wildland site.

Ambient Temperature

As expected, ambient temperature was higher in the suburbs than in the wildlands in 2003 and 2004, but this temperature difference appears to be driven by higher minimum ambient temperature in the suburbs. This finding is consistent with predictions of the urban heat island effect (Fan and Sailor 2005). However, urban heat islands normally consist of a temperature difference of 1-2° C, and we found a temperature difference of approximately 5° C in the subset of nests used in the predation risk experiment. Since ambient temperature was taken only at treatment nests within each site, ambient temperature data were not always taken simultaneously

in each site. Thus, these results do not accurately represent site-specific differences in ambient temperature.

The correlation between ambient and nest temperatures in the predation risk experiment suggests that the wire sensor woven into the nest failed to adequately quantify nest microclimate during the incubation period. Since temperature data were taken when the female was incubating, ambient and nest temperatures during the night should be significantly different from each other. During incubation, nest temperatures should never fall below the temperature necessary for normal embryonic development (34° - 36° C). Because ambient and nest temperatures were highly correlated we excluded nest temperature data for the predation risk experiment. We found that ambient temperature during the incubation period had no effect on partial hatching failure.

Embryos are particularly sensitive to overheating (Lundy 1969), and we expected temperatures greater than 38° C to have the greatest effect on hatching failure during short exposure periods, such as off-bouts for feeding during incubation. Ambient temperature rarely reached or exceeded the upper critical temperature limit (38° - 40° C) for avian eggs; therefore, maximum ambient temperature did not affect partial hatching failure rates.

From 2005-07 ambient temperature also was higher in the suburbs than in the wildlands because of higher minimum ambient temperatures. Unlike the predation risk experiment, the egg viability experiment accurately quantified site-specific differences in ambient temperature and found a 1 - 2° C minimum ambient temperature difference between sites, as predicted by the urban heat island effect (Fan and Sailor 2005). Consistent with the predation risk hypothesis, ambient temperature did not affect partial hatching failure during the laying period.

Predation Risk Hypothesis

Nest attentiveness during the incubation period does not appear to influence partial hatching failure in the Florida Scrub-Jay. The marginally significant effect of fewer off-bouts increasing partial hatching failure rates suggests that females maintaining the nest microclimate at temperatures that promote normal embryonic development (e.g., normal incubation temperatures) experience increased hatching failure. Thus, this result appears to be spurious rather than biologically relevant. We believe that increased hatching failure with fewer off-bouts occurs as a consequence of suburban females both taking fewer off-bouts and having higher rates of hatching failure.

In the predation risk experiment, scrub-jays altered nest attentiveness strategies to increased human activity. For the purposes of this study, increased human activity was meant to simulate an increase in the perception of nest predation risk (Frid and Dill 2002). As perception of nest predation risk increased, scrub-jays appeared to decrease the frequency of off-bouts but did not change the length of these off-bouts. This finding is in contrast to those of Conway and Martin (2000), who noted that female passerines decreased the frequency but increased the duration of off-bouts with increased risk of nest predation, in an effort to balance maintaining endogenous female reserves during incubation and minimizing nest activity. Suburban jays might be able to decrease the length of off-bouts while also decreasing the frequency of off-bouts because these jays have access to *ad libitum* sources of high-quality anthropogenic food (Schoech and Bowman 2003). The strategy employed by females in the experimental suburban treatment - short, infrequent off-bouts - appears to be an ideal strategy to minimize nest predation and maximize time on the nest; however, this strategy may not be viable unless reliable and predictable food sources are available to the female, allowing her to meet her nutritional

demands in a minimum amount of time. Scrub-jays did not alter nest attentiveness strategies during incubation between control suburban and wildland treatments, which suggests that suburban females do not perceive an increased risk of nest predation. Although human activity is higher in the suburbs, humans tend not to persecute Florida Scrub-jays, and many residents provide food for these relatively tame birds. Many of the scrub-jays in our suburban site are tame enough to land on residents' heads and hands. Human residents also rarely venture into the dense patches of oak scrub in which jays nest, so relatively little human activity occurs near nest sites. Thus, suburban scrub-jays may have no reason to perceive increased human activity in the suburbs as an increase risk of nest predation.

Egg Viability Hypothesis

Contrary to the predation risk experiment, nest attentiveness during the laying period appeared to influence rates of partial hatching failure. As predicted by the egg viability hypothesis, hatching failure was higher in the suburbs, where females lay larger clutches that are exposed to warmer ambient temperatures. As ambient temperatures increased seasonally in both sites, scrub-jays reduced exposure of clutches, possibly to minimize rates of hatching failure in first-laid eggs. As expected, we found that hatching failure in both sites increased as clutches reached and exceeded 3 days of exposure. Therefore, scrub-jays should begin incubation prior to reaching this 3-day threshold for maintaining egg viability. Because scrub-jays lay one egg per day, females should initiate incubation upon laying the third egg (e.g., between day 2 and day 3). Suburban scrub-jays began incubation after 2.40 (\pm 0.08) days of exposure, and wildland jays began incubation after 2.54 (\pm 0.09) days of exposure. Even though suburban clutches appeared to experience less exposure to ambient temperatures than wildland clutches, first-laid eggs that

failed to hatch in the suburbs experienced greater exposure to ambient temperatures than first-laid eggs that hatched successfully in the suburbs. Consistent with egg viability hypothesis predictions, exposure to ambient temperatures did not explain hatching failure in second, third, or fourth-laid eggs in the suburbs, which experienced less than 2 days of exposure to ambient temperatures.

The negative effect of ambient temperature on egg viability should be exacerbated as ambient temperature increases seasonally (Cooper et al. 2005). Contrary to our predictions, hatching failure did not increase seasonally because females appeared to change their incubation behavior. As ambient temperatures increase, females might maintain low rates of hatching failure by reducing exposure of first-laid eggs to ambient temperatures by either reducing clutch sizes or initiating incubation earlier or both. Scrub-jays decrease clutch size as the season progresses, a pattern that parallels the seasonal reduction in clutch size seen in many bird species (Klomp 1970).

The small sample size ($n = 44$) associated with our incubation onset data did not allow us to test predictions of incubation onset directly; however, we tested incubation onset indirectly using other, correlated parameters (e.g., apparent incubation days and embryonic development) for which we had more robust sample sizes. Our incubation onset data suggested that females that initiated incubation at clutch completion had an 18-day incubation period, and females that began incubation prior to clutch completion had apparent incubation periods less than 18 days. Thus, females appeared to begin incubation 0.5 days earlier relative to clutch completion by the end of the season. Additionally, the seasonal increase in embryonic development in first-laid eggs coupled with early-laid eggs showing greater development than late-laid eggs suggests that female scrub-jays began incubation earlier in the laying period as the season progressed. These

data strongly suggest that females are modifying their incubation behavior as ambient temperatures warm to decrease the exposure of their eggs and to maintain relatively low rates of hatching failure in first-laid eggs.

Hatching failure is pervasive among birds, and a negative relationship exists between latitude and hatching failure (Koenig 1982). Increased hatching failure with prolonged exposure to ambient temperatures prior to the onset of incubation has been shown in at least five species of birds where it has been studied (Beissinger et al. 2005), and our results on the Florida Scrub-Jay add another species and emphasize the potential ubiquity of time constraints on egg viability in explaining broad patterns of hatching failure. The egg viability hypothesis predicts that hatching failure should increase when eggs experience prolonged exposure to warm ambient temperatures (> physiological zero; 27° C), as occurs at lower latitudes, and Koenig (1982) clearly showed a latitudinal gradient in hatching failure. Therefore, declining egg viability might be an explanation for higher hatching failure at lower latitudes. Future research should examine whether increased hatching failure, especially at lower latitudes, can be explained by predictions of the egg viability hypothesis, and whether females are modifying incubation behavior to reduce the negative effects of egg viability.

CHAPTER TWO: EARLY INCUBATION ONSET MINIMIZES HATCHING FAILURE AT A COST OF INCREASING HATCHING ASYNCHRONY²

Abstract

Hatching failure is pervasive among birds and incurs an energetic and reproductive cost to breeding individuals. Thus, adaptive behaviors should evolve that minimize hatching failure. According to the egg viability hypothesis, hatching failure increases as eggs experience prolonged exposure to warm ambient temperatures. Therefore, females in warm environments should decrease exposure of first-laid eggs to ambient conditions by beginning incubation earlier in the laying period or laying smaller clutch sizes or both. Beissinger et al. (2005) showed that three days is the minimum amount of exposure necessary to increase hatching failure in other passerine species. Consistent with predictions of the egg viability hypothesis, Florida Scrub-Jays begin incubation prior to clutch completion in 4-egg clutches. Scrub-jays also begin incubation prior to clutch completion in clutches of three eggs or less, but this occurs only at the end of the season when ambient temperatures are warmer. As expected, beginning incubation prior to clutch completion results in greater hatching asynchrony, increased competitive size asymmetries between nestlings, and nonrandom loss of nestlings within nests because of starvation of the last-hatched young (brood reduction).

Hatching asynchrony and subsequent brood reduction often are explained as an adaptive strategy to quickly match brood sizes to local food resources. Under these adaptive explanations for asynchronous hatching patterns, brood reduction should occur early in the nestling period to

² This chapter will be submitted as a co-authored manuscript. I will be the primary author and I will include the following co-authors, respectively; Reed Bowman, Raoul K. Boughton, Michelle A. Rensel, and Stephan J. Schoech

avoid allocating limited food resources to last-hatched young that eventually will starve.

Alternatively, the egg viability hypothesis predicts that hatching asynchrony and brood reduction occur as a byproduct of maintaining egg viability. Thus, brood reduction should occur later in the nestling period in asynchronously hatching nests. We showed that brood reduction occurs late in the nestling period, as predicted by the egg viability hypothesis. However, brood reduction is greater in asynchronously hatching nests only in our suburban site, where the abundance of high-quality nestling food is lower. Therefore, we suggest that hatching asynchrony and brood reduction may be uncoupled in the Florida Scrub-Jay. Asynchronous hatching patterns do not appear to be an adaptive strategy to increase the rapidity of brood reduction, but may be a byproduct of minimizing hatching failure in first-laid eggs, which may carry a cost of increased brood reduction in food-limited environments.

Introduction

Hatching failure has significant fitness consequences for birds; approximately 10% of all avian eggs laid survive incubation but fail to hatch (Koenig 1982). Hatching failure is caused by lack of fertilization or embryo mortality (Lundy 1969). Several social and ecological factors may influence patterns of hatching failure including egg size, clutch size, latitude, diet, nest type and social organization (Koenig 1982). Of these factors, social organization and latitude appear to be the most significant in affecting hatchability. Hatching failure increases with increasing social organization and a negative relationship exists between hatching failure and latitude. The egg viability hypothesis states that prolonged exposure to warm ambient temperatures prior to the onset of incubation, as might be experienced by birds laying large clutches and nesting in lower latitudes, increases hatching failure (Stoleson and Beissinger 1997).

Birds typically lay one egg per day but the onset of incubation relative to clutch completion varies among species (Arnold et al. 1987; Clark and Wilson 1981). Some birds begin incubation only when the clutch is completed and their eggs typically hatch synchronously, within 24 hours. Other birds begin incubation prior to clutch completion and their eggs hatch asynchronously, with a minimum of 24 hours between the hatching of the first and last-laid eggs. Within species, the degree of hatching synchrony may vary both with clutch size and date of clutch initiation (Hebert 2002). Hatching tends to be more synchronous in smaller clutches laid earlier in the season, a pattern that closely follows predictions of the egg viability hypothesis.

Asynchronous hatching patterns in birds often are explained as an adaptive strategy to increase the number or quality of young produced within a nest (Stoleson and Beissinger 1997). Most hypotheses concerning hatching asynchrony focus on the roles of food availability and nest predation, especially during the nestling stage (Lack 1954; Clark and Wilson 1981). An alternative explanation of asynchronous hatching patterns in birds is proposed by the egg viability hypothesis (Arnold et al. 1987; Stoleson and Beissinger 1997). Unlike previous hypotheses suggesting that the adaptive benefits of hatching asynchrony occur late in the nesting cycle (matching brood size to food for nestlings), the egg viability hypothesis emphasizes the role of constraints imposed on eggs prior to incubation. Specifically, this hypothesis states that egg viability declines with increased exposure to ambient temperatures between physiological zero (24° to 27° C) and normal incubation temperatures (36° to 38° C) (Arnold et al. 1987). Physiological zero is the temperature above which development is initiated in the embryo (Cook et al. 2003). Therefore, prolonged exposure to temperatures high enough to promote development but below the temperature necessary for normal development can cause abnormal embryo development and mortality (Lundy 1969; Hebert 2002). Beissinger et al. (2005) showed

that three days is the minimum amount of exposure necessary to increase hatching failure in first-laid eggs. According to the egg viability hypothesis, hatching asynchrony may result when adults begin incubation earlier in the laying sequence to minimize hatching failure in first-laid eggs that may be exposed to long periods of ambient temperature (as would occur in larger clutches) or increased ambient temperature (as occurs at lower latitudes or later in the season because ambient temperatures are warmer) (Arnold et al. 1987).

