PRUDENT MOTHERS? PATERNAL INVESTMENT, FEMALE REPRODUCTIVE STRATEGIES AND OFFSPRING DEVELOPMENT IN THE BARN OWL (*TYTO ALBA*)

By

MEGAN L. SEIFERT

A dissertation submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY
(Zoology)

WASHINGTON STATE UNIVERSITY
School of Biological Sciences

December 2007

To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of Megan Lee Seifert find it satisfactory and recommend that it be accepted.

____
Chair

____

____

ACKNOWLEDGEMENTS

I would like to thank my husband for his unconditional support and help—with fieldwork, editing, and in so many other ways. I would like to thank my parents who always encouraged and supported me—especially in my academic aspirations. A big "thank you" goes to my supportive family, especially my grandmother who is always incredibly patient and kind, and my siblings for putting up with my erratic communication needs. Thanks also to Kitty and Barry Tuscano for general support and help in the field. I would like to thank Kimberly and Finnegan King for being wonderful roommates and providing lots of laughs and support during my last semester. There are too many friends who have listened to me, encouraged me, and supported me along the way to list here. Just know that I am grateful.

Steve Simmons deserves special thanks for teaching me how to handle barn owls, and for providing critical help with field logistics and fieldwork. Without his assistance, my field data would never have been collected.

I would like to thank all of my field assistants: BJ Greenburger, Courtney Cummings, Danielle Clay, Ross Crandall, Ann Graham, and Beckett Hills, as well as lab assistants Brian Skjei and Kristin Thompson for volunteering their time to help make my project possible. I thank Barley Hyde for entering data and commenting on my first two chapters. I would like to thank the many landowners who let me use their barn owl boxes, especially the Gallo Wine Company, which also provided me with field housing for two seasons.

I would like to thank Mark Stanback, an amazing mentor, friend, and colleague. Without Mark's help I would not be working with barn owls, and probably would have dropped out of graduate school long ago! My thanks go to him for always being willing to write recommendations, read proposals, listen and offer advice. I would like to thank my lab mates, especially: Letty Reichart, for teaching me how to extract DNA and do sexing reactions, Jeremey Egbert for all of his help fixing problems in the lab, and Michelle Beck and Jeremy for all of their advice and support along the way. I would like to thank my committee, Patrick Carter and Daniela Monk, for all their help. Thanks to Marc Evans for helping me figure out how to analyze my complex data and for being a patient statistics teacher. Lastly, I would like to thank Mike Webster and Hubert Schwabl for all of their help, advice, and for always finding the money for me to get my lab work done.

My work was supported by an Animal Behavior Society graduate grant, a James R. King Fellowship (Department of Biology, Washington State University), and a McNeil Award (Department of Biology, Washington State University). I also received travel support from the American Ornithologists Union to present some of my research.

PRUDENT MOTHERS? PATERNAL INVESTMENT, FEMALE REPRODUCTIVE STRATEGIES AND OFFSPRING DEVELOPMENT IN THE BARN OWL (*TYTO ALBA*)

ABSTRACT

by Megan L. Seifert, Ph.D.
Washington State University
December 2007

Chair: Hubert Schwabl

Parental investment directly affects offspring survival. If this investment is not equal across offspring, unequal or differential survival will occur among progeny. In birds, mothers affect the success of the nest and of individual offspring by clutch/brood size, egg size, brood sex ratio (especially in size dimorphic species), maternally derived yolk hormones, and nestling size hierarchies (caused by laying interval and onset of incubation). In the barn owl (*Tyto alba*), males provide their partners with food throughout egg laying and halfway through the nestling phase. By measuring male food delivery, we were able to quantify food resources available to females during reproduction and relate food to reproductive investment. We measured clutch size, brood size, egg mass, yolk hormones, and sex ratio differences within and among broods to examine the differing ways in which females invest in their offspring. Brood sex ratios were not related to current food resources, but more resources allowed parents to produce more offspring and fledge more offspring successfully. Population sex ratios did not follow Fisher's theory of equal allocation; parents fledged equal numbers of male and female offspring in spite of sexual size dimorphism and a significantly male biased hatching sex ratio. Barn owl mothers differentially allocated yolk hormones to eggs. The first 3-4 eggs (depending on clutch size) laid had increasing androgen levels with each successive egg and then androgen levels decreased in later laid eggs. Eggs laid later in the laying order were more likely to be male, partially supporting the sharing-out hypothesis. We found higher survival in early-hatched than in late-hatched nestlings. Male and female offspring had similar circulating plasma androgens and corticosterone concentrations. At an age when tarsus growth had reached its maximum, nestling testosterone levels were positively related to body condition. Circulating corticosterone levels increased with nestling age. Additionally, later-hatched nestlings had higher corticosterone levels; corticosterone was negatively related to body condition at two different points of the nestling phase. These results support the hypothesis that corticosterone plays a role in facilitating honest signaling of nestling need to parents. Thus parental decisions influence offspring fitness in a variety of ways.

TABLE OF CONTENTS

CHAPTER FOUR: PLASMA TESTOSTERONE AND CORTICOSTERONE LEVELS OF DEVELOPING BARN OWLS (*TYTO ALBA*): RELATIONSHIP WITH SEX, BROOD SIZE, HATCHING POSITION, AND CONDITION.....85

LIST OF TABLES

LIST OF FIGURES

DEDICATION

In memory of my father, Charles Seifert, who worked hard to put me through high school and college. He always encouraged me to work a little bit harder, but was never disappointed by my accomplishments.

CHAPTER ONE

INTRODUCTION

BACKGROUND: REPRODUCTIVE OPTIMIZATION

One of the most active areas of behavioral ecology research is the investigation of adaptation toward optimal reproductive strategies. Reproductive optimization states that parents should be selected to optimize their reproduction in response to current environmental conditions. In species with parental care, parents should try to raise as many offspring as possible without compromising their own survival or the condition of their offspring (Forbes and Mock, 2000). However, parents shouldn't produce fewer offspring than they can raise in their current environment. Natural selection should favor parents that produce the optimal number of offspring for their environment (Daan et al., 1990) with the optimal amount of investment in each offspring according to its likelihood of future reproduction (Carranza, 2004).

Environmental resources are often unpredictable. In many species of animal, this necessitates starting reproduction before the quantity and quality of resources that will be available during reproductive functions and rearing can be known. Thus parents may not be able to optimally match their reproductive output to current conditions. In such cases, parents must make tradeoffs between the risk of wasted investment through overproduction of offspring, and the risk of producing too few offspring and failing to maximize their output. One potential parental tactic is the strategic overproduction of offspring, leading to deliberate sibling hierarchies and brood reduction. Mock and Forbes (1995) found that parents that overproduce offspring can still produce a group of advantaged progeny that do well, despite the lower fitness and survival of disadvantaged offspring. Sibling competition can become a mechanism for resource competition within the nest, creating a "survival of the fittest" environment. In times of abundant resources, all offspring may survive; however, when food is not readily available, only the most fit individuals in the brood will survive (Kozlowski and Stearns, 1989; Forbes and Mock, 1998; Mock and Parker, 1998a; Forbes and Mock, 2000). There are energetic costs to sibling competition for both parents and offspring, especially when siblings are evenly matched (Forbes and Mock, 1998). Siblings spend energy on competition that could otherwise be directed to growth, thus reducing fitness; parents waste energy because brood reduction via sibling competition can take a long time. Over the duration, the parents provision all offspring, even though those that will eventually die, offering no reproductive value. One way that parents can accelerate offspring competition toward brood reduction is by pursuing a strategy of enhancing the competitive abilities of certain offspring, also known as parental favoritism.

Parental favoritism occurs when parents enhance the condition and/or fitness of some offspring at the expense of others. The adaptive significance of parental favoritism, and also the mechanisms underlying it, have been the focus of numerous studies over the past several years (Mock and Parker, 1998b). Parental favoritism may be a mechanism to increase fitness by dedicating resources to offspring that are most likely to survive and offering them the best chance for future reproductive success. Most studies of parental favoritism have focused on egg size as a measure of energetic investment (e.g. Williams, 1994; Muller et al., 2005), and behavioral strategies of parents, such as preferential feeding of some young and asynchronous incubation, both of which can influence sibling rivalry (see review by Magrath, 1990).

Birds make excellent models for studying reproductive optimization because there are many stages of development and mechanisms of allocation that are subject to parental manipulation. Clutch size, egg quality, egg hormone content, asynchronous incubation of the eggs (resulting in an asynchronously hatching, age-structured brood of siblings), and sex ratio are all potential ways in which maternal favoritism can foster or mitigate brood hierarchy. Females can differentially allocate resources and hormones to individual eggs even before they are laid. The discovery that the eggs of a clutch contain variable concentrations of maternally derived anabolic steroid hormones (Schwabl, 1993a) has suggested a subtle physiological mechanism for parental favoritism by hormonal effects on nestling growth and competitive behavior (Schwabl et al., 1997b).

The order in which offspring of different sex and quality are laid into a clutch can affect the success of each progeny and, in turn, the reproductive success of the parents. Sexual size dimorphism, sex ratio, brood size, hatching asynchrony, and laying order can all have a profound effect on each nestling's within-brood environment. Females may be able to influence some of these variables by laying eggs of difference size, varying the contents of the eggs, such as maternal hormones, and the sex ratio of and order in which the eggs in a clutch are laid. For example, the amount of energy that each offspring receives in the form of egg mass is easy to quantify. It is also possible to quantify egg constituents, such as the maternally derived yolk androgens that each offspring receives.

In various bird species there are differing degrees of sexual size dimorphism. When one sex is larger than the other, the size disparity may create variable competitive abilities among siblings. If nestlings of the smaller sex are laid before any of the larger sex, it may help them compete. On the other hand, if they are laid after the larger sex they may be disadvantaged. Thus, the order in which eggs of different sexes are laid can be a form of parental favoritism in birds.

The timing of incubation initiation determines the presence and degree of sibling hierarchies for each species. For instance, where females begin incubation as soon as the first egg is laid, and keep laying one egg/day until the clutch is complete, eggs hatch one day apart and first-hatched nestlings will have a clear size and competitive advantage throughout nestling development. Nestlings from later-laid eggs will become runts and will be less likely to survive until fledging. Hatching asynchrony is a tool that parents may use to pace sibling competition. The sooner after laying incubation begins, the longer the laying interval, and the larger the clutch size, the more pronounced the resulting brood size hierarchies will be.

I investigated parental optimization strategies in a wild population of barn owls (*Tyto alba*). I examined several mechanisms of parental optimization: hatching asynchrony, sex ratios among and within broods, yolk hormone concentrations and variation in egg mass as a measure of energy content. I also followed up clutches to see how these factors affect nestling condition, survival, and their circulating hormone levels.

MECHANISMS OF REPRODUCTIVE OPTIMIZATION

Hatching Asynchrony

There are many ways that parents can handicap some offspring and favor others. In birds, one strategy is to establish an age-structured brood hierarchy by beginning incubation before all eggs are laid. This results in a nest environment in which, at any given time during nesting, first-hatched offspring are older and larger than later-hatched offspring. The size and age hierarchies that result from hatching asynchrony benefit earlier-hatched nestlings at the expense of later-hatched nestlings. It has been proposed that these hierarchies help shorten the duration of sibling competition by hastening the death of the least competitive nestling(s) (e.g. Mock et al., 1987; reviewed by Magrath, 1990). Therefore, the order in which eggs are laid can have profound consequences for the fitness and survival of resulting nestlings.

Sex Ratio

Another potential parental strategy is facultative adjustment of offspring sex. Although the physiological mechanism for facultative adjustment of sex ratio in birds has not been discovered, many studies have shown extreme sex ratios correlated to food availability or other environmental factors (see review by Pike and Petrie 2003), suggesting the possibility of maternal sex ratio manipulation.

Selection favors an equal overall investment in sons and daughters (Fisher, 1930). Sex allocation theory predicts that parents should produce females and males in equal numbers and provide equal parental care. In species with sexual size dimorphism, Fisher's theory predicts that parents should produce more of the less costly sex to achieve equal investment. Whereas Fisher's theory makes sex ratio predictions at the population level, each individual breeding pair (nest) is affected by its own current environmental conditions (i.e. food resources). Hence, at the level of individual pairs, there is the possibility that sex ratios will deviate from those predicted by Fisher (1930) based on each set of parents' condition and success at providing food for their offspring. Consequently, the Trivers-Willard hypothesis (1973) proposes that mothers in better condition should invest more by producing the more costly sex, while mothers in poor condition should produce the less costly sex. This theory was originally designed to explain maternal investment in polygynous ungulates, like red deer that produce a single offspring at a time, and may be less appropriate for making predictions about species that produce many offspring per reproductive bout. Myers (1978) updated the Trivers-Willard hypothesis to account for species with multiple offspring per litter (brood) and little variance in future reproductive success. Her hypothesis also predicts that females in good condition should bias offspring sex ratio toward the more costly sex, while females in poor conditions should bias offspring sex ratio toward the less expensive sex. Her argument is that, in species producing multiple offspring, parental success is based on the number of offspring successfully produced. Hence, sex ratio biasing should occur early in development. Her rationale is that because the alternative—differential mortality—entails a loss of reproductive potential for parents, it is not a likely mechanism of sex ratio biasing. However, overproduction of the less costly sex is an attempt to maximize reproductive output by minimizing offspring mortality and producing as many offspring as possible.

In 1986 Clutton-Brock reported sex ratio variation in birds (Clutton-Brock, 1986). Since then, numerous studies have shown sex ratio variation in many different avian species (reviewed by Pike and Petrie, 2003). Birds provide excellent model systems to study facultative adjustment of offspring sex ratio because females are the heterogametic sex (ZW), and therefore it is the female's egg, and not the male's sperm, that determines the sex of the offspring. In addition, with the evolution of molecular sexing, in some cases even un-hatched eggs can be sexed, allowing researchers to determine primary sex ratio (the laying of male and female eggs). To date, most studies have examined the sex ratios at hatching or fledging and the potential adaptive benefits of different sex ratios (reviewed by Pike and Petrie, 2003).