Although initiating incubation prior to clutch completion might minimize rates of hatching failure, early onset of incubation also incurs a cost of increasing hatching asynchrony. If asynchrony is large, earliest-hatched nestlings may be fed before their siblings hatch, thus producing competitive size asymmetries among nestlings (Stoleson and Beissinger 1997). The resulting size asymmetry occasionally results in brood reduction or the nonrandom loss of nestlings because of starvation because last-hatched young lose within-nest contests for food with their older, bigger siblings. Most hypotheses suggest that initiating incubation early is an adaptive behavior to increase hatching asynchrony and increase the rapidity of brood reduction when food is limiting, thereby reducing the brood to a size suitable to the available resources (Lack 1954; Stoleson and Beissinger 1997). In contrast, the egg viability hypothesis suggests that hatching asynchrony might occur as a byproduct of minimizing hatching failure in first-laid eggs. According to the egg viability hypothesis, hatching asynchrony should increase with increasing clutch size, especially in warmer environments.

Previous studies of the egg viability hypothesis have focused mainly on brood manipulations to study the effects of increased egg exposure to ambient temperature on the hatchability of eggs (Veiga and Vinuela 1993; Arnold 1993; Stoleson and Beissinger 1999). Research on the Florida Scrub-Jay (*Aphelocoma coerulescens*) suggests that females laying

larger clutches may begin incubation earlier in the laying sequence when exposed to warm ambient temperatures to decrease exposure of first-laid eggs and minimize hatching failure (Aldredge et al., *in prep*). We conducted a natural experiment investigating the effects of variation in incubation behavior to offset the negative effects of egg viability on patterns of hatching asynchrony and brood reduction.

Previous research on the Florida Scrub-Jay suggests that hatching failure is higher in a suburban population than in a nearby wildland population because of constraints on egg viability (Aldredge et al., *in prep*). Suburban scrub-jays breed earlier and lay a larger median clutch size than wildland jays. The site-specific difference in clutch size appears to be driven by clutch initiation date because early-nesting females lay larger clutches in both sites (Bowman 1998). Because scrub-jays begin incubation at clutch completion (Woolfenden and Fitzpatrick 1996), first-laid eggs in the larger clutches of suburban jays should experience greater exposure to ambient temperature than subsequent eggs in the same clutch, leading to higher rates of hatching failure. Additionally, the time required to lay the 4-egg median clutch sizes in the suburbs is at or above the 3-day threshold for maintaining egg viability, whereas the time required to lay the 3-egg median clutch sizes in the wildlands is below this 3-day threshold (Beissinger et al. 2005; Aldredge et al., *in prep*). Consistent with egg viability hypothesis predictions, hatching failure is higher in first-laid eggs in the suburbs, and hatching failure also appears to decrease with increasing laying order (Aldredge et al., *in prep*). As ambient temperatures increase seasonally female scrub-jays in both sites appear to begin incubation earlier in the laying sequence to decrease exposure of clutches to potentially deleterious temperatures and minimize hatching failure in first-laid eggs.

Although scrub-jay eggs typically hatch within a 24-hour interval, we hypothesize that females will modify their incubation behavior to offset the negative effects of egg viability. We predict that females will begin incubation prior to reaching the 3-day threshold for maintaining egg viability in other passerines (Beissinger et al. 2005). Since scrub-jays typically lay one egg per day, females should begin incubation at or near clutch completion in 3-egg clutches and prior to clutch completion in 4-egg clutches.

We hypothesize that this pattern of incubation onset will influence patterns of hatching asynchrony and brood reduction. We predict that females that initiate incubation prior to clutch completion will have asynchronously hatching nests. If asynchronous hatching patterns are driven by behaviors that maintain egg viability, hatching will be synchronous in 3-egg clutches, which occur below the 3-day threshold for maintaining egg viability, and hatching will be asynchronous in 4-egg clutches, which occur above this 3-day threshold (Beissinger et al. 2005). Similarly, we predict that increased hatching asynchrony will increase rates of brood reduction within nests. If hatching asynchrony is an adaptive mechanism to quickly match brood sizes with food availability, then brood reduction will occur early in the nestling period. Alternatively, if asynchronous hatching patterns occur as a byproduct of maintaining egg viability, brood reduction will occur later in the nestling period. Lastly, if female scrub-jays follow predictions of the egg viability hypothesis by beginning incubation earlier in the laying period to minimize hatching failure in first-laid eggs in larger clutches, brood reduction should be higher in asynchronous nests (i.e., 4-egg clutches) than in synchronous nests (i.e., 3-egg clutches).

Methods

Study Organism and Study Sites

The Florida Scrub-Jay is a cooperative breeder and the only bird species endemic to Florida. Scrub-jays lay 3-5 egg clutches and begin incubation with the ultimate (last-laid) or penultimate (second to last-laid) egg (Woolfenden and Fitzpatrick 1996). The incubation and nestling periods both last approximately 18 days, but the incubation period is less variable than the nestling period. Florida Scrub-Jays are dependent on the fire-maintained xeric oak scrub habitat characteristic of the Lake Wales Ridge in central Florida. Over 85% of Florida's original oak scrub habitat has been destroyed through conversion to urban or suburban areas and agriculture (Peroni and Abrahamson 1985). This habitat destruction has been instrumental in the loss of over 80% of the Florida Scrub-Jay population (Bowman 1998).

Our research was conducted on two populations of Florida Scrub-Jays in south-central Florida. One population in Placid Lakes Estates (27 15'N, 81 25'W) occurs in fragmented patches of oak scrub habitat within a residential development. This suburban population has been studied intensively over the past 16 years (Bowman 1998; Bowman and Woolfenden 2001). Ambient temperatures in the suburban site are 1-2° C warmer than our wildland site (Aldredge et al., *in prep*), a pattern consistent with the urban heat island effect (Fan and Sailor 2005). Suburban scrub-jays also begin breeding earlier each year and lay larger clutches than jays in the wildlands (Bowman 1998). The wildland population occurs at Archbold Biological Station, 10 km south of the suburban site, where the scrub is fire-maintained and occurs in large contiguous blocks. This wildland site has been studied intensively over the past 18 years (Schoech 1996).

Onset of Incubation

We found and monitored 350 nests during the 2005-2007 breeding seasons, which lasted from late February until early June. All nests were found prior to egg-laying and followed until either the entire nest failed or some young successfully fledged. Each egg was weighed within 24 hours of being laid to determine initial egg weight, and laying order was identified by marking eggs with an indelible marker. We recorded hatching synchrony by visiting nests at least once daily from seventeen days after clutch completion until all eggs were hatched (see *Hatching Asynchrony*); each nestling was marked in the sequence in which it hatched.

HOBO thermocouples (HOBO H8 Pro Series, Onset Computer Corporation, Bourne, Massachusetts, USA) were placed within 2m of sites known to have been selected by jays for nesting to quantify site-specific differences in ambient temperature at nest sites. Thermocouples were placed in three-sided housings approximately 2m above the ground to avoid ground irradiance from the white sand and each housing faced north to avoid direct exposure to sunlight. Ambient temperature was recorded every fifteen seconds and each thermocouple was moved to a new nest site every six days. Two thermocouples were used in each site and both thermocouple temperatures in each site were averaged to avoid the confounding effect of variation in ambient temperature because of individual housing placement.

On the day the first egg was laid, we placed thermoprobes (HOBO H12-001, Onset Computer Corporation, Bourne, Massachusetts, USA) in each nest. Thermoprobes were gently pushed through the nest lining at the bottom of the nest until the sensor was just exposed at the interface of the eggs and lining. Thermoprobes collected temperature data every minute from laying of the first egg until three days after clutch completion. The onset of incubation was apparent because nest temperature closely tracked ambient temperature until the onset of

incubation when nest temperature became stable between 31° and 36° C (Fig. 1). Artificial eggs, which could give a more accurate estimate of incubation temperature, were not used because scrub-jays reject artificial eggs (Fleischer and Woolfenden 2004).

Hatching Asynchrony and Brood Reduction

Beginning seventeen days after clutch completion, we checked nests four times a day (every 3-4 hours) to determine the order and timing of hatching. Hatching synchrony was recorded as the time, in 12-hour increments, elapsed between the first-hatched and last-hatched egg. Entire nests hatching within one visit (i.e., one hatching interval) were recorded as a zero and the variable increased by one unit every twelve hours until hatching was complete. We marked all nestlings within each nest according to hatching order and followed them through the entire nestling period. We identified hatching order by marking nestlings' toenails with red nail polish and recording from which egg each nestling hatched, when known. We revisited nests, reapplied nail polish, and recorded the amount of brood reduction five days after the first egg hatched. Eleven days after the first egg hatched, all nestlings within a nest were banded, morphological measurements taken, and brood reduction recorded.

As part of the long-term demographic work on both populations, we also visited nests daily starting seventeen days after the first egg hatched to record how many nestlings successfully fledged. Nestlings still alive eighteen days after the first egg hatched, even if they were still in the nest, were recorded as successfully fledged. We did not test the relationship between hatching synchrony and brood reduction when the young fledge (day 18) because of the error in quantifying brood reduction at this time. Nestlings are capable of fledging 2-3 days prior to fledging, and checking for individual nestlings that might have fledged could prompt

remaining nestlings to prematurely fledge, thereby reducing survivorship. Once some fledging has occurred within a nest, it is difficult to determine whether nestlings that disappear died because of brood reduction or were depredated following departure from the nest.

Statistical Analysis

Although clutch size in Florida Scrub-Jays ranges from 2-5 eggs, the median clutch size is four eggs in the suburban site and three eggs in the wildland site, thus only 3 and 4-egg clutches were used in subsequent analyses. Because the sample size associated with our incubation onset data was small ($n = 44$) we used the relationship between incubation onset and apparent incubation days (days between last-laid egg and first-hatched egg) to calculate incubation onset for the entire 2005-08 dataset ($n = 183$), which we used for all of our incubation onset analyses. We used ANCOVA to examine the relationship between incubation onset, site, clutch size, and clutch initiation date. Only the main effects were significant, thus we reran the ANCOVA separately for each main effect. We also used ANCOVA to examine the relationship between hatching synchrony, site, clutch size, and clutch initiation date. For all other analyses, parametric tests were used when data fit basic statistical assumptions. All values are reported as means (± 1 SEM).

Results

Onset of Incubation

The onset of incubation was determined for 44 nests during the 2006-2007 breeding seasons; 35 nests in the suburbs and 9 nests in the wildlands. Incubation onset was positively correlated with number of apparent incubation days (days between last-laid egg and first-hatched

egg) (Spearman's $\rho = 0.418$, $n = 33$, $p = 0.016$; Fig. 9). Scrub-jays initiating incubation at clutch completion had an apparent incubation period of 17.89 days; scrub-jays that began incubation prior to clutch completion had a shorter incubation period, but eggs still hatched approximately 18 days after incubation was initiated. Females in both sites began incubation earlier in the laying period as the season progressed (ANCOVA; $F_{(1,178)} = 5.382$, $p = 0.021$). After accounting for this early incubation onset, we found significant main effects of site and clutch size on incubation onset. Suburban females began incubation earlier in the laying period than wildland females (ANCOVA; $F_{(1,180)} = 15.563$, $p < 0.001$), and females laying 4-egg clutches began incubation earlier in the laying period than females laying 3-egg clutches (ANCOVA; $F_{(1,180)} = 20.281$, $p < 0.001$).

Hatching Asynchrony and Brood Reduction

Hatching synchrony was determined for 119 nests during the 2006 and 2007 breeding seasons; 59 nests in the suburbs and 60 nests in the wildlands. Nests were considered synchronous if at most 24 hours elapsed between the first and last-hatched eggs. Conversely, nests were considered asynchronous if more than 24 hours elapsed between the first and last-hatched eggs. A strong negative relationship existed between incubation onset and hatching synchrony ($F = 12.974$, $df = 5$, $p < 0.001$; Fig. 12). Females whose nests hatched asynchronously (> 24 hrs between first and last-hatched eggs) began incubation $1.76 (\pm 0.21)$ days before clutch completion and females whose nests hatched synchronously (< 24 hrs between first and last-hatched eggs) began incubation $0.42 (\pm 0.11)$ days before clutch completion. Hatching asynchrony increased in both sites as the season progressed (ANCOVA; $F_{(1,101)} = 9.256$, $p = 0.003$). After accounting for this seasonal increase in hatching asynchrony, we found a

significant main effect of clutch size (ANCOVA; $F_{(1,101)} = 13.701, p < 0.001$) and a 2-way interaction between site and clutch size (ANCOVA; $F_{(1,101)} = 11.345, p = 0.001$; Fig. 13). Hatching asynchrony was greater in 4-egg clutches than in 3-egg clutches. Scrub-jays in both sites appeared to reduce clutch sizes as the season progressed. Hatching asynchrony appeared to be similar in 3 and 4-egg clutches in the wildlands, but hatching asynchrony increased at different rates for each clutch size. Alternatively, hatching asynchrony was greater in 4-egg clutches than in 3-egg clutches in the suburbs, but hatching asynchrony appeared to increase at similar rates in each clutch size.

Hatching synchrony had no effect on brood reduction by day 5 of the nestling period (Fisher's Exact $_{(1-\alpha)}$, $p = 0.421$), but brood reduction was higher in asynchronous nests by day 11 of the nestling period (Fisher's Exact $_{(1-\alpha)}$, $p = 0.037$). Day 11 brood reduction appears to be driven by site-specific differences in brood reduction. Hatching synchrony had no effect on brood reduction by day 11 of the nestling period in the wildlands (Fisher's Exact $_{(1-\alpha)}$, $p = 0.558$), but brood reduction was higher in asynchronous nests by day 11 of the nestling period in the suburbs (Fisher's Exact $_{(1-\alpha)}$, $p = 0.036$) (Fig. 14). Brood reduction also was higher in 4-egg clutches than in 3-egg clutches in the suburbs by day 11 of the nestling period (Fisher's Exact $_{(1-\alpha)}$, $p = 0.036$). Clutch size did not influence day 11 brood reduction in the wildlands (Fisher's Exact $_{(1-\alpha)}$, $p = 0.532$).

Discussion

Scrub-jays appear to vary incubation onset to reduce exposure of clutches to warm ambient temperatures and minimize the negative effects of declining egg viability. Previous research on the Florida Scrub-Jay showed that hatching failure is higher in first-laid eggs in the

suburbs, where eggs may be exposed to ambient temperatures for longer periods than in the wildlands because clutch sizes are larger (Aldredge et al., *in prep*). This research also showed that as ambient temperatures increased seasonally the apparent incubation period (time elapsed between the last-laid egg and first-hatched egg) fell below the scrub-jay's 18-day incubation period and early-laid eggs began development prior to late-laid eggs, suggesting that scrub-jays in both sites were beginning incubation earlier in the laying sequence, possibly to reduce exposure and minimize hatching failure in first-laid eggs in warm environments. In this paper, we showed that apparent incubation periods and incubation onset were highly correlated. Females with an apparent incubation period less than 18 days began incubation prior to clutch completion. As predicted, females in both sites began incubation prior to clutch completion when laying 4-egg clutches, where egg exposure is at or above the 3-day threshold for maintaining egg viability. Females laying 3-egg clutches began incubation near clutch completion. Regardless of clutch size, females began incubation closer to clutch completion at the beginning of the season, when ambient temperatures were lower, and began incubation earlier relative to clutch completion as seasonal ambient temperatures warmed. However, females laying 4-egg clutches always began incubation earlier than females laying 3-egg clutches, a pattern consistent with predictions of the egg viability hypothesis.

Suburban females began incubation earlier in the laying sequence than wildland females. Although ambient temperatures are higher in the suburbs than in the wildlands, which may cause females to begin incubation earlier in the laying period, the site effect appears to be a consequence of the larger median clutch size in the suburbs. Minimum ambient temperatures are 1-2° C higher in the suburbs than in the wildlands, a pattern consistent with the urban heat island effect (Aldredge et al., *in prep*). Therefore, temperature differences between sites should be

greater at the beginning of the season when minimum ambient temperatures (e.g., nighttime temperatures) are lowest. This leads to the prediction that differences in the onset of incubation between suburbs and wildlands should be greatest early in the season. Alternatively, seasonal declines in clutch size occur more slowly in the suburbs, leading to the greatest difference in median clutch size at the end of the breeding season. This leads to the contrasting prediction that differences in the onset of incubation between suburbs and wildlands should be greatest late in the season. Suburban females appeared to begin incubation even earlier in the laying sequence as the season progressed than did wildland females. Thus, the greater difference in incubation onset between sites later in the season appears to follow predictions of site-specific differences in clutch size driving patterns of incubation onset.

Although females modify incubation behavior to maximize the number of eggs that hatch, early incubation onset incurs a cost of increased hatching asynchrony. Hatching synchrony appeared to closely follow patterns of incubation onset. Females whose entire clutch hatched within one hatching interval began incubation $0.22 (\pm 0.21)$ days after clutch completion. Females whose clutch took approximately 24 hours (1 day) between the first and last-hatched eggs began incubation $0.79 (\pm 0.15)$ days before clutch completion, and females whose clutch took approximately 48 hours (2 days) between the first and last-hatched eggs began incubation $1.64 (\pm 0.46)$ days before clutch completion.

Incubation onset began earlier and hatching became more asynchronous as the season progressed and ambient temperatures warmed. Even though incubation onset was affected only by the main effects of site and clutch size, hatching asynchrony was influenced by clutch size and its interaction with site. This occurred because hatching spread is similar in 3 and 4-egg clutches in the wildlands, but hatching spread is shorter for 3-egg clutches than for 4-egg

clutches in the suburbs. Consistent with the egg viability hypothesis, clutch size in wildland jays appears to conform to the predicted tradeoff between beginning incubation earlier in large clutches in warm environments and maintaining low rates of hatching asynchrony by laying smaller clutch sizes and modifying incubation behavior to minimize brood reduction within nests. Wildland jays primarily lay 4-egg clutches until hatching spread approaches 24 hours (e.g., asynchronous hatching) when they appear to reduce clutch size to 3 eggs. This pattern also could be explained by other life history factors, such as declining reproductive value of late-hatched offspring or the cost of reproduction causing seasonal reductions in clutch size (Sockman et al. 2006). However, this pattern appears to match *a priori* predictions of the egg viability hypothesis and should be investigated further. Suburban jays, on the other hand, maintain a larger median clutch size throughout the season, which minimizes the seasonal difference in clutch size despite the associated cost of increased hatching asynchrony. Nestling recruitment is lower in our suburban population because of stochastic factors, such as increased hatching failure and higher post-fledging mortality (predation) (Bowman, unpub. data); therefore, females laying larger clutch sizes in the suburbs, regardless of season, may have a higher probability of producing more young that survive to reproduce.

Hatching asynchrony may result in brood reduction, a strategy often explained through its adaptive value in quickly matching brood sizes with food resources for nestlings (Lack 1954). Unlike adult scrub-jays that eat an omnivorous diet, scrub-jay nestlings, like most passerines, rely on lepidopteran and orthopteran larva (Stallcup and Woolfenden 1978) to maintain the high growth rates necessary during the nestling and post-fledging periods (Woolfenden 1978). Larva abundances appear to increase as the season progresses (Bowman unpub. data; Fig. 15), and previous research shows that lepidopteran larva abundances are lower in the suburbs than in the

wildlands (Shawkey et al. 2004). Therefore, if hatching asynchrony and brood reduction occur as an adaptive mechanism to quickly match brood sizes to local food resources, we predict that 1) hatching asynchrony should be greater at the beginning of the season when food is limiting and 2) hatching asynchrony should be greater in the suburbs than in the wildlands because of reduced arthropod abundances. Additionally, patterns of brood reduction should occur because of hatching asynchrony. Therefore, brood reduction 1) should be higher in asynchronously hatching nests and 2) should occur early in the nestling period to minimize feeding nestlings that eventually will starve. We already have shown that hatching asynchrony was greatest at the end of the season when ambient temperatures were warmest. Therefore, hatching asynchrony appears to occur as a byproduct of maintaining egg viability. Patterns of brood reduction initially appear to be consistent with predictions of the egg viability hypothesis; brood reduction was higher in asynchronously hatching nests and brood reduction occurred relatively late post-hatching. However, brood reduction is higher in asynchronously hatching nests only in the suburbs, where nestling food is limiting. Therefore, hatching asynchrony and brood reduction may be uncoupled in the Florida Scrub-Jay; hatching asynchrony occurs to maintain viability of first-laid eggs in warm environments, while brood reduction is higher in asynchronously hatching nests but occurs mainly as a consequence of limited food for nestlings.

This uncoupling of hatching asynchrony and brood reduction may be a primary reason why studies have shown equivocal support for Lack's brood reduction hypothesis (Clark and Wilson 1981; Magrath 1990; Stoleson and Beissinger 1997). If our intent had been to specifically test the brood reduction hypothesis, we might have concluded that 1) nests with asynchronous hatching patterns experience greater brood reduction and 2) brood reduction occurs because of limited food availability. It is only after close scrutiny of an alternative

hypothesis (e.g., the egg viability hypothesis) that we see that 1) hatching asynchrony appears to occur as a byproduct of minimizing hatching failure in first-laid eggs in warm environments, and 2) brood reduction is more frequent in asynchronously hatching nests late in the nestling period, as predicted by the egg viability hypothesis, but brood reduction is highest where food is limiting, as predicted by adaptive brood reduction hypotheses. Therefore, asynchronous hatching patterns do not appear to be set up as an adaptive mechanism to increase the rapidity of brood reduction in the Florida Scrub-Jay, but occur as a byproduct of maintaining egg viability.

Although incubation behavior and hatching asynchrony appear to closely follow seasonal increases in ambient temperature, incubation behavior does not appear to follow small fluctuations in ambient temperature. If incubation behavior was based primarily on ambient temperature, larger site-specific differences in incubation onset should have occurred at the beginning of the season when differences in minimum ambient temperatures were greatest. However, suburban females appeared to begin incubation earlier in the laying period than wildland females at the end of the season when ambient temperatures were similar between sites but minimum ambient temperatures in both sites were warmer. Incubation behavior in both sites appeared to follow predictable seasonal patterns rather than track short-term variations in ambient temperature. Therefore, scrub-jays may use seasonal cues that are more predictable than ambient temperature to regulate incubation behavior because of the strong correlation between ambient temperature and season. Previous research has shown that prolactin, the hormone responsible for regulating avian incubation behavior, increases continuously with season, suggesting incubation behavior may not be affected directly by temperature fluctuations, but may be regulated by the endocrine system using other, more predictable environmental cues, such as photoperiod (Sockman et al. 2006). Many species exhibit a seasonal increase in basal prolactin

levels (Meijer et al. 1990; Wingfield and Goldsmith 1990; Chakraborty 1995; Silverin and Goldsmith 1997; Sharp et al. 1998; Maney et al. 1999; Sockman and Schwabl 2001). Therefore, a seasonal increase in early incubation onset might be widespread among birds, possibly to offset declining egg viability as ambient temperatures increase seasonally.

We have shown that an early onset of incubation in large clutches may occur mainly to maintain viability of first-laid eggs in warm environments. However, initiating incubation early incurs a cost of increasing hatching asynchrony and possibly brood reduction. This can ultimately have profound effects on avian clutch size as birds must initiate incubation early to minimize hatching failure in warm environments, but also begin incubation close to clutch completion to synchronize hatching within nests and minimize brood reduction. The seasonal and latitudinal gradients in avian clutch size are consistent with egg viability hypothesis predictions; clutch size decreases with advancing season and decreasing latitude, respectively (Lack 1968; Klomp 1970; Cooper et al. 2005), possibly because of a concomitant increase in ambient temperatures. Scrub-jays, especially wildland scrub-jays, appear to exhibit a seasonal reduction in clutch size consistent with the seasonal and latitudinal trends in many bird species. As temperatures warm, suburban and wildland females initiate incubation earlier in the laying period, possibly to reduce exposure and minimize hatching failure in first-laid eggs. However, early incubation onset increases hatching asynchrony and possibly brood reduction. Thus, females can lay smaller clutches and begin incubation closer to clutch completion, which reduces exposure of first-laid eggs while minimizing hatching asynchrony. As predicted, wildland females appeared to begin laying 3-egg clutches as hatching synchrony approached 24 hours, possibly to reduce exposure of first-laid eggs and minimize hatching asynchrony within nests. Therefore, our results suggest that *a priori* predictions of the egg viability hypothesis may

explain seasonal trends in avian clutch size as females balance the tradeoff between minimizing exposure of first-laid eggs to warm ambient temperatures and minimizing hatching asynchrony and possibly brood reduction. This reduction in clutch size with warmer ambient temperature also may explain the latitudinal trend in avian clutch sizes (Cooper et al. 2005). Therefore, future research should examine whether these *a priori* predictions explain the seasonal, and potentially latitudinal, trends in avian clutch size. Through controlled experiments on other species, future research can test the relative importance of factors that regulate incubation onset by separating potential correlated environmental effects, such as ambient temperature and season.

CONCLUSIONS

Although our results indicate that hatching asynchrony occurs as a byproduct of maintaining egg viability in first-laid eggs, we wanted to examine predictions of an additional hypothesis to determine whether hatching asynchrony could be explained by other factors. Namely, is hatching asynchrony an adaptive mechanism to increase the rapidity of brood reduction? This led to specific *a priori* predictions for both hypotheses (i.e., adaptive brood reduction vs. egg viability). If hatching asynchrony is an adaptive mechanism to increase the rapidity of brood reduction (Hypothesis 1 [H1]) we predict that; P1.1) brood reduction will be higher in asynchronously hatching nests early in the nestling period, P1.2) hatching asynchrony patterns will track seasonal patterns of nestling food availability, and consequently P1.3) brood reduction patterns will track seasonal patterns of nestling food availability. Alternatively, if hatching asynchrony and brood reduction occur as a byproduct of maintaining egg viability [H2] we predict that P2.1) brood reduction will be higher in asynchronously hatching nests later in the nestling period, P2.2) hatching asynchrony patterns will track seasonal patterns of ambient temperature, and consequently P2.3) brood reduction will track seasonal changes in ambient temperature.