Yolk Hormones

Maternally derived steroid hormones, such as the androgen testosterone in egg yolks, influence nestling development (see a review by Groothuis et al., 2005). The most common pattern of variation of yolk androgens in a clutch is higher concentrations in the later-laid eggs, for example in canaries (*Serinus canaria*), kestrels (*Falco sparverius*) and black-headed gulls (*Larus ridibundus*) (Eising et al., 2001; Schwabl, 1993b; Sockman and Schwabl, 2000). Other species, such as cattle egrets (*Bubulcus ibis*), show the opposite pattern; they have decreasing androgen concentrations with laying order (Schwabl et al., 1997a). Since these yolk androgens increase growth and begging in canary (*Serinus canaria*), starling, and gull nestlings (Schwabl, 1993b; 1997; Lipar et al., 1999; Eising et al., 2001; Lipar, 2001; Eising et al., 2003), the differential levels of androgens in the eggs of a clutch has been proposed as a mechanism of maternal favoritism and a means to mitigate or enhance the effects of hatching asynchrony (Schwabl et al., 1997a).

Egg Mass

Avian egg size (and presumably resource content) sometimes varies predictably with laying order; for example, in gulls and terns, egg size decreases with laying position (e.g. Nager et al., 2000; Fletcher and Hamer, 2004). Passerine eggs, in contrast, tend to increase in size with laying order (e.g. Clark and Wilson, 1981; Slagsvold et al., 1984). Nestlings hatching from larger eggs can experience increased growth and fitness (Williams, 1994; Christians, 2002), presumably because larger eggs have more resources that can provide advantages to resulting nestlings. Like yolk steroids, egg mass variation across the clutch can either mitigate or enhance the effects of hatching asynchrony. Mothers can potentially mitigate nestling size hierarchies by increasing the mass of later-laid eggs or increase the nestling size hierarchy by decreasing egg mass in later laying positions.

THE EFFECT OF FOOD ON FEMALE STRATEGY

In many birds, the rate of food delivery to nestlings is a critical factor in the reproductive success of a pair. Quantifying the number of offspring produced in relation to food availability can be difficult. However, in some birds, such as some species of hornbill and the barn owl, females are completely reliant on their mates for food during the laying, incubation, and some of the nestling stage. In these species, the amount of food delivered to the nest can readily be quantified to determine the amount of food available to mothers. It is then possible to determine the effect of food on, for example, clutch size, sex ratio, and egg mass. In species with sexual size dimorphism, offspring sex ratio can be an important variable in parental energy expenditure because it is often more costly to raise offspring of the larger sex. Therefore, when food supplies are low, it may be beneficial for mothers to produce more of the smaller sex. However, if food availability is unpredictable, females sometimes lay more eggs than the number of offspring they will be able to raise. Because producing the greatest number of healthy offspring is critical to a parent's fitness, it has been hypothesized that extra eggs are a form of insurance (Ricklefs, 1977). In years when food is abundant, parents are able to feed and raise all their offspring; in other years the least fit, or smallest offspring (those hatching from "insurance" eggs) will die.

CONSEQUENCES FOR NESTLINGS

Circulating Nestling Plasma Hormones

Levels of circulating testosterone and other androgens are thought to be higher in nestlings facing increased levels of competition. Higher levels of testosterone are thought to suppress nestling immune systems, increase stress hormone levels (e.g. Olson and Kovacs, 1996; Muller et al., 2003; Fargallo et al., 2007), and negatively affect molt, growth, and fat deposition (see Ketterson et al., 1996; Groothuis et al., 2005). Fargallo et al. (2007) found that experimentally elevated levels of testosterone negatively impacts nestling immunity, coloration and growth. Moreover, nestlings with higher levels of testosterone are more competitive and beg more in the nest (Goodship and Buchanan, 2006; Quillfeldt et al., 2006). Therefore, increased levels of testosterone may be beneficial if they help a nestling beg, but they can also be detrimental to its survival. I measured testosterone levels in nestling barn owls and related them to hatching order and clutch size, the factors that likely influence competition among siblings.

The adrenalcortical system releases glucocorticoids, primarily corticosterone, a metabolic and stress hormone. Birds under stress due to lack of food, competition, or potential predation, have increased corticosterone levels (e.g. Kitaysky et al., 2001). According to Kitaysky et al., nestlings with higher levels of corticosterone beg more than nestlings with lower levels, and corticosterone levels increase when nestlings are in poor condition (2001). Therefore, smaller, competitively disadvantaged nestlings are predicted to have higher levels of corticosterone than their larger dominate siblings.

Nestling Survival

Although parental reproductive optimization strategy does not always include the survival of all offspring, it is beneficial for each individual offspring to adopt tactics that improve its odds of survival. In some cases, parental favoritism plays a role in a parental strategy known as brood reduction. For example, parents can preferentially feed some nestlings, and perhaps the mother can enhance the growth of certain offspring by allocating more resources to their eggs or bestowing eggs with more anabolic hormones. The less favored nestlings are then more likely to develop slower and die.

SUMMARY

In birds, environmental conditions influence parents' reproductive strategies, and parents can affect the success of individual offspring, as well as the whole clutch, through variation in clutch size, extent of sibling hierarchies in a brood (via onset of incubation and preferential food delivery), maternally derived yolk androgens, egg size, and brood sex ratio. Each of these variables is likely to affect the success of individual offspring as well as the entire nest. The interactions between some or all of these variables may be more telling of parents' investment or favoritism toward certain offspring than any one by itself (reviewed by Sockman et al., 2006). Many studies have examined just one or two of these strategies, but important information may be missed by thinking of them as individual qualities rather than a suite of behavioral and physiological traits that make up parental reproductive strategy. I investigated a suite of parental strategies, including egg mass, nestling sex, laying order, and yolk hormones in a bird species with unique natural history traits.

STUDY SPECIES

The barn owl (*Tyto alba*) is a nocturnal raptor that is found on every continent except Antarctica. The barn owl is an excellent model species for addressing questions about how parental strategies optimize their reproductive output and how they affect their nestlings. Barn owls have extreme hatching asynchrony, caused by long inter-egg intervals (2-7 days), and an onset of incubation with the first egg. Barn owls are reverse sexually size dimorphic (RSSD, females larger than males), making females potentially more costly to raise than males. Barn owls are locally plentiful and willingly nests in man-made boxes, thus reducing concerns of insufficient sample size and eliminating nest searching. Barn owls are large enough to tolerate the taking of periodic blood samples and, likewise, their eggs are sufficiently large to collect yolk samples from without endangering development.

From egg laying until halfway through brooding, female barn owls are completely reliant on their mates for food. Females remain in the nest box and their mates bring them food at dusk and again before dawn. It is thought that better quality males are able to obtain more food, making provisioning an obvious signal of male quality for females (Wallace, 1948). Even though barn owls are large birds (females 500-700g and males 400-500g), the male alone must supply the female with enough energy to produce relatively large clutches. The clutch size ranges from 3-12 eggs; each egg weighs between 20 and 31 grams (Seifert, pers. obs.). Thus, females must invest a lot of energy to produce a clutch. The barn owl also provides us with an opportunity to study nest development over an extended period of time. In most bird species, egg laying, incubation, and the nestling phase through fledging last only a few weeks; however, barn owls have a 30-day incubation stage and a 60-day nestling phase. After approximately 30 days of brooding, females begin leaving the nest to hunt, and both parents feed their nestlings until fledging. In barn owls, the exact fledging date is hard to determine because nestlings continue to roost in their nest box for up to two weeks after their first flight. During that time adults teach their nestlings to hunt; afterward there is little to no parental care.

STUDY SITE

We choose to study a large population of barn owls in the Central Valley of California near Merced (37.3° N, 120.5° W). Because much of the Central Valley is intensively farmed, and because rodents are abundant agricultural pests, farmers in the region have opted for free rodent control in the form of numerous barn owls nest boxes. Even though barn owls are declining in much of their range, the species is healthy in California, presenting a large wild population.

PURPOSE AND GENERAL APPROACH

The overall purpose of this study was to investigate parental optimization in relation to current food supply and its effects on nestling survival, growth, and androgen and stress hormone levels. I hypothesized that parental optimization strategies would be pronounced in the barn owl because of its large clutches, extreme hatching asynchrony, and RSSD. The resulting competitive sibling size hierarchies in barn owl nests would likely have important impacts on nestling condition and survival. This study was conceived to help elucidate some of the effects of parental reproductive strategies on nestling survival and fitness.

I conducted 3 studies that comprise chapters 2-4, all done with free-living barn owls. In chapter 2, I investigate the population sex ratio at hatching (primary) and fledging (secondary) to test Fishers hypothesis (1930) of equal investment in male and female offspring. I also examine how sex ratios of each nest vary in relation to the food supplied to the female by the male in order to test Myers (1978) hypothesis of adjustment of primary sex ratio to the current environmental conditions.

In chapter 3, I investigate maternal allocation of resources to barn owl eggs. I report on how egg mass, sex, and yolk androgens change with laying order. I test the sharing out hypothesis (Carranza, 2004), stating that females should invest more resources in the offspring that are most likely to survive because those investments will have the greatest payoff. I also examine how hatching position affects nestling survival.

In chapter 4, I report on how nestling laying position and sex affect circulating plasma androgen and corticosterone levels. This study compliments the chapter on parental allocation (chapter 3), because it examines the physiological response to parental favoritism. I test the male disadvantage hypothesis—that male nestlings have higher testosterone levels than female nestlings, leading to increased male nestling mortality. I examine how testosterone levels are affected by sex, brood size and laying position, maternal tactics that influence the degree of sibling competition. Finally, I test components of the honest signaling hypothesis that corticosterone is related to nestling body condition, which is a result of degree of competition, which is itself a result of parental reproductive strategies and food supply.

ATTRIBUTION

Chapters 2-4 will be submitted for publication as original manuscripts. On each of these manuscripts I will be the first author. Co-authors on my studies include my advisors Hubert Schwabl and Mike Webster, and my husband Ambrose Tuscano. Ambrose will be an author for help with data collection, help editing, and help with brainstorming ideas for the project as well as the writing. On chapter 2, Mark Stanback will also be an author for discussing my research and chapters with me. On chapters 3 and 4, Marc Evans will be a co-author for his help and statistical advice. As first author on each of these papers I conducted the vast majority of each study. I obtained the field funding for these projects, and my advisors helped me with lab funding. I made contact with ranchers to get permission to use their nest boxes, I was the primary field researcher, although many volunteers, including my husband, gave me assistance in the field. I entered my data and analyzed it with the help of Marc Evans. I did all of my own laboratory hormone work, most of my DNA extractions (I had help from two undergraduates that I trained), and all of my own PCR sexing reactions. I also did the writing of all of the manuscripts, which were commented on by Ambrose Tuscano, Patrick Carter, Mark Stanback, Hubert Schwabl, and Mike Webster.

WORKS CITED

Carranza J, 2004. Sex allocation within broods: the intrabrood sharing-out hypothesis. Behavioral Ecology 15:223-232.

Christians JK, 2002. Avian egg size: variation within species and inflexibility within individuals. Biological Reviews 77:1-26.

Clark AB, Wilson DS, 1981. Avian breeding adaptations: hatching asynchrony, brood reduction, and nest failure. Quarterly review of biology 56:253-277.

Clutton-Brock TH, 1986. Sex ratio variation in birds. Ibis 128:317-329.

Daan S, Dijkstra C, Tinbergen JM, 1990. Family planning in the kestrel (*Falco tinnunculus*): the ultimate control of covariation of laying date and clutch size. Behaviour 114:83-116.

Eising CM, Eikenaar C, Schwabl H, Groothuis TG, 2001. Maternal androgens in black-headed gull (Larus ridibundus) eggs: consequences for chick development. Proceedings of the Royal Society of London Biology 268:839-846.

Eising CM, Muller W, Dijkstra C, Groothuis TGG, 2003. Maternal androgens in egg yolks: relation with sex, incubation time and embryonic growth. General and Comparative Endocrinology 132:241-247.

Fargallo JA, Martinez-Padilla J, Toledano-Diaz A, Santiago-Moreno J, Davila JA, 2007. Sex and testosterone effects on growth, immunity, and melanin coloration of nestling Eurasian kestrels. Journal of Animal Ecology 76:201-209.

Fisher RA, 1930. The Genetical Theory of Natural Selection. Oxford: Clarendon Press.

Fletcher KL, Hamer KC, 2004. Offspring sex ratio in the common tern *Sterna hirundo*, a species with negligible sexual size dimorphism. Ibis 146:454-460.

Forbes L, Mock D, 1998. Parental optimism and progeny choice: when is screening for offspring quality affordable? Journal of Theoretical Biology 192:3-14.

Forbes S, Mock DW, 2000. A tale of two strategies: life-history aspects of family strife. Condor 102:23-34.

Goodship NM, Buchanan KL, 2006. Nestling testosterone is associated with begging behaviour and fledging success in the pied flycatcher, *Ficedula hypoleuca*. The Proceedings of the Royal Society B 273:71-76.

Groothuis TGG, Muller W, von Engelhardt N, Carere C, Eising C, 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. Neuroscience and Biobehavioral Reviews 29:329-352.

Ketterson ED, Nolan V, Cawthorn MJ, Parker PG, Ziegenfus C, 1996. Phenotypic engineering: Using hormones to explore the mechanistic and functional basis for phenotypic variation in nature. Ibis 138:70-86.

Kitaysky AS, Kitaiskaia EV, Wingfield JC, Piatt JF, 2001. Dietary restriction causes chronic elevation of corticosterone and enhances the stress response in red-legged kittiwake chicks. Journal of Comparative Physiology B: Biochemistry Systematics and Environmental Physiology 171:701-709.

Kozlowski J, Stearns SC, 1989. Hypotheses for the production of excess zygotes: models of bet-hedging and selective abortion. Evolution 43:1369-1377.

Lipar JL, 2001. Yolk steroids and the development of the hatching muscle in nestling European starlings. Journal of Avian Biology 32:231-238.

Lipar JL, Ketterson ED, Nolan V, 1999. Intra-clutch variation in testosterone content of red-winged blackbird eggs. Auk 116:231-235.

Magrath RD, 1990. Hatching Asynchrony in altricial birds. Biological Reviews 65:587-622.

Mock DW, Forbes L, 1995. The evolution of parental optimism. Trends in Ecology and Evolution 10:130-134.

Mock DW, Lamey TC, Ploger BJ, 1987. Proximate and ultimate roles of food amount in regulating egret sibling aggression. Ecology 68:1760-1772.

Mock DW, Parker G, 1998a. Siblicide, family conflict and the evolutionary limits of selfishness. Animal Behaviour 56:1-10.

Mock DW, Parker GA, 1998b. Siblicide, family conflict and the evolutionary limits of selfishness. Animal Behaviour 56:1-10.

Muller W, Dijkstra C, Groothuis TGG, 2003. Intersexual differences in T-cell mediated immunity of blackheaded gull chicks (Larus ridibundus) depend on the hatching order. Behavioral Ecology and Sociobiology 55:80-86.