We have shown that brood reduction is higher in asynchronously hatching nests late in the nestling period (P2.1; Fig. 14), as predicted by the egg viability hypothesis. However, brood reduction is higher in asynchronous nests only in the suburbs where food is limiting, which suggests that brood reduction may be influenced by the availability of nestling food. Fortunately, this system is ideal for examining *a priori* predictions of both hypotheses. Previous research shows that nestling food (lepidoptera and orthoptera abundances) is lower at the beginning of the season than at the end of the season (Bowman unpub. data; Fig. 15). This leads to the prediction

from Lack's hypotheses of adaptive brood reduction that P1.2) hatching asynchrony and P1.3) brood reduction should be greater at the beginning of the season than at the end of the season as females increase asynchronous hatching patterns to increase the rapidity of brood reduction. Alternatively, ambient temperatures increase as the season progresses. This leads to the alternative prediction of the egg viability hypothesis that P2.2) hatching asynchrony and P2.3) brood reduction should be greater at the end of the season when females begin incubation earlier in the laying period to minimize hatching failure in first-laid eggs. Our results indicate that hatching asynchrony is higher at the end of the season (Fig. 13), as predicted by the egg viability hypothesis (P2.2). Therefore, we examined seasonal patterns of brood reduction by running a binary logistic regression using site, clutch size and clutch initiation date as our independent variables to see whether brood reduction occurs as an adaptive mechanism to match brood sizes with local food resources [H1] or occurs as a byproduct of maintaining egg viability [H2]. Site and clutch size were used in our models because we wanted to test whether either of these factors were more important in explaining patterns of brood reduction.

None of the variables examined (site, clutch size, or clutch initiation date) explained brood reduction early (by day 5) in the nestling period. However, a 2-way interaction existed between site and clutch initiation date ($p = 0.014$; Fig. 16) late (by day 11) in the nestling period. Brood reduction by day 11 of the nestling period was higher at the beginning of the season in both sites, but brood reduction was higher in the suburbs than in the wildlands. The frequency of brood reduction in the wildlands decreased more rapidly than in the suburbs, which resulted in a very low frequency ($\sim 2\%$) of brood reduction in the wildlands by the end of the season, but still moderate rates ($\sim 15\%$) of brood reduction in the suburbs at the end of the season. A 2-way interaction also existed between clutch size and clutch initiation date ($p = 0.050$; Fig. 17). Day 11

brood reduction was higher in 4-egg clutches than in 3-egg clutches in both sites, even though day 11 brood reduction was higher in 3-egg clutches at the beginning of the season. Brood reduction in 3-egg clutches appeared to decrease to very low levels as the season progressed ($p = 0.066$), whereas brood reduction in 4-egg clutches did not change with advancing season ($p = 0.883$).

Consistent with a correlation between rates of brood reduction and availability of nestling food, brood reduction was higher at the beginning of the season in both sites when food availability was lowest. Even though hatching asynchrony appeared to explain patterns of brood reduction, brood reduction was highest when hatching asynchrony was lowest. Hatching asynchrony only explained patterns of brood reduction in the suburbs, where nestling food appears to be limiting (Shawkey et al. 2004). Florida Scrub-Jays primarily have synchronously hatching nests, but some variation exists in hatching spread. Natural variations in hatching asynchrony might not be great enough in scrub-jay nests to increase rates of brood reduction because food availability swamps any effect of hatching asynchrony. Thus, hatching asynchrony may exacerbate brood reduction, but this occurs mainly when food is limiting. When food is really limiting, brood reduction is going to be much higher, even if hatching asynchrony is low. Therefore, brood reduction in the Florida Scrub-Jay appears to be explained by patterns of nestling food availability, thus scrub-jays should track variations in nestling food to minimize rates of brood reduction within nests.

Onset of breeding differs between suburban and wildland jays. As predicted, wildland jays appear to track annual variations in larva abundances, whose growth rates are strongly influenced by ambient temperature (Perrins 1979). In the wildland site, onset of breeding varies between years by as much as 4-6 weeks (Bowman et al. 1998). However, some wildland jays

appear to mistime the initiation of breeding because brood reduction is highest at the beginning of the season when larva abundances are lowest. This may occur because birds use proximate cues (i.e., warm ambient temperatures) instead of actual arthropod abundances to accurately predict when larva will be adequate for raising young (van Noordwijk et al. 1995). Early-nesting females in the wildlands might mistime reproduction when cold spells occur after the initiation of breeding, which uncouples the relationship between the proximate cue (i.e., warm ambient temperatures) and emergence of arthropods.

Wildlands jays typically begin breeding later than suburban jays (Bowman 1998). In the suburbs, adult scrub-jays utilize *ad libitum* sources of anthropogenic food that is of high quality for adults but appears to be of low quality for feeding nestlings. Access to these predictable food sources advances mean laying date in suburban scrub-jays and leads to larger median clutch sizes (Reynolds et al. 2003; Schoech et al. 2008). Onset of breeding also is less variable between years in the suburbs than in the wildlands. Suburban scrub-jays begin nesting in late February or early March; thus, suburban eggs begin hatching in mid- to late-March, well before the peak in nestling food abundances (Fig. 15). Therefore, not only are larva abundances reduced in the suburbs, but suburban jays appear to mistime reproduction by laying their largest clutches so that nestling feeding occurs prior to the increase in nestling food abundances.

As Perrins (1970) suggested, this mistiming may occur because suburban females begin breeding as soon as they are physiologically capable, which is advanced in the suburbs because of access to *ad libitum* sources of anthropogenic food. Suburban females possibly begin breeding before enough food is available for feeding nestlings because females that begin breeding earlier in the season produce more young than females that breed later in the season, even after accounting for the costs of losing last-hatched young to brood reduction. Therefore, suburban

females do not appear to track food resources to determine when to initiate breeding, which may occur because 1) the cues used to match the presence of nestlings to the increase in nestling food abundance are unreliable in the suburbs or 2) some factor other than food is driving onset of breeding in suburban scrub-jays.

Seasonal patterns of brood reduction also differed by clutch size in both sites. Females laying 3-egg clutches at the beginning of the season had higher rates of brood reduction than females laying 4-egg clutches. However, the frequency of brood reduction in 3-egg clutches decreased dramatically and 3-egg clutches had very low rates of brood reduction by the end of the season, whereas brood reduction occurred in approximately 30% of 4-egg clutches throughout the season. This pattern suggests that females laying 3-egg clutches at the beginning of the season may be of poorer quality (e.g, poor body condition, young, or inexperienced) than females laying 4-egg clutches, which would explain the smaller clutch size and higher rates of brood reduction. In addition, if larva abundance is low at the beginning of the season, these females may have greater difficulty feeding their young than better quality females. Alternatively, the constant rate of brood reduction throughout the season in 4-egg clutches suggests that it is difficult to fledge more nestlings, regardless of food availability. This is further supported by brood reduction patterns in broods of 5 nestlings, where brood reduction occurs in 100% of nests.

I conclude that patterns of hatching asynchrony and brood reduction appear to be uncoupled in the Florida Scrub-Jay. Hatching asynchrony occurs when females begin incubation prior to clutch completion, which appears to be an adaptive strategy to minimize hatching failure in early-laid eggs. Therefore, hatching asynchrony does not occur to increase the rapidity of brood reduction but occurs mainly as a byproduct of maintaining egg viability in early-laid eggs.

Brood reduction appears to be weakly affected by patterns of hatching asynchrony, except in the suburban site where scrub-jays experience a concomitant decrease in food for nestlings.

Therefore, brood reduction appears to occur because of limitations in food for young. My results might elucidate why equivocal support has been found for the adaptive nature of asynchronous hatching patterns in explaining rates of brood reduction. It appears that food limitation is a major determinant in rates of brood reduction, but females do not appear to be adaptively setting up dominance hierarchies within nests to increase the rapidity of brood reduction. Therefore, scrub-jays may maximize their fitness by modifying patterns of incubation onset to 1) maximize the number of eggs that hatch successfully and 2) maximize the number of young that fledge successfully.

APPENDIX: TABLES AND FIGURES

Table 1: Akaike's Information Criterion (AIC) analysis using binary logistic regression examining factors influencing whole hatching failure

Model	-2 Log likelihood	K	ΔAIC_i
yr + sit + cl init + help + cl size	331.996	6	0.00
yr + sit + cl init + cl size	341.849	5	1.12
yr + sit + help + cl size	335.112	5	1.43
sit + cl init + help + cl size	335.422	5	1.84
yr + sit + cl size	337.51	5	2.74
sit + cl init + cl size	335.839	5	2.80
yr + cl init + help + cl size	345.088	4	3.51
sit + cl size	350.378	4	4.30
yr + cl init + cl size	338.74	4	6.41
cl init + help + cl size	345.561	4	6.41

Model variables: sit = site; yr = year; help = presence of helpers; cl init = clutch initiation date; cl size = clutch size

Table 2: Akaike's Information Criterion (AIC) analysis using binary logistic regression examining factors influencing partial hatching failure

Model	-2 Log likelihood	K	ΔAIC_i
sit + cl size	2295.307	6	0.00
yr + sit + cl size	2296.902	5	1.60
sit + cl init + cl size	2297.068	5	1.76
yr + sit + help + cl size	2297.575	5	2.27
sit + cl init + help + cl size	2297.723	5	2.42
yr + sit + cl init + cl size	2298.666	5	3.36
yr + sit + cl init + help + cl size	2299.289	4	3.98
sit	2300.583	4	5.28
sit + help	2301.11	4	5.80
yr + sit	2302.247	4	6.94

Model variables: sit = site; yr = year; help = presence of helpers; cl init = clutch initiation date; cl size = clutch size

Table 3: (from LeClair 2005) Multiple binary logistic regression examining the influence of predation risk experiment factors on partial hatching failure

Variable	<i>df</i>	<i>p-value</i>	<i>R</i>²
Frequency of off-bouts	1	0.06	0.16
Duration of off-bouts	1	0.94	0.16
Proportion 24-hours off nest	1	0.83	0.16
Maximum ambient temperature	1	0.74	0.001

Table 4: Forward binary logistic regression showing the influence of apparent incubation days and site on partial hatching failure

Variable	β	SE	<i>p</i>-value	R^2
Apparent incubation days	-285.301	80.754	< 0.001	0.108
Site	-1.189	0.468	0.011	0.176
Constant	15.662	4.612	0.001	

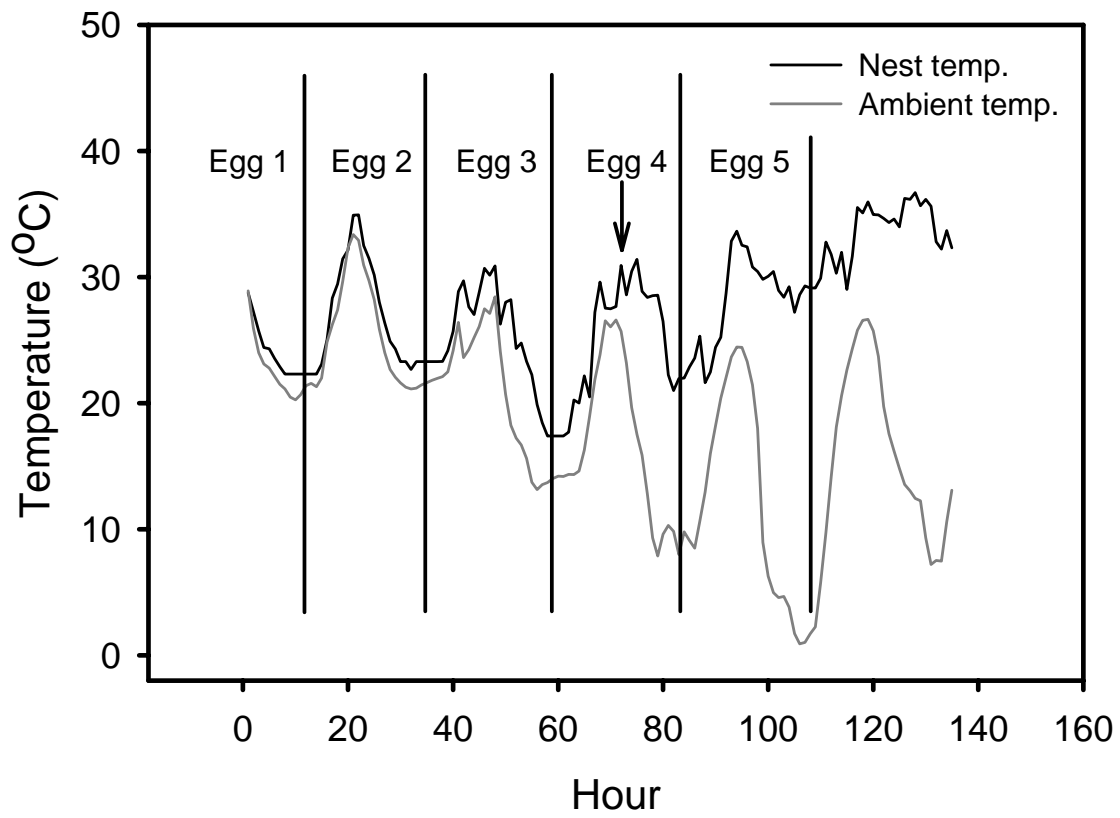


Figure 1: Example of the relationship between ambient and nest temperatures during the laying and beginning of the incubation periods

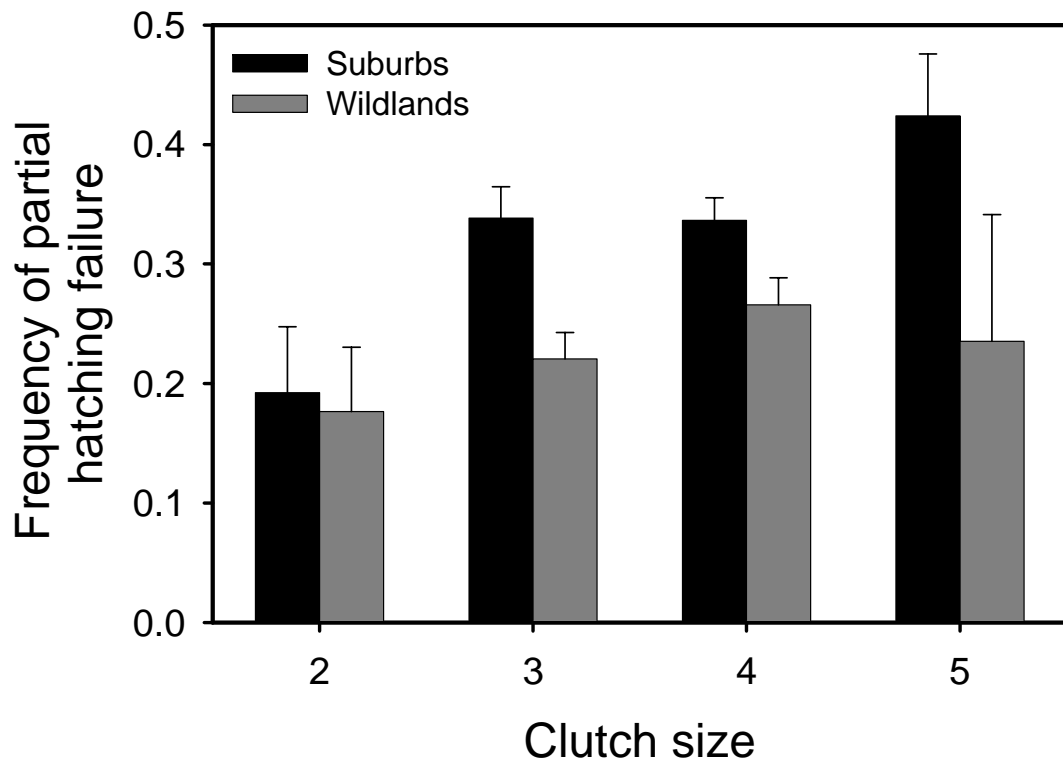


Figure 2: Relationship between rates of partial hatching failure and clutch size in wildland and suburban Florida Scrub-Jays

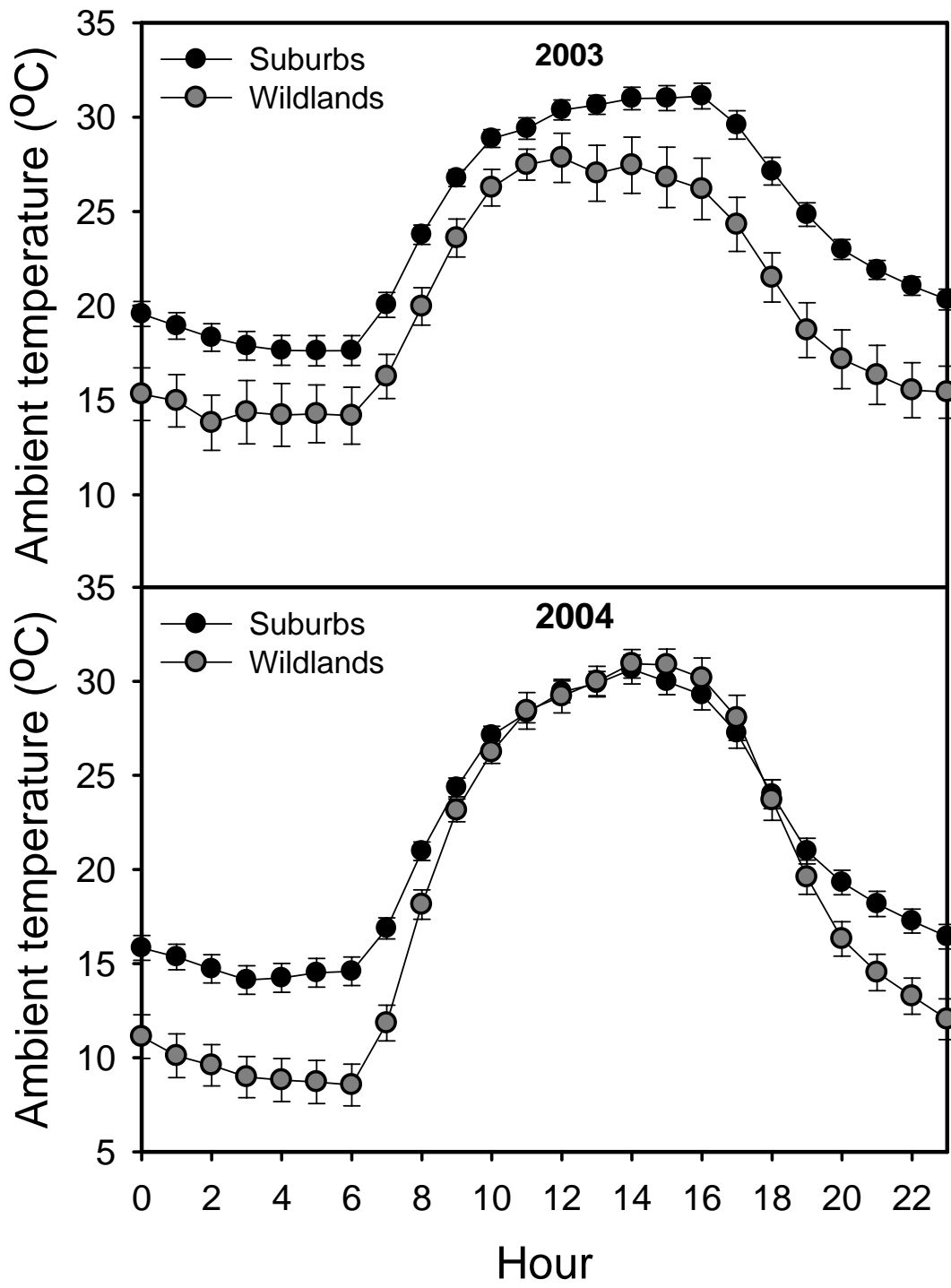


Figure 3: (from LeClair 2005) Mean daily ambient temperatures in the suburbs and wildlands during the breeding season in 2003 and 2004

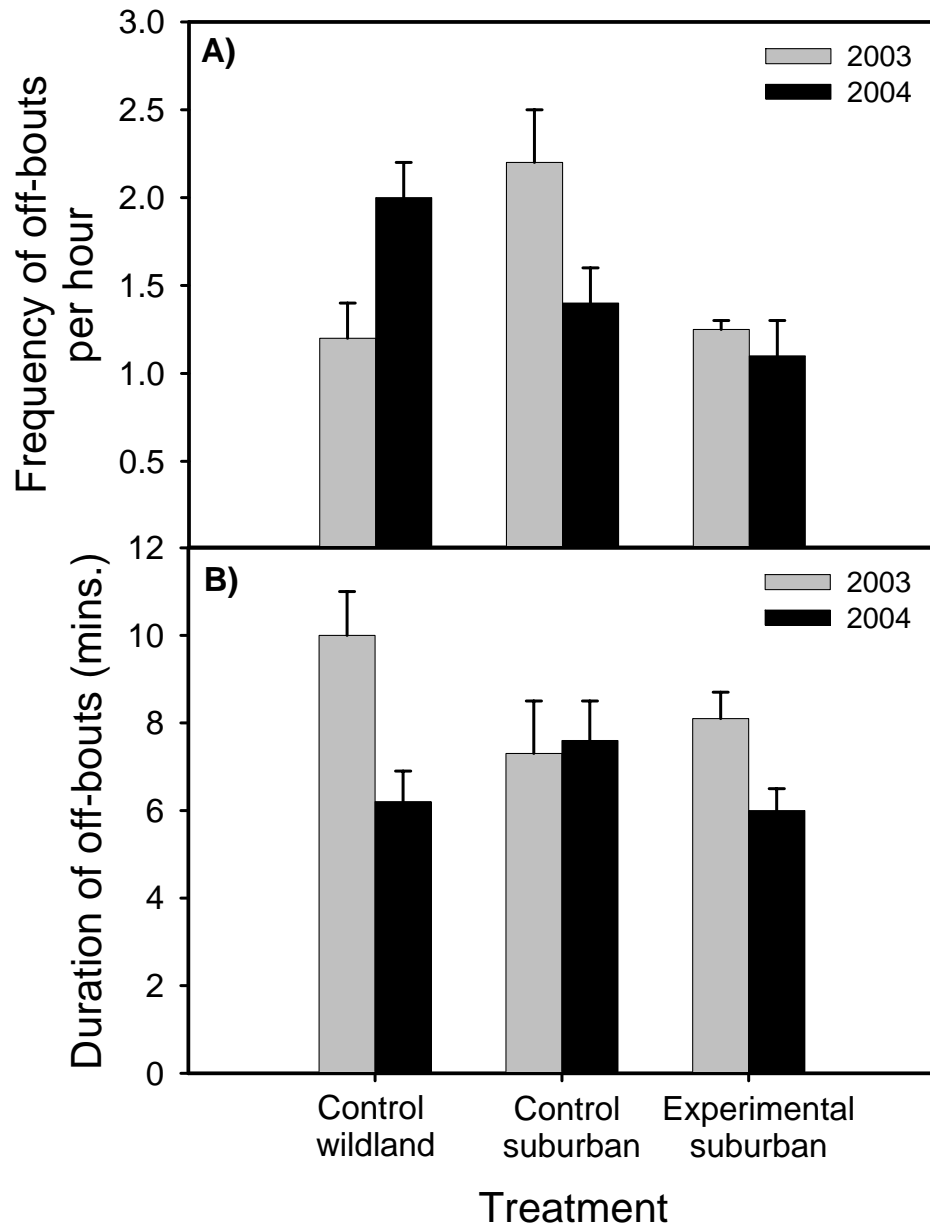


Figure 4: (from LeClair 2005) Treatment differences in **A**) frequency and **B**) duration of off-bouts during the predation risk experiment in 2003 and 2004

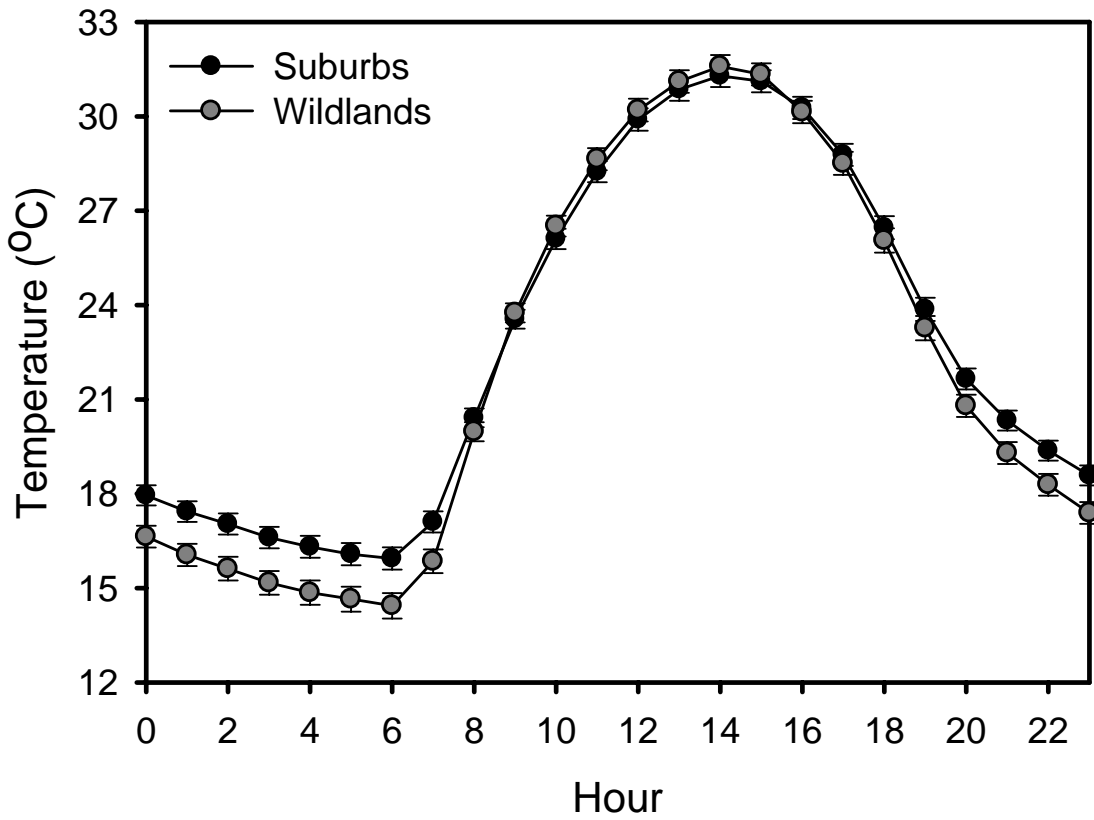


Figure 5: Mean daily ambient temperature in the suburbs and wildlands during the breeding season from 2005-2007