Muller W, Groothuis TGG, Eising CM, Daan S, Dijkstra C, 2005. Within clutch co-variation of egg mass and sex in the black-headed gull. Journal of Evolutionary Biology 18:661-668.

Myers JH, 1978. Sex ratio adjustment under food stress: maximization of quality or numbers of offspring? The American Naturalist 112:381-388.

Nager RG, Monaghan P, Houston DC, 2000. Within-clutch trade-offs between the number and quality of eggs: experimental manipulations in gulls. Ecology 81:1339-1350.

Olson NJ, Kovacs WJ, 1996. Gonadal steroids and immunity. Endocrinology Reviews 17:369-384.

Pike TW, Petrie M, 2003. Potential mechanisms of avian sex manipulation. Biology Reviews 78:553-574.

Quillfeldt P, Massello JF, Strange IJ, Buchanan KL, 2006. Begging and provisioning of thin-billed prions, *Pachyptila belcheri*, are related to testosterone and corticosterone. Animal Behavior 71:1359-1369.

Ricklefs RE, 1977. On the evolution of reproductive strategies in birds: reproductive effort. American Naturalist 111:453-478.

Schwabl H, 1993a. Yolk Is a Source of Maternal Testosterone for Developing Birds. Proceedings of the National Academy of Sciences of the United States of America 90:11446-11450.

Schwabl H, 1993b. Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Science USA 90:11446-114450.

Schwabl H, 1997. The contents of maternal testosterone in house sparrow *Passer domesticus* eggs vary with breeding conditions. Naturwissenschaften 84:406-408.

Schwabl H, Mock DW, Gieg JA, 1997a. A hormonal mechanism for parental favouritism. Nature 386:231-231.

Schwabl H, Mock DW, Gieg JA, 1997b. A hormonal mechanism for parental favouritism. Nature 386:231.

Slagsvold T, Sandvik J, Rofstad G, Lorentsen O, Husby M, 1984. On the adaptive value of intra-clutch egg size variation in birds. AUK 101:685-697.

Sockman KW, Schwabl H, 2000. Yolk Androgens reduce offspring survival. Proceedings of the Royal Society of London Biology 267:1451-1456.

Sockman KW, Sharp PJ, Schwabl H, 2006. Orchestration of avian reproductive effort: an integration of the ultimate and proximate bases for flexibility in clutch size, incubation behaviour, and yolk androgen deposition. Biological Reviews 81:629-666.

Trivers RL, Willard DE, 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90-91.

Wallace GJ, 1948. The Barn Owl. Mich. Agr. Exp. Sta. Tech. Bull.:208.

Williams TD, 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biological Reviews 68:35-59.

CHAPTER TWO

OFFSPRING SEX RATIOS AND RESOURCES IN THE BARN OWL (*TYTO ALBA*)

ABSTRACT

Parents should maximize their reproductive effort in response to current environmental conditions by varying the number of offspring they produce and, if there are different costs to raising sons and daughters, by manipulating the sex ratio of their offspring. Many different hypotheses have been proposed to explain sex ratio variation observed in nature. One of the most common is Fisher's hypothesis of equal allocation. Other hypotheses have suggested that parents should bias offspring sex ratios, as well as number of offspring, based on the availability of resources. Fisher's theory is not mutually exclusive with these newer theories, but at times Fisherian selection may be obscured by resource-dependent strategies. Moreover, because Fisher's theory makes predictions about total investment across the period of offspring dependence, biased parental investment patterns may be obscured if the primary sex ratio (of eggs/zygotes) is itself biased. We used the barn owl (*Tyto alba*), a bird with reversed sexual size dimorphism, to: examine the effect of environmental conditions on patterns of parental investment, including investment in male and female offspring. We found that females adjusted clutch size according to food resources provided by the male parent, but did not vary offspring sex ratio based on current environmental conditions. Although fledging sex ratio did not differ from parity, the population sex ratio at hatching was male biased. Underproduction of the more costly sex may be a mechanism by which parents can equalize investment in the sexes and simultaneously minimize wasted resources.

INTRODUCTION

Natural selection should favor parents that produce the optimal number of offspring for their environmental conditions (Daan et al., 1990). In species with parental care, parents should try to raise as many offspring as possible without compromising their own survival or the condition of their offspring (Forbes and Mock, 2000). Differential costs between male and female offspring may affect the number of offspring that a parent can raise. For example, if males are twice as costly to produce as female offspring, then parents may be able to produce only one male for every two female offspring. Individuals of the larger more costly sex may experience increased mortality during the nestling phase because their greater resource requirements make them more susceptible to starvation (Teather and Weatherhead, 1988; 1989; 1994). Thus, sexual size dimorphism can potentially lead to complex patterns of sex ratio allocation by parents.

In many bird species, clutch size is widely variable (reviewed by: Godfray et al., 1991). One explanation for clutch size variation within a population is the selection pressure of reproductive optimization favoring individuals that lay an appropriate number of eggs for their nesting environment (Lack's Clutch Size) (Lack, 1947). If mother birds can predict the resources that will be available to their nestlings, they should adjust the size of their clutches to maximize their reproductive success (e.g. Korpimaki and Hakkarainen, 1991; Hakkarainen et al., 1997; Korpimaki and Wiehn, 1998; Dunn et al., 2000; Zanette et al., 2006; Kleindorfer, 2007). Another critical factor in the optimization of parental investment is brood sex ratio. Especially in species where sons and daughters have different costs and benefits, parents should be under selective pressure to optimize the sex ratio of their broods (reviewed by: Benito and Gonzalez-Solis, 2007).

Fisher's hypothesis (1930) of equal allocation is one of the most thoroughly tested and frequently cited theories in population biology. This hypothesis is based on the fact that, in sexual species, every offspring has one mother and one father, so the average reproductive success of the rarer sex will always be greater than that of the more common sex. Consequently, frequency-dependent selection favors equal total investment by parents in their sons and daughters. When the sexes are equally costly to produce, Fisher's hypothesis predicts an equal sex ratio at independence. However, in sexually size dimorphic (SSD) and reverse sexually size dimorphic (RSSD) species, there are often different costs to raising sons and daughters (Anderson et al., 1993; Reidstra et al., 1998; Weimerskirch et al., 2000; Laaksonen et al., 2004), but see (Torres and Drummond, 1999; Brommer et al., 2003). Thus, Fisher's (1930) hypothesis predicts that when one sex is more costly, the sex ratio at independence will be biased toward the less costly sex.

An important assumption of Fisher's hypothesis is that all parents have an equal ability to raise offspring—a faulty supposition for many populations and species. Myers (1978), building on the work of Trivers and Willard (1973), proposed that the sex ratio produced by individual parents will depend on parental condition and/or resources available to parents, as well as the differential costs of raising sons and daughters. Specifically, Myers' (1978) hypothesis posits that, if parental reproductive success is defined by the number of offspring successfully produced, and there are differential costs to raising sons and daughters, then females in good condition (or with superior resources) should bias offspring sex ratio toward the more costly sex, whereas females in poor condition (or with inferior resources) should bias offspring sex ratio toward the less costly sex. Moreover, Myers' (1978) hypothesis proposes that such sex ratio biasing should occur early in development because producing more of the less costly sex constitutes a more efficient mechanism for biasing sex ratios than does differential mortality. Differential mortality is costly to parents because, for some amount of time, they invest in offspring that will not fledge, and which thus represent no reproductive value.

Myers' hypothesis assumes that parents have the ability to bias the primary sex ratio of their offspring to match current environmental conditions. While the mechanism for adjusting primary sex ratio (at laying) has not yet been discovered, there is compelling evidence that birds can adjust offspring sex ratio in relation to their food resources and/or condition (see review by Pike and Petrie, 2003). In the last four years, several studies have found sex ratios related to levels of circulating maternal stress hormones (corticosterone) and in some cases maternal testosterone (Veiga et al., 2004; von Engelhardt et al., 2004; Love et al., 2005; Rutkowska and Cichon, 2006; Pike and Petrie, 2006). Resource-dependent sex ratios have been noted in a number of different bird species (Howe, 1977; Roskoft and Slagsvold, 1985; Bednarz and Hayden, 1991; Olsen and Cockburn, 1991; Dzus et al., 1996; Lessells et al., 1996; Kalmbach et al., 2001; Arnold and Griffiths, 2003), however there are many other studies that show no differences in sex ratios in relation to resources. Our study investigated both clutch size and offspring sex ratios in relation to food resources to determine whether food resources affect clutch size or brood sex ratio. We examined sex ratio not only at fledging, as in many previous studies (reviewed by Benito and Gonzalez-Solis, 2007), but also at hatching to determine whether biases arise from overproduction of one sex or from differential mortality.

We investigated clutch size and offspring sex ratio biasing in the barn owl (*Tyto alba*), a species that produces large, variable clutches (3-12 eggs, Seifert, pers. obs.). The barn owl's unusual nesting behavior makes it an excellent species in which to examine the effects of environmental conditions on reproductive strategy. From a few days before egg laying until about half way through the nestling phase (approximately 60 days in total), females do not hunt, instead relying on their mates to provision them and their nestlings. Thus, much of the success of a pair's reproductive efforts depends on the results of male hunting in relation to the total resource demands of the female and her nestlings. Moreover, in barn owls, male courtship behavior involves stockpiling rodents in potential nesting cavities. The size of these larders varies substantially among males (Seifert, pers. obs.), such that females may be able to use male courtship feeding/stockpiling behavior as a predictor of future resource availability. Accordingly, females mated to males with high rates of food delivery should produce larger clutches and go on to fledge more offspring than those mated to males with low rates of food delivery. Finally, barn owls also exhibit pronounced RSSD, with adult females being about 25% heavier than males. Female barn owls seem to be more costly than males for parents to produce because they grow larger and heavier during their nestling phase (Seifert et al., in prep.). Given differential costs between the sexes, barn owl parents should manipulate brood size and sex ratios in order to maximize reproductive output in relation to population sex ratio and resource availability.

Because barn owl males are smaller than females, in order for barn owl parents to invest equally in the sexes, as Fisher predicts, the population must be male-biased. If parents invest equally in the sexes, they might not be able to adequately provision offspring of the larger sex, resulting in increased female mortality. If Fisher's hypothesis holds true we predict a male biased sex ratio at fledging. If Myers's hypothesis holds true in barn owls, we predict that females mated to low delivering males will produce male-biased clutches, whereas females mated to high delivering males will produce female-biased clutches at both hatching and fledging.

METHODS

We studied a nest-box population of barn owls on agricultural ranches in a 20-mile radius of Merced, CA. Barn owls readily nest in boxes, making it convenient to find their nests and collect data on a regular basis. In central California they initiate nests from late December until early May and are almost exclusively single brooded (Simmons pers. comm.; Seifert, pers. obs.). Clutch sizes range from 3-12 eggs (Marti et al., 2005; Seifert, pers. obs.). Females initiate incubation with the laying of the first egg. Males do not incubate, but do provision females before and during egg laying and throughout incubation; females do not forage independently during this period. Once nestlings hatch, males continue to deliver all food until nestlings are approximately one month old—after which both adults provision the nestlings.

We checked nest boxes from the second week in January until the middle of May in 2005 and 2006. We checked boxes every four days until there were signs of nest initiation, (i.e. an adult roosting in the box, and/or a stockpile of rodents in the box), after which we checked boxes every other day throughout egg laying (barn owls do not lay more than one egg every other day, (Marti et al., 2005)). We numbered, weighed (to the nearest 0.5g), and measured (length and width; to the nearest 0.1mm) each egg after it appeared in the nest. We assumed that egg laying had finished when a new egg did not appear in the nest for 8 days, and at that point we checked the boxes every 4 days until 26 days had passed since the first egg was laid. We then checked boxes every other day until all the nestlings had all hatched or 40 days had passed from the day the last egg was laid. As each nestling hatched we measured its wing length (to the nearest 0.5 mm), body mass and the left tarsus (to the nearest 0.5 mm). We gave each nestling a colored plastic band until we could fit it with an adult size 6 USFWS metal band (usually around day 10). From each nestling we collected a blood sample from the brachial wing vein on the first day it was found and stored samples in lysis buffer at 4ºC for molecular analysis (below).

Male Provisioning

We monitored male food delivery at nest boxes to determine food resources available to females during egg production and again during the nestling phase. We determined the amount of food brought by the male with infrared cameras installed inside nest boxes. We recorded male delivery to nests three times during egg laying and three times during the two weeks after all nestlings had hatched; each recording session was for a total of six hours beginning at 9:30 pm. We analyzed videos to determine number and type of food delivered (voles, gophers, mice, other rodents, and songbirds). A previous study (Simmons, unpub. data) determined the local average mass of each type of prey, and we used these mass estimates and the number of prey items delivered to calculate the overall mass of the food that was delivered during each six-hour segment. For analyses we averaged the food delivery in grams over the three nights of each phase (laying and nestling).

Molecular Determination of Nestling Sex

Even though nestling barn owls are sexually dimorphic in both size and color, we used a molecular sexing technique to obtain information about brood sex ratio before any offspring died or disappeared from the nest. For these analyses we used only nests that had greater than 80% hatching success. We extracted DNA from blood samples using a standard phenol-cholorform extraction (Westneat, 1990). To determine nestling sex we followed the protocol of Kahn et al. (1998), using primers 1237L and 1272H. We used adults of known sex to optimize PCR conditions and in each PCR run we included a known adult of each sex to verify our results. PCR reactions consisted of 40 cycles at 94°C for 60 seconds, 55°C for 60 seconds and 72°C for 45 seconds. The total reaction volume was 10µl and included 1µl of DNA and 1X PCR buffer (Applied Biosystem), 0.15 mM dNTP mix, 0.5 uM of each primer, 2mM of $MgCl_2$ and 2.5U/ul of Taq.

Statistics

To test whether the population sex ratio at hatching and at fledging differed from parity, we performed a one-sample z-test, using Neuhauser's (2004) method because we have clustered data from each brood. We used a multiple regression procedure in SAS to test whether the hatching sex ratio was related to the (we only used nests that had greater than 80% hatching success) the average food delivery (in grams) during egg laying, and Julian date. In another multiple regression we tested to see if clutch size was related to food delivery and Julian date. We used a multiple regression in SAS to test whether the fledging sex ratio was related to food delivery during the nestling phase, and Julian date. In another multiple regression we tested to see if the number of offspring fledged was related to the food delivery during the nestling phase and Julian date. We performed another multiple regression to test whether the proportion of offspring fledged was related to food delivery, the number of eggs that were laid in the nest and Julian date. Finally, we used a standard linear regression model (Type III) to test whether male food delivery at egg laying/incubating was related to delivery during the nestling stage.