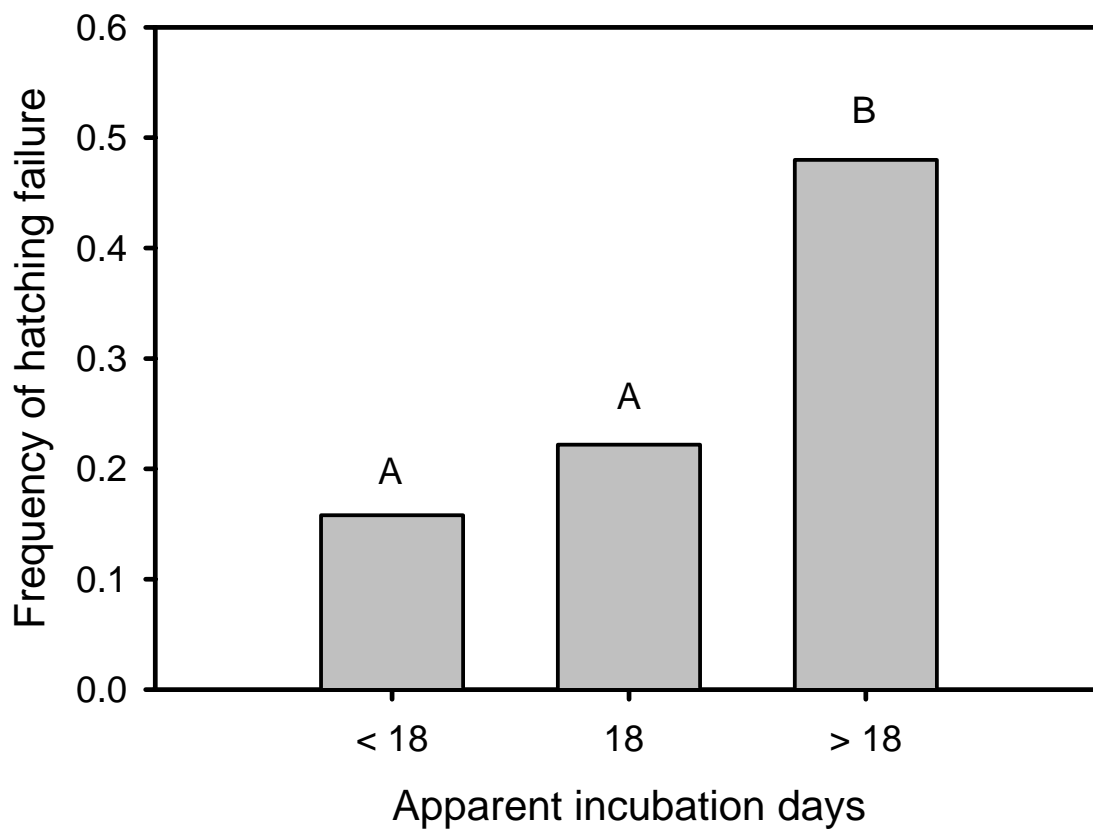


Figure 6: Relationship between frequency of partial hatching failure and number of apparent incubation days

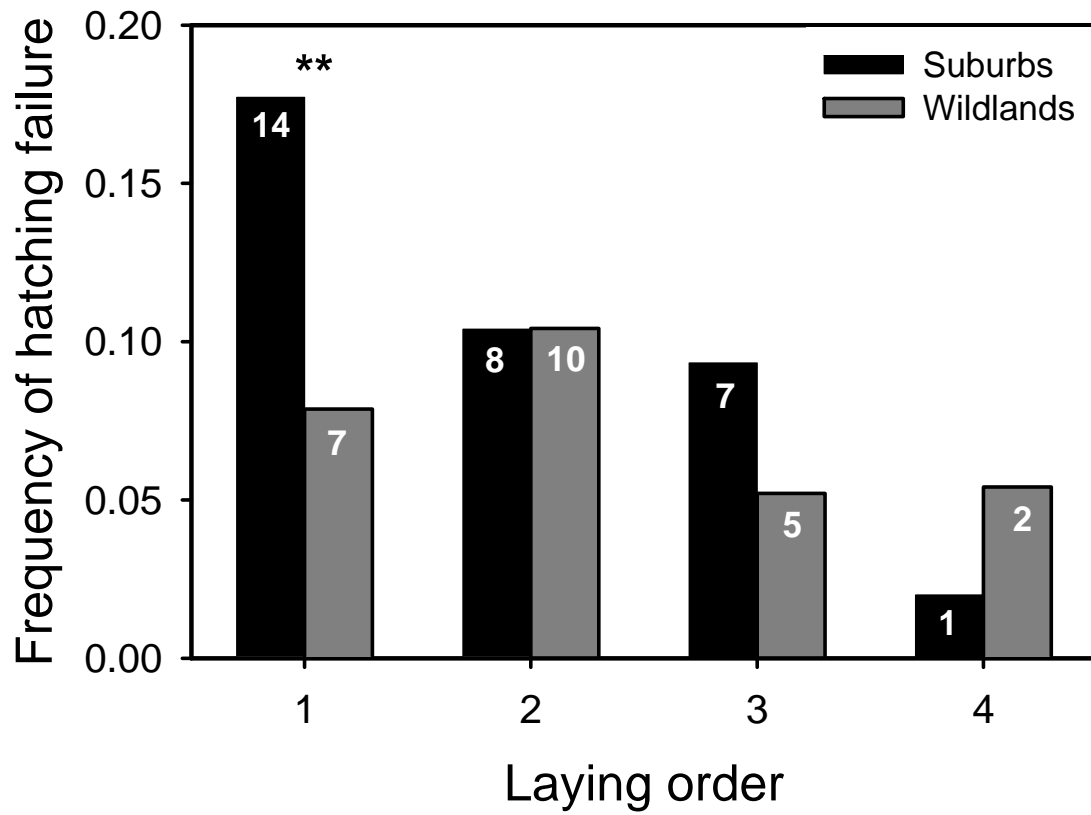


Figure 7: Relationship between partial hatching failure and laying order in the suburbs and wildlands