RESULTS

The mean hatching sex ratio for both years of the study was 57% male (n = 49 broods), which differed significantly from parity (one-sample z-test: $z_{49} = 1.9508$, $P = 0.025$). Separating years, the mean sex ratio at hatching differed significantly from parity in 2006 (58% male, n = 25 broods, one-sample z-test: $z_{25} = 1.6659$, $P = 0.047$) but not in 2005 (56% male, n = 24 broods, one-sample z-test: $z_{24} = 1.1021$, $P = 0.131$).

In contrast to the hatching sex ratio, the mean sex ratio at fledging was 52% male (n = 43 clutches) across all years, which did not differ significantly from parity (one-sample z-test z_{43} =0.4721, $P = 0.318$). Fledging ratios for two years followed a similar pattern to hatching ratios: in 2005, 51% of fledglings were male and in 2006 54% were male. We used the same nests for both sets of data (Figure 2.1); the difference in sample size results from those nests that fledged no offspring (9 in 2006). Thus, our results indicate that male nestlings experienced higher mortality during the nestling phase.

Although males delivered food at a higher rate during the nestling than laying/incubation phase, the rate of food delivery by each male during the egg phase was positively related to his food delivery during the nestling phase ($\beta = 0.839$, $t = 3.86$, $P = 0.001$, df = 1, Figure 3.2). We found that the sex ratio at hatching was not related to male food delivery during egg laying ($\beta = 0.000$, $t = 1.55$, $P = 0.140$, df = 1, Table 2.1A, Figure 2.3A) but, the number of eggs laid was positively related to food delivery during egg laying ($\beta = 0.01$, $t = -2.49$, $P = 0.021$, df = 1, Table 2.1B, Figure 2.3B). Sex ratio at fledging was not associated with male provisioning rate during the nestling stage ($\beta = 2.16$, $t = 0.02$, $P = 0.988$, df = 1, Table 2.2A, Figure 2.4A), however, the number of offspring fledged was positively related to the rate of food delivery during the nestling phase ($\beta = 89.09$, $t = 2.47$, $P = 0.027$, df = 1, Table 2.2B, Figure 2.4B). Also, the proportion of offspring that fledged was positively related to the average prey delivery to the nest ($\beta = 0.001$, $t = 3.20$, $P = 0.005$, df = 1, Table 2.3, Figure 2.4C), and negatively related to the number of eggs that were in the nest ($\beta = -0.140$, $t = -2.30$, $P = 0.033$, df = 1, Table 2.3, Figure 2.4D).

DISCUSSION

Our results show that female barn owls do seem to adjust clutch size in accordance with food resources. The positive correlation between male provisioning during egg laying and number of eggs laid suggests that barn owl mothers are able to base reproductive investment on current or future resource availability as a means to maximize clutch size. This finding is consistent with the predictions of reproductive optimization theory and life history theory on clutch size (Lack, 1947). We found a strong relationship between male provisioning during egg laying/incubation and nestling phases, which suggests that an individual male's provisioning changed in a predictable way over the nesting cycle. This finding suggests the possibility that mother barn owls use early signs of their mate's provisioning rate (e.g. stockpile size) as a predictor of future provisioning rates, and adjust clutch size accordingly.

Some previous studies have shown that birds can adjust clutch size, and consequently the number of offspring they fledge, in relation to the current environment (e.g. Korpimaki and Hakkarainen, 1991; Korpimaki and Wiehn, 1998; Dunn et al., 2000; Zanette et al., 2006). For example, in a different raptor species, Korpimaki and Wiehn, (1998) found that average clutch size varied with seasonal food availability. However most of these studies used indirect methods to assess food resources available for feeding nestlings. In contrast, our study determined directly the amount of food delivered to the nest during laying in a wild bird. Because male food delivery during egg laying appears to be a good indicator of food delivery during the nestling phase (Figure 2.2), a female can use courtship feeding as an indicator of future food supplies and adjust clutch size accordingly. Maximization of clutch size can be critically important in species that have altricial young and reproduce more than once in a lifetime, as it allows parents to invest in an appropriate number of offspring in a given reproductive bout without sacrificing their future reproductive output by overextending themselves (Lack, 1947). Moreover, we found that number of young fledged was associated with male provisioning rates, because provisioning rates affected both the number of eggs laid (Figure 2.3B) and the proportion of the resulting nestlings that fledged (Figure 2.4C). Thus, our results suggest that parent barn owls do make decisions about offspring number in relation to food resources. Although we do find that the proportion of offspring fledged was negatively related to clutch size (Figure 2.4D), indicating that larger clutches do have more offspring die than smaller clutches. However, with more food more offspring fledge per nest and a higher proportion of offspring fledge from each nest.

A review by Benito and Gonzalez-Solis (2007) found that RSSD species have male biased sex ratios at hatching and fledging, supporting Fisher’s (1930) hypothesis. When plotted onto their continuum of size dimorphism and offspring hatching sex ratio, barn owls fit their regression line well. However, their study found that in RSSD species (only 3 other studies) there is increased mortality for female nestlings, making fledging sex ratio even more male biased. Likewise, in SSD species, there tends to be elevated levels of male mortality. Benito and Gonzalez-Solis’s findings support Fisher’s prediction that when sons and daughters are unequally sized parents produce more of the smaller sex by the end of dependence; their results suggest the sex ratio bias is achieved via increased mortality for offspring of the larger sex (Emlen, 1997). The tendency of the larger sex of offspring to suffer higher mortality rates is often explained by increased susceptibility to adverse conditions and starvation (e.g. Teather and Weatherhead, 1994).

Our results counter this prediction: in barn owls the male biased hatching sex ratio shifted closer to parity by fledging (Figure 2.4B). Because we used the same nests at hatching and fledging there seems to be higher survival rates of female nestlings than male nestlings during the nestling stage. Lower mortality the larger sex, runs counter to the findings of Benito and Gonzalez-Solis (2007) . We may see higher female survival in nestlings because they may sex out-compete the smaller males for food. Future research should examine competitive sibling interactions to determine whether, in fact, intra-brood competition could be responsible for increased mortality of the smaller sex during the nestling phase, as reported in the great tit (*Parus major*) (reviewed in Oddie, 2000). We may also see differential mortality due to a sex ratio bias in the laying order (Seifert et al., in prep.).

Because barn owl females are the more costly sex to raise (Seifert et al., in prep.), we expected sex ratios to be male biased at fledging (Fisher, 1930). Instead, we found that offspring sex ratios were male biased at hatching but at fledging did not differ from parity (Figure 2.1). Fisher's (1930) theory does not make explicit predictions about sex ratio at hatching, although primary sex ratio is one way for parents to equalize investment between sons and daughters. Fisher's hypothesis does not take into account energy expense at egg laying or the potential costs of raising offspring that do not fledge. Accordingly, testing sex ratio biases at fledging can potentially result in misleading conclusions about parental investment in sons and daughters. Thus, a simple examination of fledgling sex ratios is not an effective way to test equal allocation to the sexes, because as we found, nestling mortality can obscure patterns of parental investment, particularly when laying (or hatching) sex ratios are not at parity. It is possible that barn owl parents invest equally in sons and daughters, however a more detailed analysis examining the costs parents incur to produce non-surviving male offspring, would further elucidate parental investment strategies. Perhaps our results are different than those reported by Benito & Gonzalez-Solis (2007) because they only report on three RSSD species with sex ratio data at both hatching and fledging; if more species were tested, we might find that other species also have increased mortality of the smaller sex.

Our results do not support Myers's hypothesis (1978), which states that the sex ratio at hatching for a given nest should reflect environmental conditions because females should bias sex ratio to maximize the number of fledglings and minimize nestling mortality. First, we found no correlations between sex ratio at hatching or at fledging with male food delivery rate (Figures 2.3 and 2.4). Moreover, the difference between hatching and fledging sex ratios maybe due to differential mortality between the sexes, such that males (the over-represented sex at hatching) may have higher mortality rates than females. It may be that elevated male mortality is inevitable in the barn owl due to some factor such as hormones, as found in some other species (Muller et al., 2003; Fargallo et al., 2007).

One possible explanation for these results is that barn owls are incapable of facultative sex ratio adjustment, or maybe the cost difference between raising males and females is not great enough to have selected for sex ratio biasing. Alternatively, it is possible that barn owl females use cues other than male provisioning rates to adjust sex ratio. Previous studies in birds that have found offspring sex ratio biases in relation to current environment (e.g. Torres and Drummond, 1997; Velando, 2002) have examined food abundance in the territory surrounding the nest, rather than actual food delivery rates. Barn owl females might also use larger scale cues, such as current or previous food abundance in the local environment, as the basis for biasing offspring sex ratio. In agreement with this possibility, a study on another owl species found that females bias offspring sex ratio as well as clutch size based on the vole population during the season prior to breeding (Korpimaki and Wiehn, 1998). Such cues of food availability and other environmental conditions may be more important to female reproductive success, and hence sex ratio biasing, than is male food delivery.

Conclusions

The review by Benito and Gonzalez-Solis (2007) of offspring sex ratio in birds found very few (7) studies in which sex ratios were quantified at both hatching and at fledging in the same broods. That so few studies have been conducted using data from both of these critical phases of nesting is both surprising and problematic. Benito and Gonzalez-Solis's results highlight the common and potentially faulty assumption among researchers that parental investment may be accurately estimated at a single point during reproduction. For instance, parents incur costs by producing eggs and by raising offspring that don't fledge, yet these costs are not reflected in fledging data. To truly gauge parental investment, sex ratios must be calculated at both hatching and fledging.

Barn owl parents maximize the number of offspring they produce based on their current food supply, however we did not find any evidence for optimization of sex ratio in relation to food. Two potential explanations for the lack of sex bias in relation to male food delivery are that females cannot adjust offspring sex ratio facultatively, or that they use environmental cues other than mate provisioning. There was a male sex bias at hatching, but we did not find a bias at fledging, making it unclear whether females invest equally in sons and daughters as predicted by Fisher (1930).

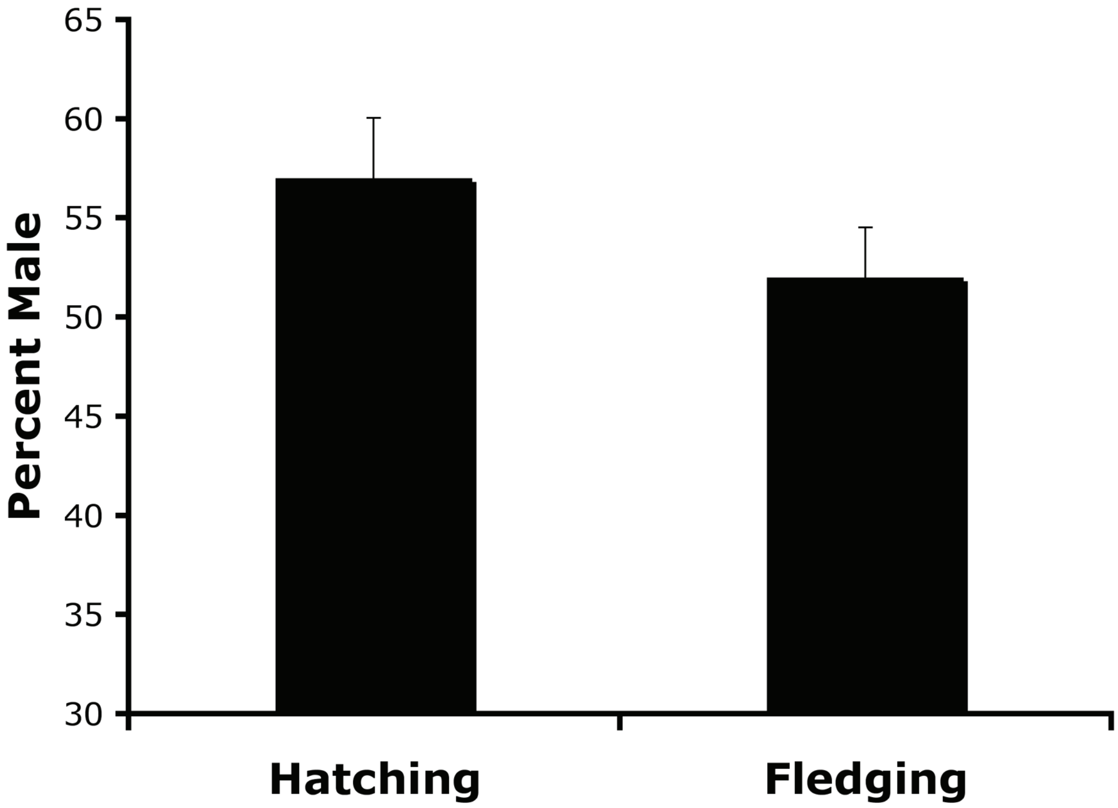

Figure 2.1. The hatching sex ratio was significantly male biased, while the fledging sex ratio was near parity. Error bars show one standard error, but statistical tests accounted for clustered data (see text). Male mortality was higher than female during the nestling phase.