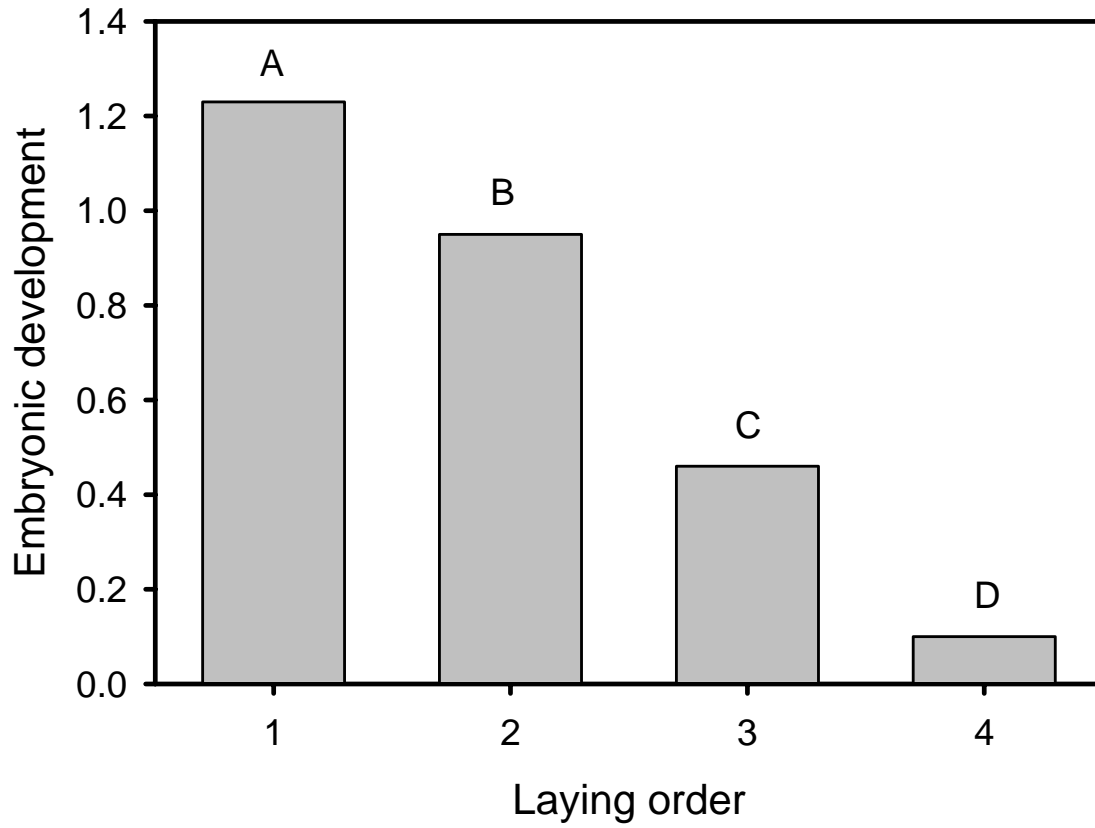


Figure 8: Relationship between embryonic development and laying order

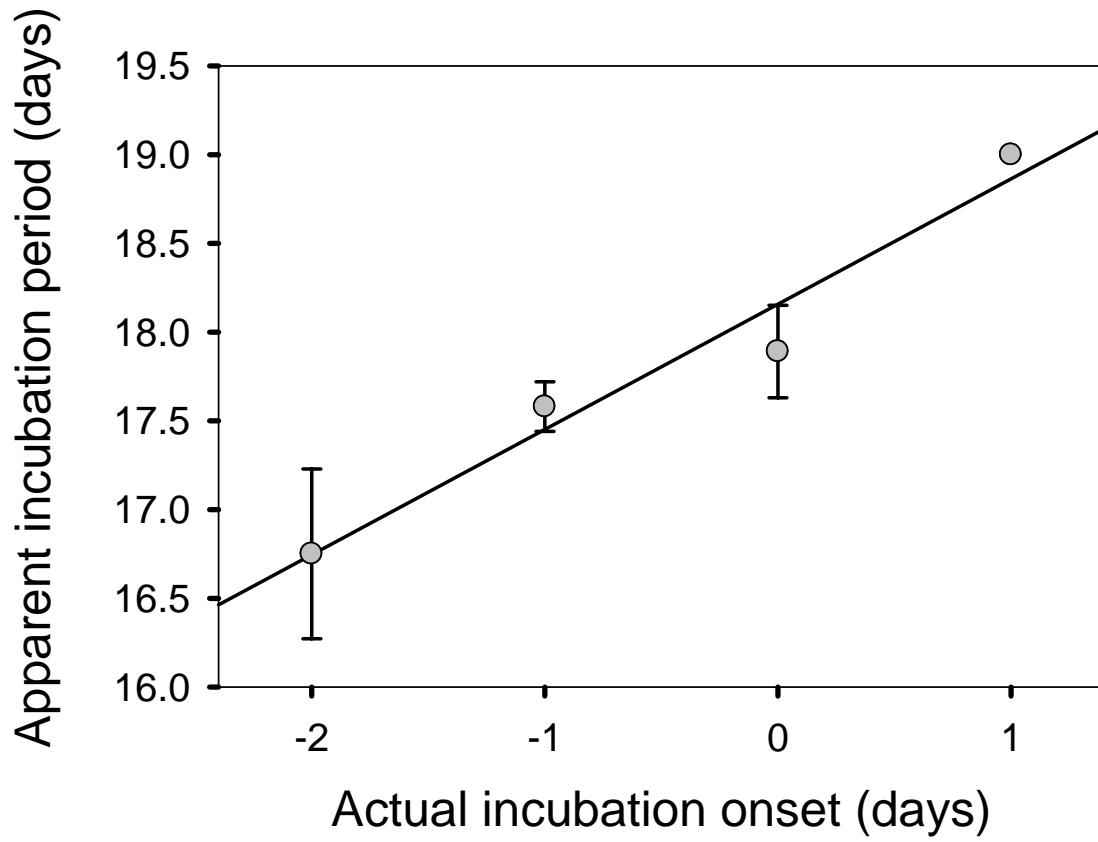


Figure 9: Relationship between apparent incubation days and incubation onset

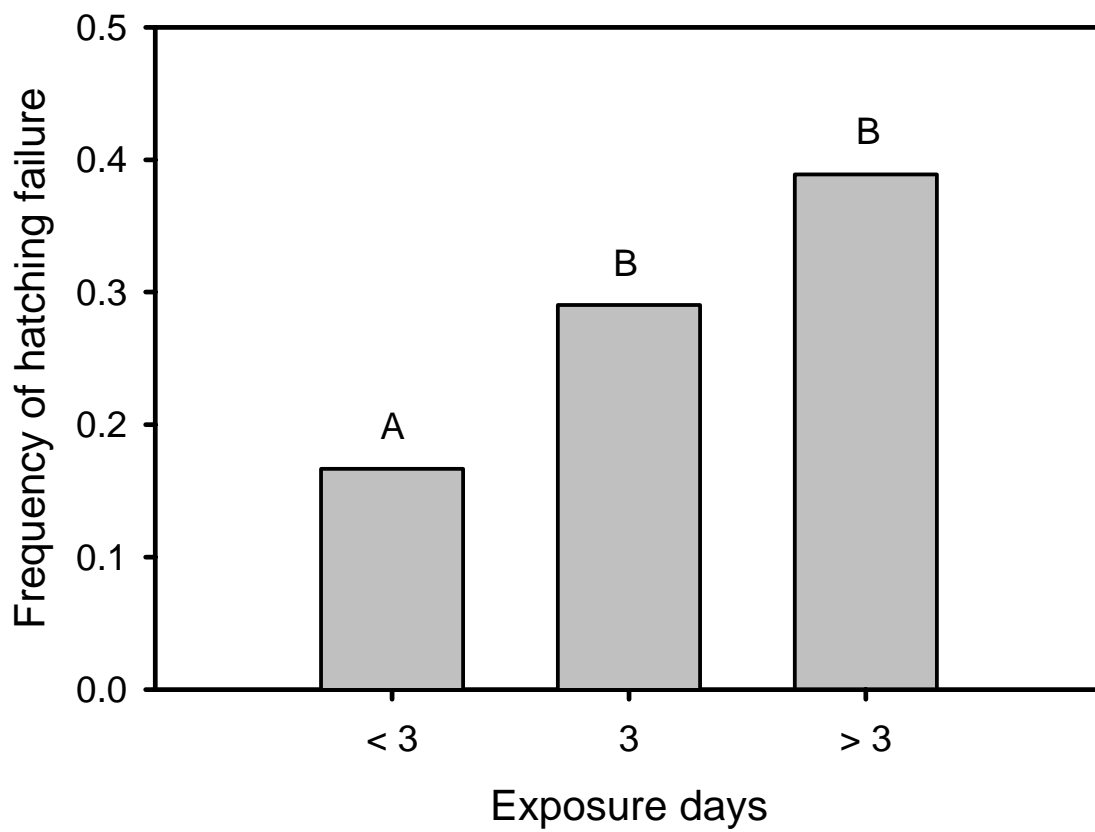


Figure 10: Relationship between partial hatching failure and exposure of clutches to ambient temperature

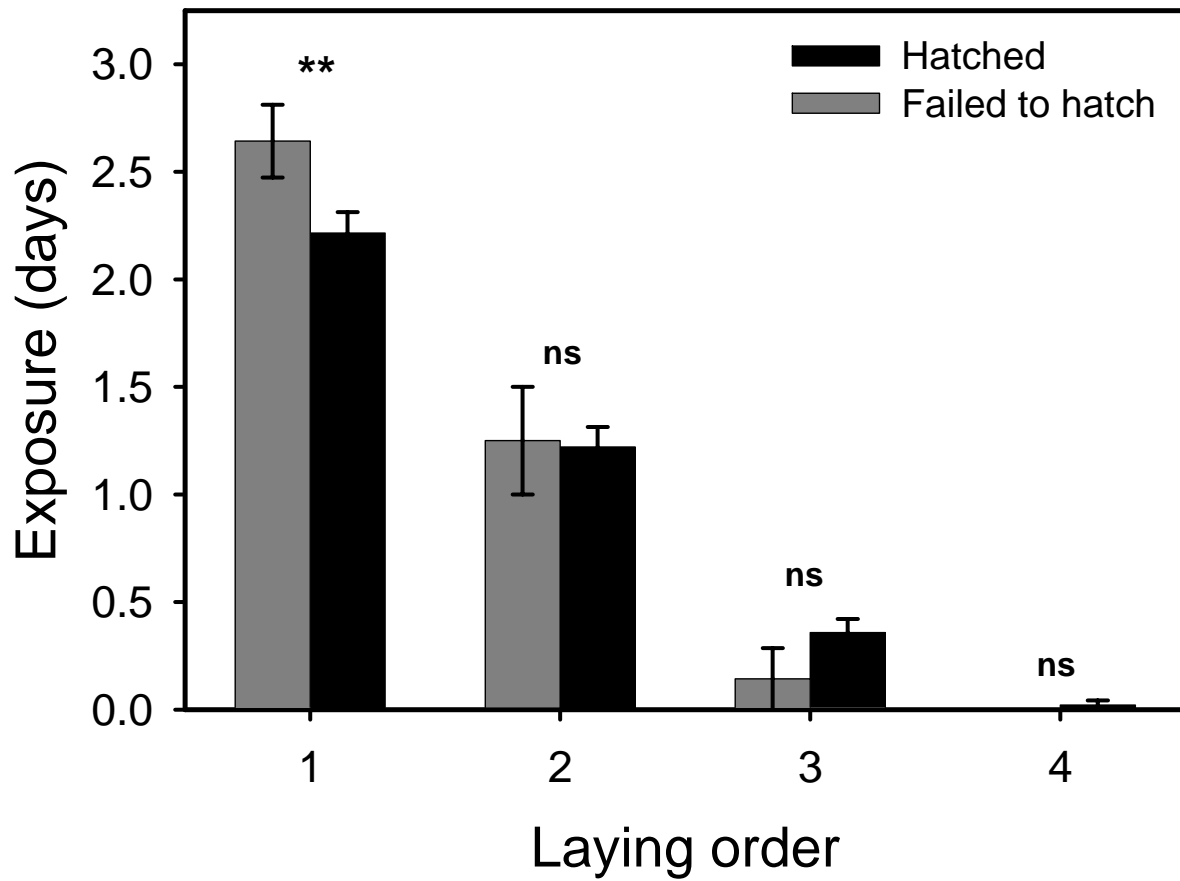


Figure 11: In suburban nests, the relationship between exposure (in days) to ambient temperatures for eggs that hatched and eggs that failed to hatch relative to laying order

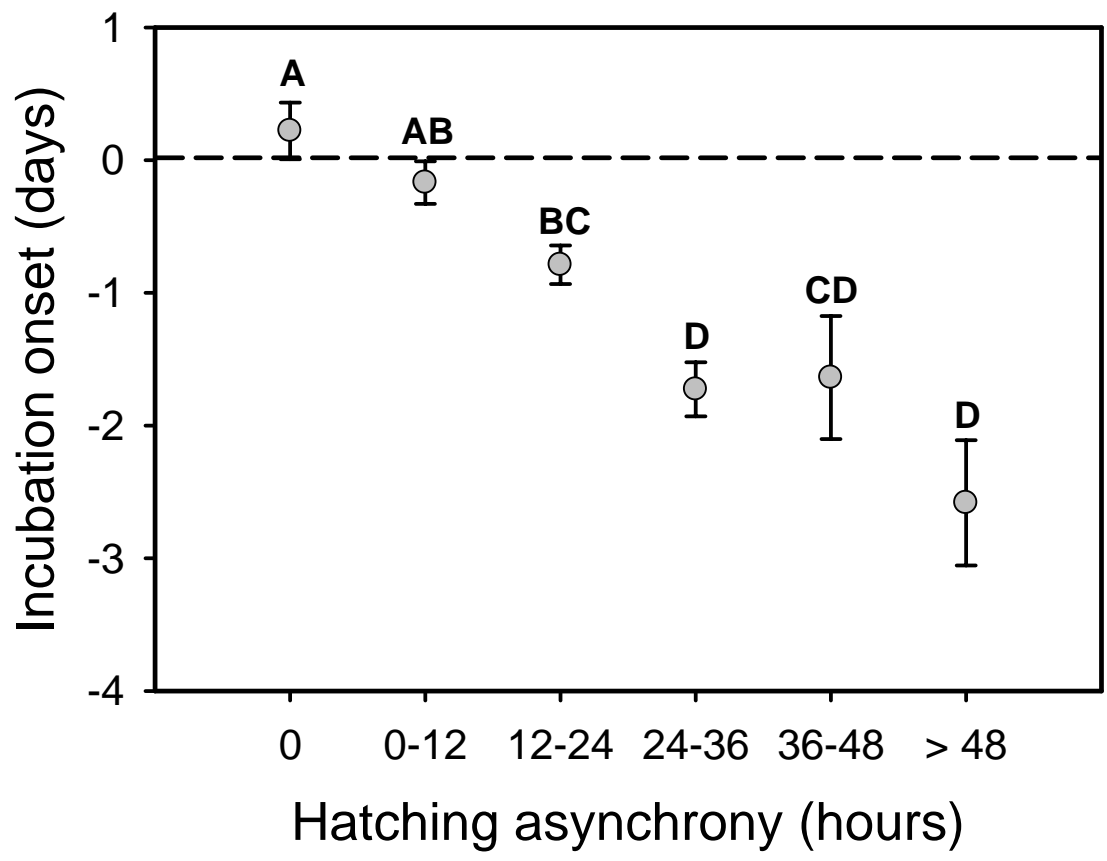


Figure 12: Relationship between incubation onset and hatching asynchrony within nests

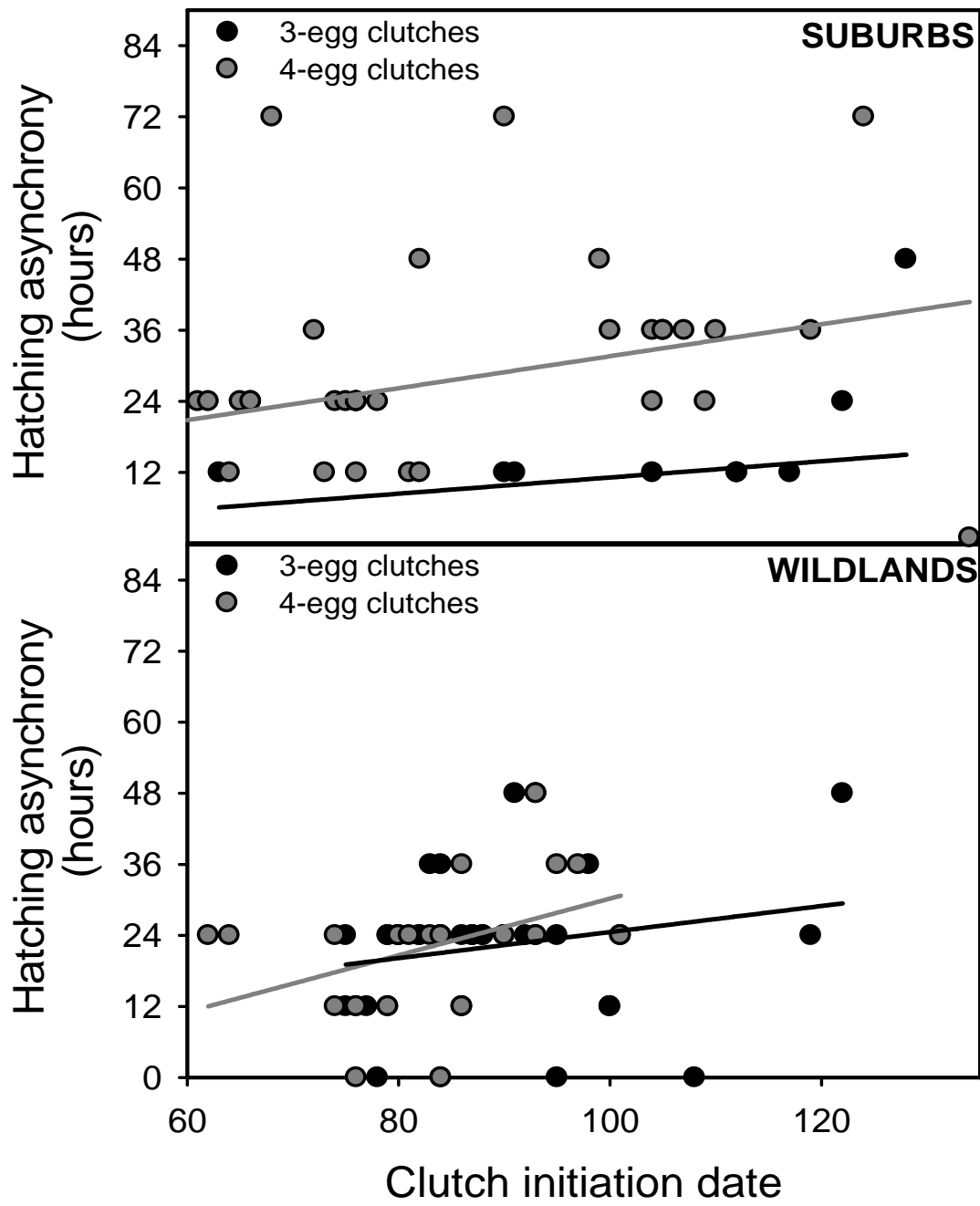


Figure 13: Seasonal patterns of hatching asynchrony in 3 and 4-egg clutches within suburban and wildland nests