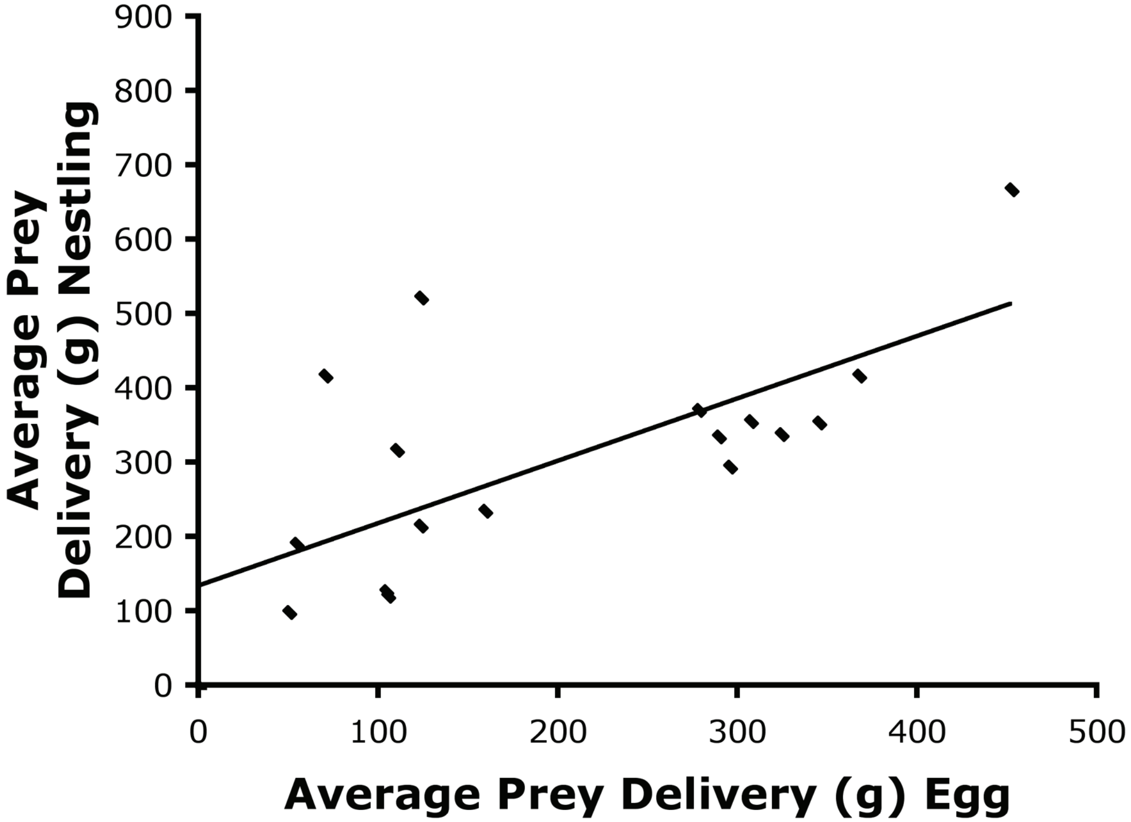

Figure 2.2. The average food delivery rate during the egg laying phase was positively related to the average food delivery rate during the nestling phase (n=19 nests).

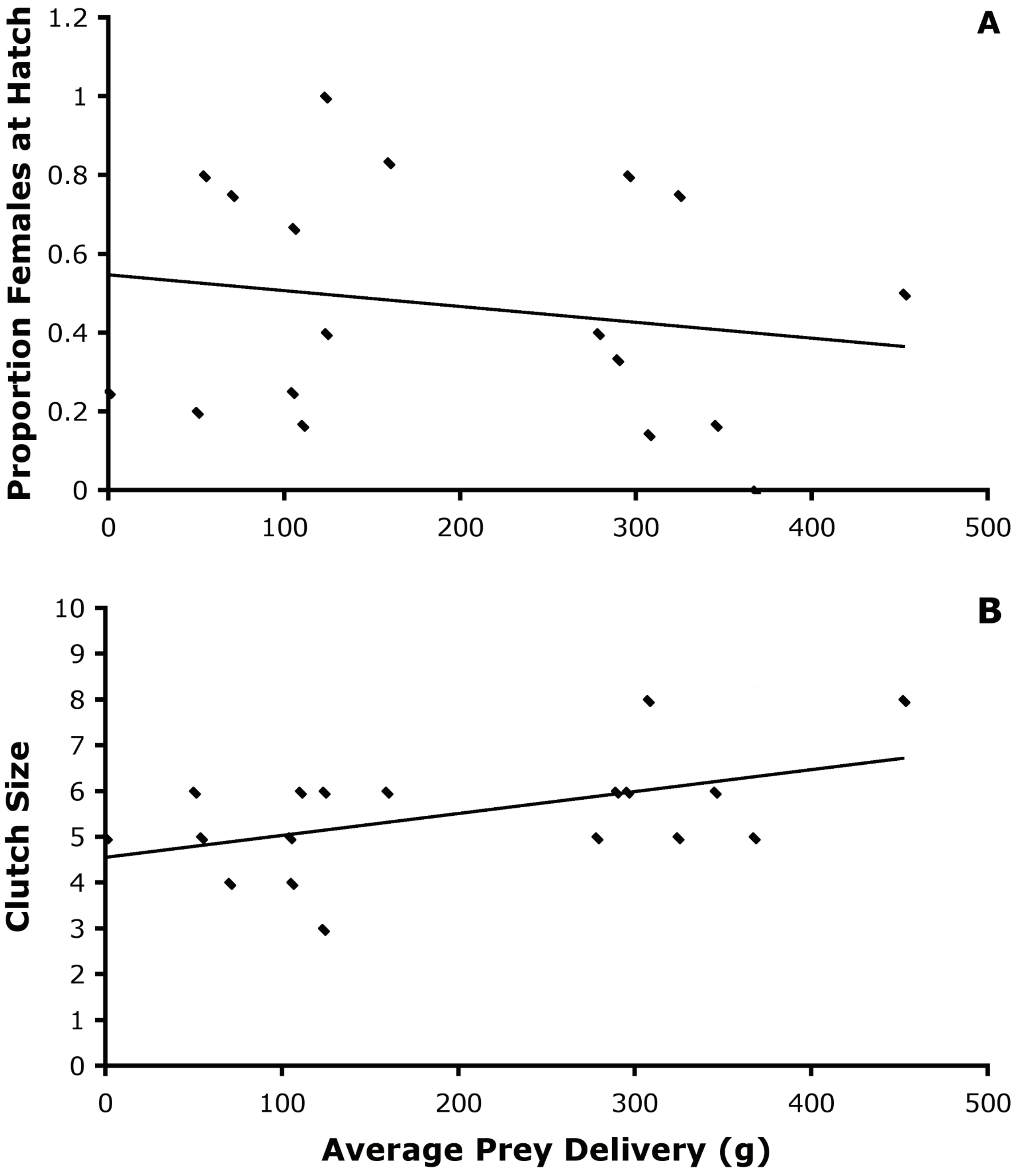

Figure 2.3 The average prey delivered during the egg laying phase in relation to the number of females or offspring in the nest. **A.** The hatching sex ratio of the nest was not related to the average mass (g) of the food delivered by the male during egg laying (n=19 clutches). **B.** The number of eggs that were laid was positively related to the amount of food delivered (n=19 clutches). In parts A-D the statistics we report come from a multiple regression, but the regression lines shown are for the univariate regressions.

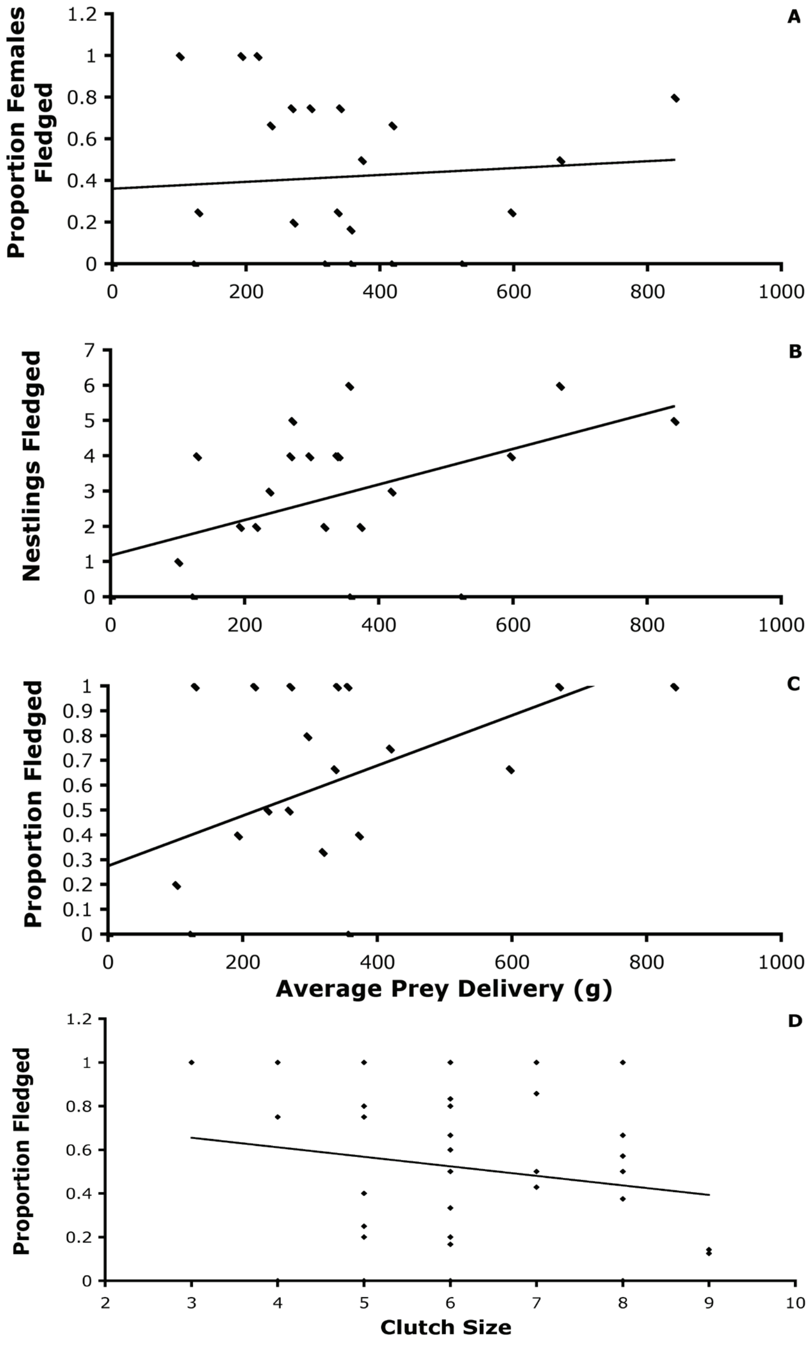

Figure 2.4. A. The sex ratio of nests at fledging was not correlated to the amount of food that was delivered during the nestling phase (n=23 broods). **B.** The number of offspring that fledged from each nest was correlated to the average mass (g) of the prey delivered by the male during the nestling phase (n=23 broods). **C.** The proportion of offspring that fledged was related to the average food delivery rate during the nestling phase (n=23 broods). **D.** The proportion of offspring that fledge was negatively related to the clutch size (n=23). Parts A through D the statistics we report come from a multiple regression, but the regression lines shown are for the univariate regressions.

Table 2.1: Table 1: **A)** Results from a regression that analyzed the relationship between sex ratio and food delivery during the egg phase, clutch size and Julian date. **B)** Results from a regression that analyzed the relationship between clutch size and food delivery during the egg phase and Julian date.

A. Sex ratio	*df*	N	ß	*t*	*P*
Food Delivery	1	19	0.000	1.550	0.140
Date	1	19	-0.003	-1.100	0.286
B. Clutch Size					
Food Delivery	1	19	0.001	2.490	0.021
Date	1	19	0.010	0.350	0.730

Table 2.2: **A)** Results from a multiple regression that analyzed the relationship between sex ratio and food delivery during the nestling phase, number fledged, and Julian date. **B)** Results from a multiple regression that analyzed the relationship between the number that fledged and the average food delivery during the nestling phase and Julian date.

A. Sex Ratio	*df*	N	ß	*t*	*P*
Food Delivery	1	23	0.000	-0.010	0.995
Julian Date	1	23	-0.006	-1.770	0.100
B. Number Fledged					
Food Delivery	1	23	0.005	3.200	0.004
Date	1	23	0.014	1.120	0.276

Table 2.3: Results from a multiple regression that analyzed the relationship between the proportion fledged and clutch size, food delivery during the nestling phase, and Julian date.

Proportion Fledged	*df*	N	ß	*t*	*P*
Clutch Size	1	23	-0.140	-2.300	0.033
Male Food Delivery	1	23	0.001	3.200	0.005
Julian Date	1	23	0.003	1.47	0.159

WORKS CITED

Anderson D, Reeve J, JEM G, Weathers W, Hutson S, Cunningham H, Bird D, 1993. Sexual size dimorphism and food requirements of nestling birds. Canadian Journal of Zoology 71:2541-2545.

Arnold K, Griffiths R, 2003. Sex-specific hatching order, growth rates and fledging success in jackdaws *Corvus monedula*. Journal of Avian Biology 34:275-281.

Bednarz JC, Hayden T, 1991. Skewed brood sex ratio and sex-biased hatching sequence in Harris' hawks. The American Naturalist 137:116-132.

Benito MM, Gonzalez-Solis J, 2007. Sex ratio, sex-specific chick mortality and sexual size dimorphism in birds. Journal of Evolutionary Biology 20:1522-1530.

Brommer JE, Karell P, Pihlaja T, Painter JN, Primmer CR, Pietiainen H, 2003. Ural owl sex allocation and parental investment under poor food conditions. Oecologia 137:140-147.

Daan S, Dijkstra C, Tinbergen JM, 1990. Family planning in the kestrel (*Falco tinnunculus*): the ultimate control of covariation of laying date and clutch size. Behaviour 114:83-116.

Dunn PO, Thusius KJ, Kimber K, Winkler DW, 2000. Geographic and ecological variation in clutch size of tree swallows. AUK 117:215-221.

Dzus EH, Bortolotti GR, Gerrard JM, 1996. Does sex biased hatching order in bald eagles vary with food resources? Ecoscience 3:252-258.

Emlen ST, 1997. When mothers prefer daughters over sons. Trends in Ecology and Evolution 12:291-292.

Fargallo JA, Martinez-Padilla J, Toledano-Diaz A, Santiago-Moreno J, Davila JA, 2007. Sex and testosterone effects on growth, immunity, and melanin coloration of nestling Eurasian kestrels. Journal of Animal Ecology 76:201-209.

Fisher RA, 1930. The Genetical Theory of Natural Selection. Oxford: Clarendon Press.

Forbes S, Mock DW, 2000. A tale of two strategies: life-history aspects of family strife. Condor 102:23-34.

Godfray HCJ, Partridge L, Harvey PH, 1991. Clutch Size. Annual Review of Ecology and Systematics 22:409-429.

Hakkarainen H, Koivunen V, Korpimaki E, 1997. Reproductive success and parental effort of Tengmalm's owls: Effects of spatial and temporal variation in habitat quality. Ecoscience 4:35-42.

Howe H, 1977. Sex ratio adjustment in the common grackle. Science 198:744-746.

Kahn N, St. John J, Quinn TW, 1998. Chromosome-specific intron size differences in the avian CHD gene provide and efficient method for sex identification in birds. The Auk 115:1074-1078.

Kalmbach E, Nager RG, Griffiths R, Furness RW, 2001. Increased reproductive effort results in male-biased offspring sex ratio: an experimental study in a species with reversed sexual size dimorphism. The proceedings of the Royal Society London B 268:2175-2179.

Kleindorfer S, 2007. The ecology of clutch size variation in Darwin's small ground finch Geospiza fuliginosa: comparison between lowland and highland habitats. Ibis 149:730-741.

Korpimaki E, Hakkarainen H, 1991. Fluctuating food-supply affects the clutch size of Tengmalms owl independent of laying date. Oecologia 85:543-552.

Korpimaki E, Wiehn J, 1998. Clutch size of kestrels: seasonal decline and experimental evidence for food limitation under fluctuating food conditions. Oikos 83:259-272.

Laaksonen T, Fargallo JA, Korpimaki E, Lyytinen S, Valkama J, Poyri V, 2004. Year- and sex-dependent effects of experimental brood sex ratio manipulation on fledging condition of Eurasian kestrels. Journal of Animal Ecology 73:342-352.

Lack D, 1947. The significance of clutch size. Ibis 89:302-352.

Lessells CM, Mateman AC, Visser J, 1996. Great tit hatchling sex ratios. Journal of Avian Biology 2:135-142.

Love OP, Chin EH, Wynne-Edwards KE, Williams TD, 2005. Stress Hormones: A link between maternal condition and sex -biased reproductive investment. American Naturalist 166:751-766.

Marti CD, Poole AF, Bevier LR, 2005. Barn Owl (Tyto alba). The birds of North America.

Muller W, Dijkstra C, Groothuis TGG, 2003. Intersexual differences in T-cell mediated immunity of blackheaded gull chicks (Larus ridibundus) depend on the hatching order. Behavioral Ecology and Sociobiology 55:80-86.

Muller W, Groothuis TGG, Eising C, Dijkstra C, 2005. An experimental study on the causes of sex-biased mortality in the black-headed gull- the possible role of testosterone. Journal of Animal Ecology 74:735-741.

Myers JH, 1978. Sex ratio adjustment under food stress: maximization of quality or numbers of offspring? The American Naturalist 112:381-388.

Neuhauser M, 2004. Tests for a biased sex ratio when data are clustered. Environmental and Ecological Statistics 11:295-304.

Oddie KR, 2000. Size matters: competition between male and female great tit offspring. Journal of Animal Ecology 69:903-912.

Olsen PD, Cockburn A, 1991. Female-biased sex allocation in peregrine falcons and other raptors. Behavioral Ecology and Sociobiology 28:417-423.

Owens IPF, Short RV, 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories on sexual selection. Trends in Ecology and Evolution 10:44-47.

Pike TW, Petrie M, 2003. Potential mechanisms of avian sex manipulation. Biological Reviews 78:553-574.

Pike TW, Petrie M, 2006. Experimental evidence that corticosterone affects offspring sex ratios in quail. Proceedings of the Royal Society B-Biological Sciences 273:1093-1098.

Reidstra B, Dijkstra C, Dann S, 1998. Daily expenditure of male and female Marsh Harrier nestlings. The Auk 115:635-641.

Roskoft E, Slagsvold T, 1985. Differential mortality of male and female offspring in experimentally manipulated broods of the rook. Journal of Animal Ecology 54:261-266.

Rutkowska J, Cichon M, 2006. Maternal testosterone affects the primary sex ratio and offspring survival in zebra finches. Animal Behaviour 71:1283-1288.

Teather KL, Weatherhead PJ, 1988. Sex-specific energy requirements of great-tailed grackle (*Quiscalus mexicanus*) nestlings. Journal of Animal Ecology 57:659-668.

Teather KL, Weatherhead PJ, 1989. Sex-specific mortality in nestling great-tailed grackles. Ecology 70:1485-1493.

Teather KL, Weatherhead PJ, 1994. Allometry, adaptation, and the growth and development of sexually dimorphic birds. Oikos 71:515-525.

Torres R, Drummond H, 1997. Female-biased mortality in nestlings of a bird with size dimorphism. Journal of Animal Ecology 66:859-865.

Torres R, Drummond H, 1999. Does large size make daughters of blue-footed booby more expensive than sons? Journal of Animal Ecology 68:1133-1141.

Veiga JP, Vinuela J, Cordero PJ, Aparicio JM, Polo V, 2004. Experimentally increased testosterone affects social rank and primary sex ratio in the spotless starling. Hormones and Behavior 46:47-53.

Velando A, 2002. Experimental manipulation of maternal effort produces differential effects in sons and daughters: implications for adaptive sex ratios in the blue-footed booby. Behavioral Ecology 13:443-449.

von Engelhardt N, Dijkstra C, Daan S, Groothuis TGG, 2004. Effects of 17-beta-estradiol treatment of female zebra finches on offspring sex ratio and survival. Hormones and Behavior 45:306-313.

Weimerskirch H, Barbraud C, Lys P, 2000. Sex differences in parental investment and chick growth in wandering albatrosses: fitness consequences. Ecology 81:309-318.

Westneat DF, 1990. Genetic parentage in the Indigo Bunting: A study using DNA fingerprinting. Behavioral Ecology and Sociobiology 27:67-76.

Zanette L, Clinchy M, Smith JNM, 2006. Food and predators affect egg production in song sparrows. Ecology 87:2459-2467.

CHAPTER THREE

THE EARLY BIRD GETS THE WORM: A TEST OF THE SHARING-OUT HYPOTHESIS AND PARENTAL ALLOCATION IN BARN OWLS (*TYTO ALBA*)

ABSTRACT

Many hypotheses have been proposed to explain how, in species with large broods/litters, parents should vary sex of and resources to different offspring to maximize reproductive output. Optimal allocation of food resources to offspring can be complicated when hatching asynchrony and size differences among progeny create competitive asymmetries. In asynchronously hatching bird species, parents can either minimize the effects of hatching asynchrony (i.e. by increasing the amount of resources (e.g. egg mass, yolk hormones, food to nestlings) to later hatched offspring) or enhance those effects (i.e. by decreasing the resources to later offspring). The sharing-out hypothesis proposes that, under such conditions, parents are selected to adjust offspring sex within the brood according to patterns of resource allocation in order to maximize reproductive output. We tested the hypothesis that parents can either mitigate or enhance hatching asynchrony, as well as the sharing out hypothesis, by examining how egg mass and yolk hormones vary with laying order and sex allocation patterns in a wild population of barn owls (*Tyto alba*), a species in which both sexual size dimorphism and extreme hatching asynchrony lead to a large size and developmental hierarchy among siblings within a brood. We found that early-hatched nestlings were more likely to survive than later-hatched nestlings. Ours is the first study to find that yolk androgen levels increase early in the laying order (first 3 eggs) and then decrease in the later laying positions, suggesting that parents follow different allocation strategies in early than in late-laid eggs. We found that eggs laid later in the laying sequence were more likely to be male than those laid earlier, which partially supports the sharing-out hypothesis. Interestingly, these last-laid male eggs were not different in size from earlier male eggs, but later-laid female eggs tended to be heavier than earlier-laid eggs. These results suggest that mothers can vary sex ratio, egg mass, and hormones across their broods.

INTRODUCTION

One major area of research in behavioral ecology focuses on understanding how parents adjust their reproductive strategies to maximize fitness. Parents are selected to maximize their reproductive output by producing as many offspring as possible with in a reproductive bout without compromising their future reproduction or offspring quality (Forbes and Mock, 2000). Means of parental reproductive optimization include differential allocation of resources to individual offspring, distribution and proportion of offspring sex, and resource distribution to members of each sex. Researchers have begun to focus on a female's ability to differentially allocate resources to different offspring even before they are born, i.e., through non-genetic maternal effects (Yolk hormones: see review by Groothuis et al., 2005; antioxidents: e.g. Royle et al., 2001; Saino et al., 2002;

egg mass: e.g. Williams, 1994; Rutkowska and Cichon, 2005). The order in which eggs of different sex and quality are laid can affect the success of each offspring and, in turn, the reproductive success of its parents. Within-brood sex ratio biases have been reported in many bird species (reviewed by Pike and Petrie, 2003), leading to speculation about the potential adaptive value of these parental manipulations. Several hypotheses (e.g. Fisher, 1930; Trivers and Willard, 1973) attempt to predict how parents should bias the sex ratio of their offspring given the relative cost of each sex and current environmental conditions (reviews in: Charnov, 1982; Frank, 1990; Clutton-Brock, 1991; Godfray and Werren, 1996; Pike and Petrie, 2003). At the population level, Fisher's hypothesis of equal allocation explains that frequency-dependent selection favors equal total investment by parents in their sons and daughters. Within a family group, Trivers and Willard's hypothesis (1973) predicts that females should bias the sex ratio of their offspring according to current environmental conditions, such as food resources and their own condition.

These theories do not, however, take into account the fact that, in species with large broods, hatching asynchrony and/or sexual size dimorphism can create size hierarchies among nestlings that affect each individual's ability to obtain food resources. As a consequence, individual offspring within a brood likely experience different environmental conditions (e.g. parental food supply and sibling competition), which may result in variable food intake. In such cases, larger nestlings are likely to out-compete smaller nestlings (e.g. Muller et al., 2005), and the resulting differences in offspring growth and development likely influence the value of individual offspring to their parents.

Because the conditions under which nestlings are reared can impact their reproductive success and survival (Bortolotti, 1986; Haywood and Perrins, 1992; de Kogel, 1997; Blount et al., 2006; Van de Pol et al., 2006), in species with hatching asynchrony, parents can potentially improve their reproductive success by favoring some nestlings over others (Forbes and Mock, 1998). For instance, differential provisioning of yolk hormones, egg mass, and food to offspring has been documented in many species (Howe, 1976; Beissinger and Waltman, 1991; Forbes and Lamey, 1996; Schwabl et al., 1997; Forbes and Mock, 1998), and can influence the success of individual offspring and potentially the parents' reproductive success (Anderson et al., 1997; Amat et al., 2001).

Asynchronous hatching (caused by incubation before all eggs have been laid) can result in a sibling age hierarchy that influences sibling competition and nestling quality (Stoleson and Beissinger, 1997; Magrath, 1990). The causes and consequences of hatching asynchrony are not fully understood; its adaptive function in parental optimization is heavily debated (Magrath, 1990; Stoleson and Beissinger, 1997). Although there are potentially many adaptive explanations for hatching asynchrony, here we consider its consequences rather than its adaptive value.

Previous research has found that parents can enhance the effects of hatching asynchrony by decreasing the resources (such as yolk hormones) allocated to each successive offspring (e.g. Schwabl et al., 1997). Conversely, other studies show that parental resources may mitigate the effects of hatching asynchrony by increasing resources to each consecutive offspring over the course of the clutch (e.g. Eising et al., 2001). While these are two very different strategies, both may be mechanisms of parental optimization. In the first strategy, parents create "insurance" offspring that they expect to raise only if their more advantaged offspring do not survive. In this case, hatching asynchrony could help maximize parental output by allowing parents to raise more offspring than normal in favorable environments (without sacrificing the quality of earlier-hatched offspring). In the second strategy, parents set up an "optimistic" brood in which they expect to raise all of their offspring. In this case parents try to minimize the effects of hatching asynchrony so that sibling competition does not cause mortality among later-hatched nestlings. An additional advantage of hatching asynchrony to parents employing the "insurance" strategy is that when environmental food resources are poor, intra-brood competition will hasten the death of disadvantaged offspring, thus minimizing parental waste (proposed by Lack, 1947; and more recently tested by Forbes et al., 2001).

Maternal resource investment in individual eggs is a possible mechanism for parents enhancing or mitigating the effects of hatching asynchrony by biasing individual nestling condition and survival. Previous studies of avian egg size found that it varies predictably with laying order; for example, in gulls and terns, egg size decreases with laying order (e.g. Nager et al., 2000; Fletcher and Hamer, 2004), while passerine eggs tend to increase in size with laying order (e.g. Clark and Wilson, 1981; Slagsvold et al., 1984). Nestlings hatching from larger eggs, presumably containing more nutrients, can experience increased growth and fitness (Williams, 1994; Christians, 2002). Therefore parents pursuing an "optimistic" strategy should increase the mass of each egg with increasing laying order and those engaging in an "insurance" strategy should decrease the mass of their eggs with advancing laying order. Studies in a number of species have found that yolk androgens increase growth rate and enlarge the hatching muscle of nestlings (For a review see Groothuis et al., 2005; but see Sockman and Schwabl, 2000). In birds, many species increase yolk androgens across the laying order (in keeping with the “optimistic” strategy), however, in the cattle egret, yolk androgens decrease with laying order (indicative of the “insurance” strategy) (Schwabl et al., 1997).

The hatching position, mass of the egg, and yolk androgens allocated to each egg can affect the future condition of the resulting nestling. The sex of each nestling can also potentially affect its future survival and food needs. Larger offspring often require more resources (Anderson et al., 1993; Reidstra et al., 1998; Torres and Drummond, 1999; Weimerskirch et al., 2000; Laaksonen et al., 2004), so when offspring are size dimorphic, the sex of each offspring may be related to the distribution of parental resources.

The sharing-out hypothesis, proposed by Carranza (2004), accounts for unequal food intake among siblings, and suggests that in sexually size dimorphic species parents may be selected to adjust offspring sex within the brood when food is unequally distributed to nestlings in a predictable way. This hypothesis assumes that high-ranking offspring (e.g., early-hatching nestlings that are larger than their later-hatched nest-mates) should receive a more predictable amount of food than lower-ranking progeny and are therefore more likely to survive, particularly in species with pronounced size asymmetries among offspring. Thus, Carranza (2004) predicts that 1) the larger sex will be produced in the laying positions that receive the most food (because they usually require more food to survive), and 2) mothers will be under the strongest selective pressure to bias the sex of their offspring in the first laying orders because these nestlings should receive the most predictable amount of food (nestlings at the end of the laying order will receive a highly variable amount of food depending on the environment and competitive abilities of their older hatched siblings). According to Carranza (2004), these predictions will be especially accurate when (a) the species is sexually size dimorphic (the larger the size dimorphism of a species, the larger the difference in food needs between male and females and, therefore, the more likely it is to produce the more costly sex in the first laying orders), and (b) the species has a pronounced hatching asynchrony (the strongest sex ratio biases towards the larger sex in the first-laid eggs should exist in species with pronounced competitive asymmetries among nestlings.). Carranza makes these predictions because the first-hatched nestlings are larger than their siblings, which makes them more likely to receive more food resources from their parents, which should be most beneficial to the larger more costly sex because they usually require more food to survive. The extra food, in turn, makes these larger offspring more likely to survive. In summary, the sharing-out hypothesis predicts that parents should bias the sex of early-laid offspring toward the more costly (larger) sex because they require more food to survive during development.