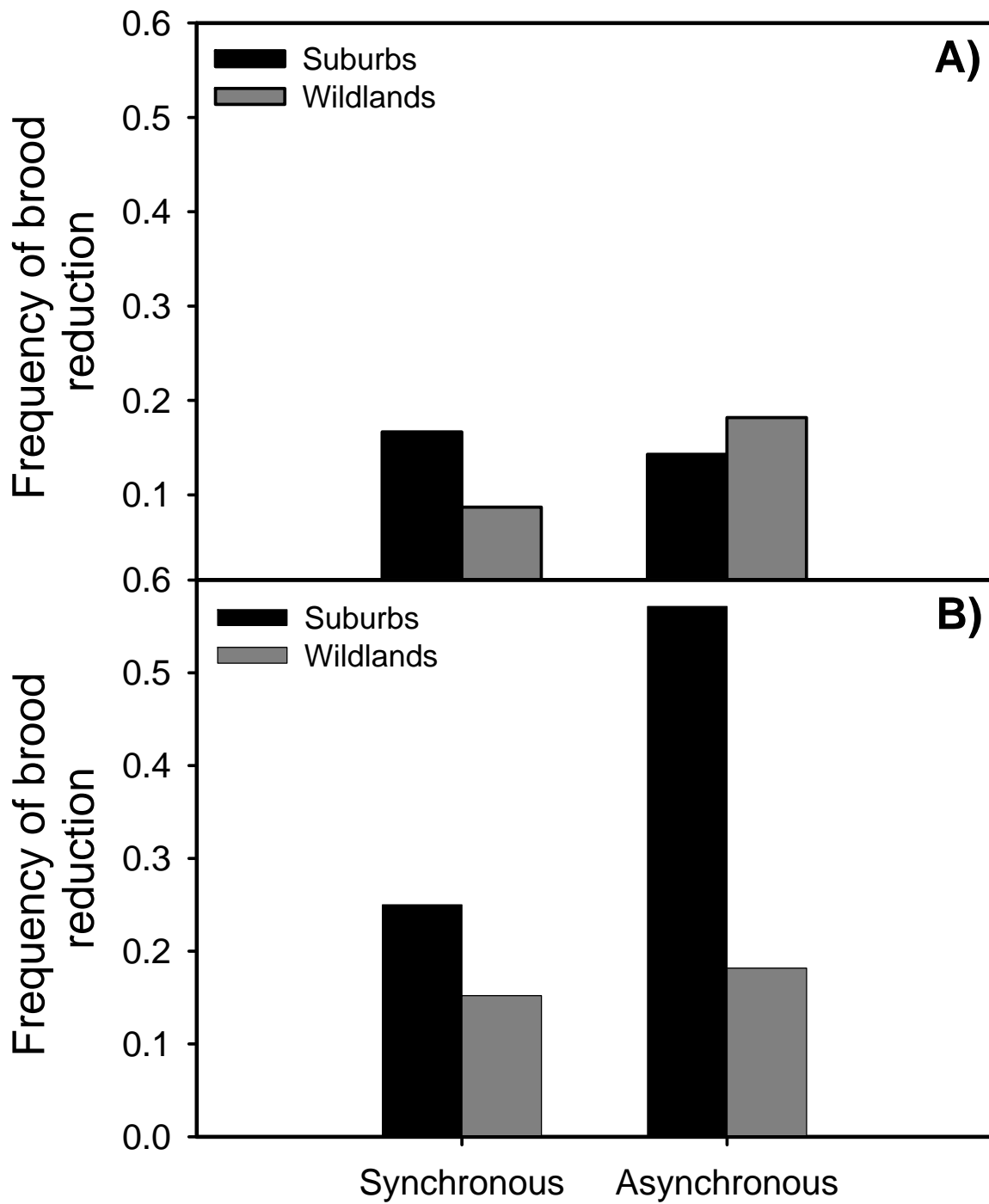


Figure 14: Site-specific differences in brood reduction **A)** early in the nestling period and **B)** late in the nestling period within synchronous and asynchronous nests

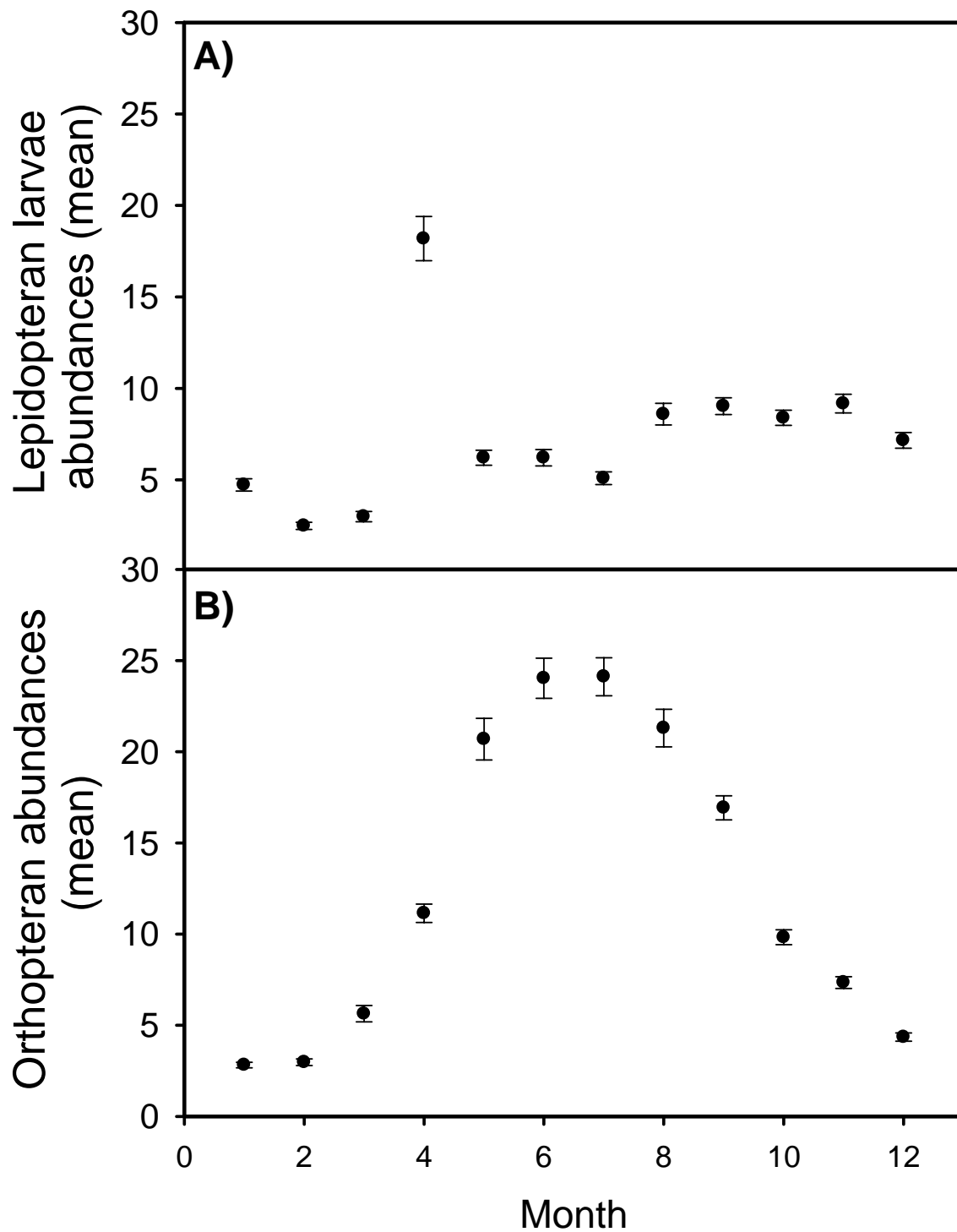


Figure 15: Mean monthly nestling food (A) lepidopteran larvae and (B) orthoptera) abundances from 1988-2000

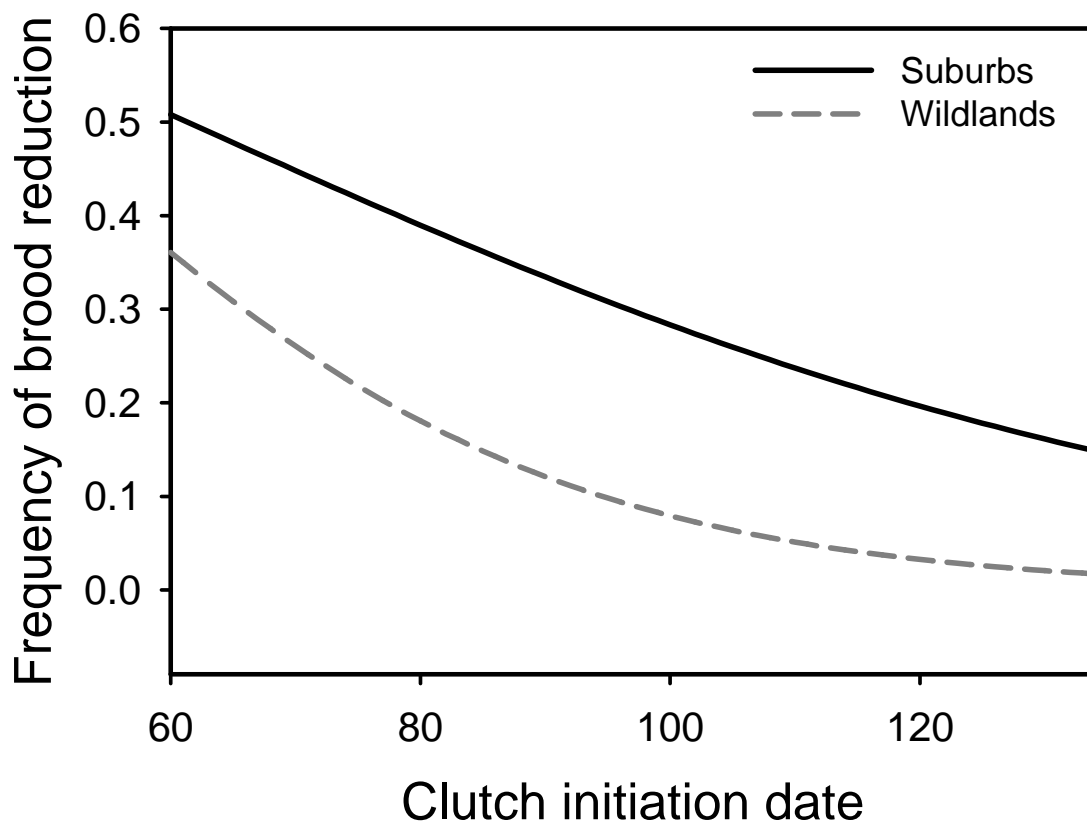


Figure 16: Differences in seasonal patterns of brood reduction between suburbs and wildlands

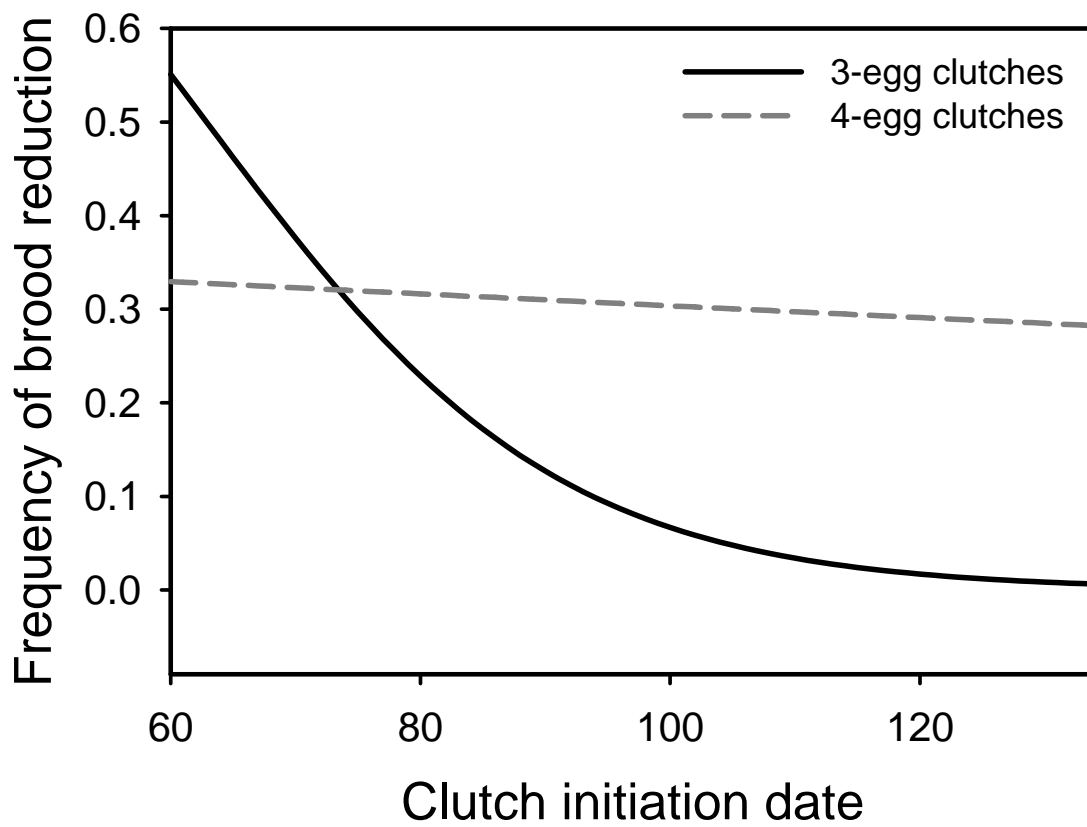


Figure 17: Differences in seasonal patterns of brood reduction between 3 and 4-egg clutches

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