We examined parental resource allocation (egg mass and yolk hormones) and tested predictions of the sharing-out hypothesis in the barn owl (*Tyto alba*), a raptor species with pronounced reverse sexual size dimorphism (RSSD), large clutch size, and pronounced hatching asynchrony. Barn owl nestlings within a brood hatch at least two days apart; sometimes up to a week separates the hatching of consecutive siblings (Seifert, pers. obs; Marti et al., 2005). Consequently, barn owl broods exhibit extreme size hierarchy, with first-hatched nestlings much larger than later-hatched siblings; female nestlings tend to be larger, and therefore more costly, than males (Seifert et al., in prep.; Marti et al., 2005). We predict that first-hatched barn owl nestlings are more likely to survive than later-hatched nestlings because of this pronounced hatching asynchrony.

To investigate how parents allocate resources to their nestlings and distribute the sex of nestlings within a brood, we examined whether barn owl parents pursue an "optimistic" strategy to try to mitigate the effects of hatching asynchrony or an "insurance" strategy to try to enhance the effects of hatching asynchrony. If barn owls engage in an "insurance" strategy, egg mass and yolk androgens will decrease with laying order. In contrast, if barn owl parents pursue an "optimistic" strategy, egg mass and yolk androgens will increase with laying order. We also tested the sharing-out hypothesis, from which we predict a female bias in the first eggs, regardless of other parental allocation strategies. This prediction arises from the fact that barn owls have pronounced hatching asynchrony and are sexually size dimorphic, and hence nestlings in the first positions should receive the most predictable amount of food. Since barn owl females are larger than males, they need more predictable food supply, and so mothers should bias the early laying order toward female offspring. However, in accordance with the sharing-out hypothesis, if parents follow an "optimistic" strategy, the female biased sex ratio should not be as strong as if parents pursue an "insurance" strategy, because in the "optimistic" scenario, first-hatched nestlings would have less of an advantage over later-hatched nestlings.

METHODS

We studied a nest-box population of barn owls on agricultural ranches in a 20-mile radius of Merced, CA (37.3° N, 120.5° W). In central California barn owls initiate nests from late December until early May and are almost exclusively single brooded (Simmons, personal communication; Seifert, pers. obs.). Clutch size ranges from 3-12 eggs (Seifert, pers. obs.). Only the female incubates the clutch starting with the first egg, which results in a large hatching asynchrony (mean: 60 hours between nestlings, range: 48 hours to 8 days) and sibling size and age hierarchy. The male feeds the female as well as the nestlings until they are approximately one month old, at which time both parents feed the nestlings (Marti et al., 2005).

We checked nest boxes from the second week in January until the middle of May in 2005 and 2006. We checked boxes every four days until there were signs of nest initiation (i.e. an adult roosting in the box, and/or a stockpile of rodents in the box), after which we checked boxes every other day for subsequent eggs because barn owls do not lay more than one egg every other day (Marti et al., 2005). On the day each egg was laid we numbered it with a non-toxic permanent marker, weighed it (to the nearest 0.01g), and measured it for length and width (to the nearest 0.1mm). To determine maternal yolk hormones, at 25 nests we took a yolk biopsy from each egg on the day it was laid according to the protocol of Schwabl (1993). We used butterfly needles to remove approximately 0.15 grams of yolk from the egg (approximately three-quarters of the butterfly needle tube). We weighed each biopsied yolk to the nearest 0.001 g and added 200 μl of water and froze it at -20° C until hormone analysis. Once there was more than an 8-day gap between the laying of successive eggs, we checked the boxes every 4 days until 26 days had passed since the first egg was laid. Because the average incubation time is 30 days, but eggs can hatch as early as 28 days, we choose to check every other day starting at 26 days. This allowed us to match hatchlings to their eggs. We then checked boxes every other day until all of the eggs had hatched or 40 days had passed from the day the last egg was laid. We measured each hatchling's wing length (to the nearest 0.5 mm), body mass, and length of left tarsus (to the nearest 0.5 mm). Each nestling was given a colored plastic band until it could be fitted with adult-size 6 USFWS metal band, usually around an age of 10 days. From each nestling we collected a blood sample from the brachial wing vein on the first day they were encountered and stored samples in lysis buffer at 4° C for molecular analysis (see below).

Hormone Analysis

We followed the previously described procedures for extraction separation and measurement of yolk androsteindione (A_4), 5α-dihydrotestosterone (5α-DHT), testosterone, corticosterone, and 17β estrodiol (E_2). Briefly, we extracted the yolk homogenates with diethyl ether/petroleum ether. We used the entire yolk sample, which as stated above, was approximately 0.15 grams. We ran the extract through diatomaceous earth columns to separate the hormones. After our first year we realized that E2 was undetectable and we stopped measuring it. We continued to measure corticosterone, however, the levels were barely detectable so we did not use them for any analyses. We analyzed yolk samples for concentrations of androgens using a radioimmunoassay (RIA) following the same methods as Schwabl (1993). We used the following antibodies: T 3003 (Wien Laboratories) for T and 5α-DHT, A 1707 (Wien Laboratories) for A_4, and B-3-163 (Esoterix Endocrinology) for corticosterone. The ^3H labeled steroids used came from Perkin Elmer Life and Analytical Sciences and were as follows NET 553 for T, NET 544 for 5α-DHT, NET 469 for A_4 for corticosterone.

The intra-assay variation for testosterone was 7.97%, for 5α-DHT it was 10.8%, for A_4 it was 7.19% and for corticosterone it was 14.6%. The inter-assay variation for testosterone was 4.15%, for 5α-DHT it was 26.8%, for A_4 it was 6.8%, and for corticosterone it was 3.67%. The average recovery efficiencies were testosterone=45%, 5α-DHT= 28%, A4= 48% and corticosterone= 36%.

Molecular Analysis of Nestling Sex

Even though nestling barn owls are sexually dimorphic in both size and color, we used a molecular sexing technique to obtain information on the sex ratio before any offspring died or disappeared from the nest. We extracted DNA from blood samples using a standard phenol-cholorform extraction (Westneat, 1990). To determine nestling sex we followed the protocol of Kahn et al (1998), using primers 1237L and 1272H. We used adults of known sex to determine the proper temperatures for our PCR and in each PCR run we used a known adult of each sex to verify our results. PCR reactions consisted of 40 cycles at 94°C for 60 seconds, 55°C for 60 seconds and 72°C for 45 seconds. We had a total reaction volume of 10µl, for which we used 1X PCR buffer (Applied Biosystems), 0.15 mM dNTP mix, 0.5 uM of each primer, 2mM of MgCl2 and 2.5U/ul of *Taq* polymerase.

Statistics

We performed all statistical tests with SAS. First, we used the SAS Proc Mixed procedure to do a general linear mixed model with the assumption that the covariance matrix was compound symmetric to determine whether egg mass varied with clutch size.

All eggs were nested within their family to eliminate pseudo-replication. Our independent variables were Julian date and clutch size.

To determine whether egg mass varies across the laying order and with sex, we again used a SAS Proc mixed procedure to do a general linear model with the assumption that the covariance structure was compound symmetric. We nested eggs within their family to control for pseudo-replication. Our independent variables were Julian date, egg order, sex and egg order by sex interaction.

To test how yolk androgens vary with laying order and clutch size, we used the Proc mixed procedure in SAS to make a general linear mixed model with the assumption that the covariance structure was compound symmetric. Our independent variables were laying order, laying order squared (to test for a quadratic change), Julian date, and clutch size. We also ran the model by re-centering the mean for laying order to remove potential effects of co-linearity between laying order and laying order squared, but it did not affect our results so we left the data un-centered.

We had low hatching success (30% on average) with eggs that had yolk biopsies and so we do not have enough information to examine difference in yolk androgen concentrations by sex.

We performed a logistic regression (also in SAS) to examine whether offspring sex varied with laying order, and whether offspring mortality was affected by sex, laying order or the interaction between the two.

RESULTS

Mortality

We found that nestlings later in the hatching order had lower survival rates than those that hatched earlier ($\chi^2 = 42.215$, $P < 0.001$, N=293, Table 3.1, Figure 3.1). We also found that males were more likely to die than females ($\chi^2 = 39.859$, $P < 0.001$, N=293, Table 3.1). However, there was no interaction between sex and hatching order, meaning that, at all hatching positions male nestlings were more likely to die than female nestlings ($\chi^2 = 0.138$, $P = 0.125$, N=293, Table 3.1).

Egg Mass

We found no differences in egg mass among nests of various clutch size ($F_{1,198} = 0.070$, $P = 0.791$, Figure 3.2). Julian date had a significant effect on egg mass ($F_{1,198} = 35.03$, $P < 0.001$, Table 3.2), so we controlled for Julian date in our analysis. Egg mass was significantly affected by egg order ($F_{1,198} = 10.46$, $P = 0.001$, Table 3.2, Figure 3.3), but not by sex ($F_{1,120} = 0.90$, $P = 0.346$, Table 3.2, Figure 3.3). There was, however, an interaction between egg order and sex ($F_{1,198} = 3.99$, $P = 0.047$, Table 3.2, Figure 3.3). Egg mass increased over the egg order, but this was mainly driven by the 11 female eggs laid into late egg orders (Figure 3.3).

Sex Ratio

Laying order was associated significantly with offspring gender ($\chi^2 = 4.239$, P = 0.039, DF= 1, N= 339, Table 3.3, Figure 3.4); although eggs laid in early positions were as likely to be male as female, those laid in later positions were more likely to be male than female.

Yolk Androgens

There was a significant effect of laying order on yolk testosterone concentration ($F_{1,134}$= 11.44, *P* = 0.001, Table 3.4a, Figure 3.5) as well as a quadratic effect, egg order squared ($F_{1,134}$= 5.77, *P* = 0.018, Table 3.4a, Figure 3.5). This suggests that testosterone levels increased and then decreased within a clutch. There was no effect of clutch size ($F_{5,25}$= 0.96, *P* = 0.4618, Table 3.4a, Figure 3.5). But there were significant interaction effects of clutch size and egg order ($F_{5,134}$= 2.69, *P* = 0.024, Table 3.4a, Figure 3.5), and clutch size and egg order squared ($F_{5,134}$= 3.38, *P* = 0.007, Table 3.4a, Figure 3.5), suggesting that different sized clutches had different patterns in the levels of androgens in each egg. There was also no effect of Julian date (F= 0.16, *P*= 0.69, Table 3.4a, Figure 3.5).

A_4 showed a similar pattern to testosterone. There was a significant effect of egg order on yolk A_4 concentration ($F_{1,134}$= 5.92, *P* = 0.016, Table 3.4b, Figure 3.6). There was a marginal effect of egg order squared ($F_{5,134}$= 2.92, P = 0.090, Table 3.4b, Figure 3.6) on A_4 concentrations, suggesting that the increase and decrease in levels within a clutch wasn't as dramatic as with testosterone. There was no effect of clutch size ($F_{5,25}$= 0.38, *P* = 0.860, Table 3.4b, Figure 3.7), and no interaction effects of egg order with clutch size for A_4 (Table 3.4b). Like A_4, there was a significant effect of egg order on the yolk 5α-DHT concentration ($F_{1,134}$= 4.01, *P* = 0.042, Table 3.4c, Figure 3.7), and a marginal effect of egg order squared ($F_{1,134}$= 2.41, *P* = 0.112, Table 3.4c, Figure 3.6), indicating that 5α-DHT levels changed with egg order, but again the increase and decrease wasn't as strong as with testosterone. There was no effect of clutch size ($F_{5,25}$= 0.28, *P* = 0.920, Table 3.4c, Figure 3.7) or any interaction of egg order with clutch size in the levels of 5α-DHT (Table 3.4c).

DISCUSSION

We found unequal survival among nestlings depending on their position in the hatching order, such that earlier-hatched nestlings were more likely to survive than later-hatched nestlings (Figure 3.1). Even at the same age (not the same point in time), earlier-hatched nestlings were much larger in structural size and mass than later-hatched nestlings (Seifert et al., in prep.). In barn owls, hatching of a single clutch can occur over a two-week period, and as a result, the oldest nestlings can be more than 200 grams (92%) heavier than their youngest siblings (Seifert, per. obs.). In addition, we found support for a sex-biased laying order, with more male eggs being laid into the later hatching positions.

Parental Investment Strategy

We found that egg mass varied with laying order and that there was a significant interaction between egg mass and sex. Because we used our variables as linear predictors, we were unable to do any pair-wise comparisons to determine the nature of these interactions. From our β values, we know that egg mass increased with laying order, but the significant interaction of sex and laying order tells us that the sexes do not show the same egg mass at each laying order. We found that later-laid female eggs were larger than later-laid male eggs (Figure 3.3). We also know that egg mass decreased throughout the season because the β for Julian date is negative (Table 3.2). We would need to do a more detailed study to determine whether the decrease in egg investment with season is due to changes in food abundance.

We found a general increase in egg mass over the laying order, however, there are only 11 females that lay larger eggs at the end of the laying order (Figure 3.3) suggesting that these females follow an "optimistic" strategy of egg content allocation. This trend appears only in female eggs, as male egg mass did not change as much with laying order (Figure 3.3). More work needs to be done before we can make a conclusive statement about which strategy females follow—if any—with egg mass allocation. Because food delivery rates can be determined in the barn owl, it would be interesting to collect more data on females that lay optimistic clutches to determine if they have more food resources than those that lay a clutch without much variation in egg mass. In other species, egg mass shows a linear pattern over the course of the laying order. In most passerine birds, egg mass tends to increase over the laying order (a pattern female barn owl eggs seem to follow) (Slagsvold et al., 1984). The fact that male barn owl eggs do not follow either pattern may indicate a differential investment in egg mass between males and females based on laying order, which is consistent with findings in gulls (Muller et al., 2005).

All bird species in which yolk androgens have been studied to date have shown either an increase in yolk androgens with laying order, a decrease with order, or no pattern at all (reviewed in Groothuis et al., 2005). In contrast to these previous studies, we found yolk androgen allocation patterns suggestive of a "hybrid" strategy, with first an increase (in the first 3 eggs) and then a decrease (in all subsequent eggs) of yolk androgens within the laying order. We believe that this previously unreported trend in yolk androgen pattern combines the "optimistic" and "insurance" strategies. In the first 3 eggs, yolk androgen levels increased from first- to later-laid, consistent with the "optimistic" strategy we defined in our introduction. This strategy may help the nestlings in positions 2 and 3 compete with their larger, eldest sibling. However, we found that there was also an increase in mortality from egg 1 to eggs 2 and 3 (Figure 3.1), which indicates that even with increased androgen levels females may not be able to completely compensate for the size differences caused by hatching asynchrony. In a previous study we found that earlier-hatched nestlings grew larger than later-hatched nestlings (Seifert et al., in prep.). More specifically, there was a decline is fledging size (wing, mass and tarsus) with each subsequent hatching position. These results suggest that yolk androgens cannot completely mitigate the size advantage that first-hatched nestlings have over all subsequent siblings. Perhaps first-hatched nestlings simply have better access to food, a competitive advantage that later-hatched siblings cannot overcome. Aside from the more obvious advantages for first-hatched nestlings, which we have already discussed, these advantaged offspring also potentially benefit from being the only progeny parents have to feed for the first 2+ days of their lives. It would be interesting to try to experimentally determine in a future study whether better early access to food may give these offspring an added advantage for growth.

Due to the barn owl's extreme hatching asynchrony, later-hatched nestlings are always much younger and smaller than their older siblings; for instance, a nestling from the fifth hatching position would be at least 10 days younger than the first-hatched nestling (Seifert, pers. obs.). In the case of later-hatched nestlings, we found that parents may adopt more of an "insurance" strategy; in general yolk androgens decreased from eggs 3 or 4 through the end of the laying order, except in 8-egg clutches, where the androgens remained at fairly low levels over the entire laying order. The decrease in yolk androgens puts the later-hatched nestlings at even more of a disadvantage in relation to their earlier-hatched siblings; not only were they smaller due to the effects of hatching asynchrony, but they also received less yolk hormones to aid in their development (reviewed by Groothuis et al., 2005). Thus, we believe that later-laid barn owl eggs represent an insurance policy for the parents—offspring that can be raised in the case of unusually high mortality among their earlier-laid siblings, but that routinely die before fledging. Most of these offspring die within the first week after they hatch (Seifert, unpub. data), which should make them less expensive to parents than those that survive longer. Because it is so unlikely that one of these later-hatched nestlings would survive without unusually high mortality among earlier-hatched siblings or an abundance of food resources in the environment, a parental strategy that decreases investment to these later nestlings can save parental resources by hastening their near-inevitable mortality if none of the early hatched nestlings die (consistent with Mock and Parker, 1998).

Sex Bias and Laying Order

We found that female barn owls biased the primary sex ratio of their clutches according to egg laying order: nestlings that hatched early were equally likely to be male or female, whereas later-hatched nestlings were more likely to be male (Figure 3.2). This result differs from our expectations based on the sharing-out hypothesis, which predicts that the stronger sex bias should exist in first-laid eggs because the resulting nestlings are those with the most predictable food supply. Because of the barn owl's pronounced hatching asynchrony and size dimorphism, we predicted a strong female bias in the early laying orders, but did not find it. Similarly, in a study of house wrens (*Troglodytes aedon*), researchers found that the latter half of the brood was female biased even though the sharing-out hypothesis predicted a very different trend—a male bias in the early-laid eggs (Albrecht, 2000). In that case, Carranza (2004) explained the sex ratio bias in the second half of the brood by noting that house wrens are monomorphic and arguing that in such species there is not any benefit to nestlings of one sex or the other being laid first. The barn owl, however, is highly sexually size dimorphic, so this argument cannot apply to our results. A possible explanation is that even though there is a size difference between males and females, it still may not be beneficial for parents to create a heavy female bias in first-hatched nestlings because males' survival may benefit equally as much as females from the extra food that preferential hatching-position confers. In addition, barn owls are monogamous and so frequency-dependent selection may ensure equal benefits to producing males or females in preferential laying orders. Alternatively, since it seems possible that male nestlings require less investment than females (Seifert et al., in prep.), these later-laid male nestlings may be more likely to survive in the disadvantaged later hatching positions because they may have more modest food requirements than females (Seifert et al., in prep.).

Carranza (2004) predicts that the sex bias should be at the beginning of the laying order because food is typically the most predictable for first-hatched offspring, but in this case, the converse may also be true: nestlings in later hatching positions may face predictably poor food conditions, and the potentially more food-demanding females may not be able to survive those conditions, resulting in a parental strategy of laying male biased insurance eggs. Our study found an offspring sex bias that seems to be ordered by the principles of Carranza's hypothesis, if not its actual predictions. Barn owl parents seem to bias their nestlings' sex in relation to the amount of food they predictably received (we believe that later-hatched nestlings received a predictably small amount of food because they almost never survive), but we suggest that the sharing-out hypothesis needs an adjustment to account for cases in which the last-laid eggs, most likely "insurance" eggs, are so disadvantaged that they consistently receive minimal food resources.

We found that males had higher mortality than female nestlings, counter to what we had predicted (males are smaller and should require less food). The higher male mortality may be caused by nestlings of the smaller sex may be more susceptible to mortality during the nestling stage because the nestlings of the larger sex out-compete them for food. We see that across all hatching positions that male nestlings have higher mortality than female nestlings (Figure 3.1). Future research should examine competitive sibling interactions to determine if, in fact, intra-brood competition could be responsible for increased mortality of the smaller sex during the nestling phase, as reported in the great tit (*Parus major*) (reviewed in Oddie, 2000).

Another likely explanation for the difference in mortality between the sexes is the preponderance of male nestlings in late-hatching positions. We know that nestlings later in the hatching order are less likely to fledge than those that hatch earlier (Seifert et al., in prep.). While mothers do not bias egg size according to sex, there seems to be a difference between male and female egg size in laying orders 6 and 7. Male eggs later in the laying order are much smaller than female eggs; our findings of significant interaction between laying order and sex (Table 3.2) support this idea. Since male eggs tend to be preferentially laid later in this sequence, our results suggest that there is female-biased investment in egg size as a function of laying order. A number of other studies report sexually size dimorphic eggs (Mead et al., 1987; Anderson et al., 1997; Cordero et al., 2000; Cunningham and Russell, 2000; Cordero et al., 2001; Blanco et al., 2003; Muller et al., 2005). We did not find an overall difference in egg mass between sexes, but the mass of female eggs tended to increase with laying order, while the mass of male eggs remained relatively stable (Figure 3.1). Due to sample size restrictions, we could not say whether female nestlings that hatch from larger eggs in later positions have better survival than females that hatch from smaller eggs in later positions. We do know that nestlings later in the hatching order were more likely to die during the nestling phase than those that hatch earlier (Figure 3.1). Male barn owl nestlings are smaller than females and require less parental investment (Seifert et al., in prep.). Therefore, it may be that female eggs, especially when laid late in the sequence, cannot survive without the extra egg mass, which could lead to female biased maternal egg mass investment late in the laying order. Alternatively, because our samples size is small for later-laid eggs, it is also possible that in these few nests, parents had more food resources and were laying a completely "optimistic" brood, but that the trend did not reach statistical significance.

As suggested by Carranza, early- and late-hatched barn owl nestlings do not seem to experience the same environmental conditions. Early-hatched nestlings have better access to food because they are larger structurally due to their much earlier hatching than later-hatched nestlings (Seifert et al., in prep.). The harsh nest environment that later-hatched males face is not the same as that experienced by earlier-hatched nestlings. Nestlings in later hatching positions face more intense competition for food because of the aforementioned size disparity between them and their older siblings. Even though siblings all have similar genetic makeup and are reared by the same parents, they may experience different environmental pressures within the nest due to the large hatching asynchrony. Thus, the laying order of a nestling may influence its phenotype.

Conclusions

In cases where birds hatch asynchronously, parents can pursue an “optimistic” strategy of resource allocation (egg mass and yolk hormones), which may help to mitigate the resulting nestling size hierarchy. On the other hand, parents can enhance the resulting nestling size hierarchy by decreasing resource allocation with an increase in laying order. In this “insurance” strategy, parents depend on the success of their first-hatched nestlings, but produce extra eggs for cases of unusually high mortality or exceptionally abundant resources, in which insurance offspring have reproductive value. There seemed to be a slight increase in egg mass with laying order, which is synonymous with our idea of an “optimistic” brood, however more work needs be done to tease out the gender effects. On the other hand, barn owl parents seem to pursue a “hybrid” strategy with their yolk hormone allocation pattern: there is an increase in yolk androgen in the first 3 eggs (those producing the nestling that are most likely to fledge), and then a decrease in yolk to each subsequent egg (although 8 egg clutches do not follow this pattern), as the likelihood of early mortality increases. We predict that other bird species with large clutches and extreme hatching asynchrony, such as parrots and hornbills, should also pursue a “hybrid” strategy of yolk hormone allocation.

We conclude that barn owls parents may be directing their investment in clutches as a means to maximize future reproductive fitness. Nestlings later in the hatching order are very likely to die before fledging. Parents may lay these extra eggs in case eggs earlier in the laying order do not hatch, or in case there are abundant food resources and they can feed and fledge more young than usual. Parents seem to be able to adjust sex ratio with laying order and potentially the egg mass for each sex by laying order, based on our finding that male eggs are similar in size across the laying order, while female eggs increase in size with laying order. Finally, we found a potentially adaptive pattern of egg yolk androgen allocation that is different from patterns observed in all other species studied to date, suggesting that barn owls and potentially species with similar breeding biology employ a subtler reproductive optimization strategy than was previously thought.

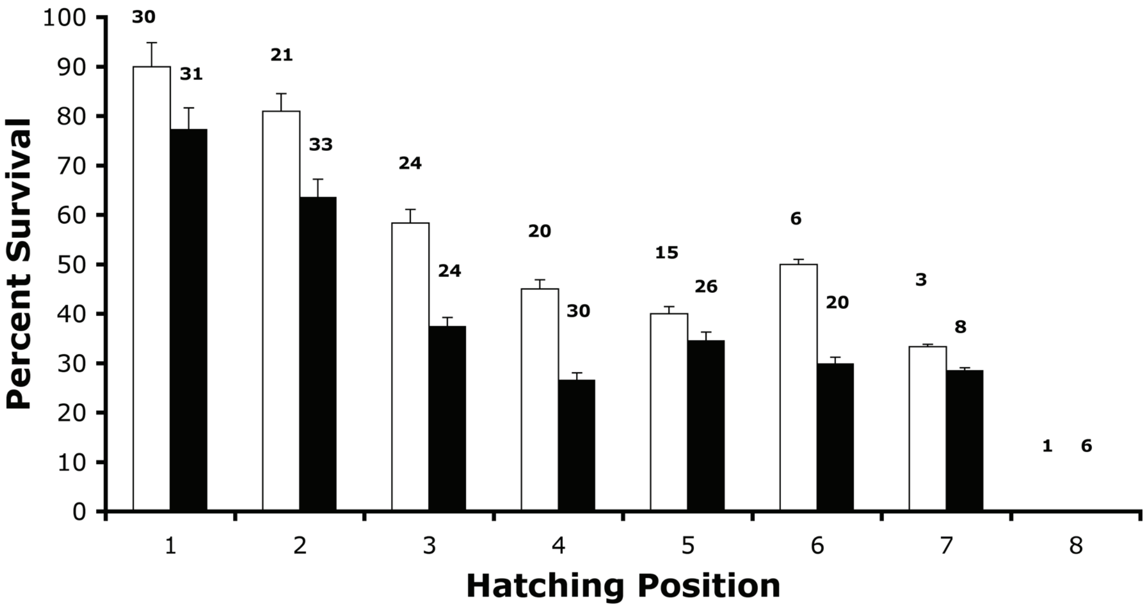

Figure 3.1. Nestlings in later hatching positions were more likely to die than those that hatch from earlier positions (N=297 nestlings). Male (closed) show lower survival than females (open) at all hatching positions. The sample size and standard error is shown for each hatching position.

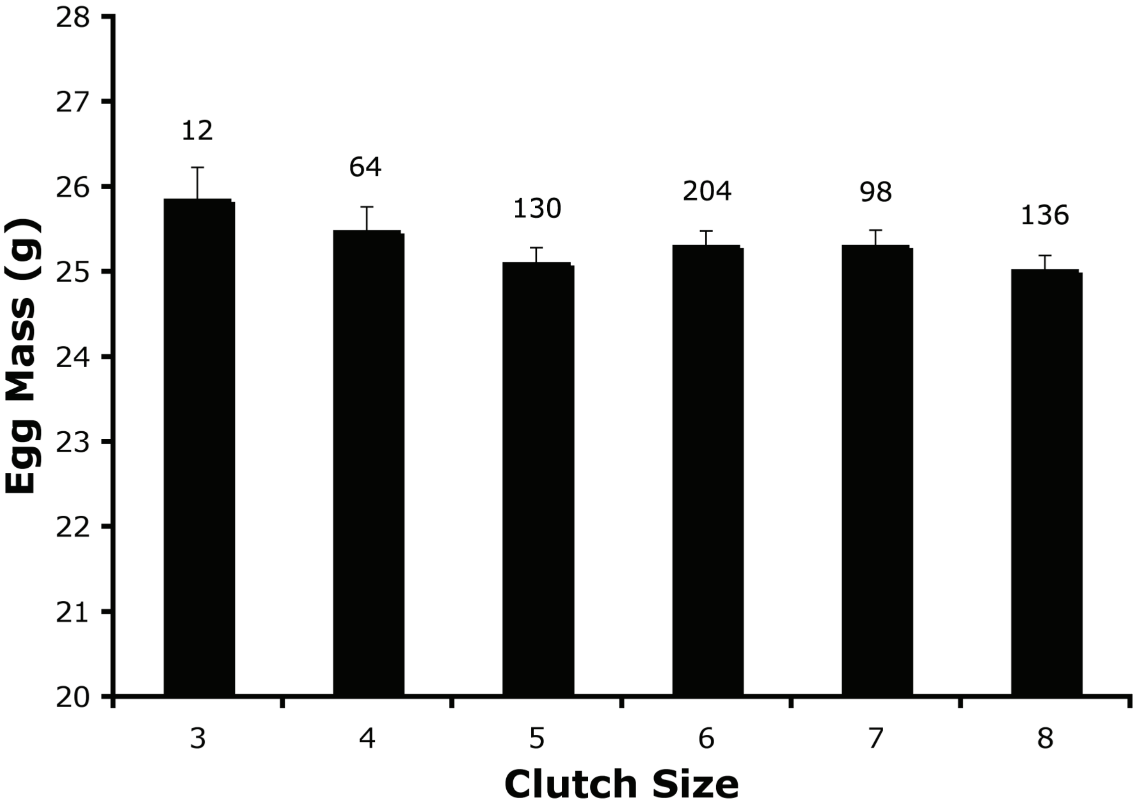

Figure 3.2. There was no difference in egg mass with clutch size. Females that produced more eggs did not make them smaller than females that produced fewer eggs. The standard error is shown for each mean and the number of eggs used to calculate the mean is shown above each bar.

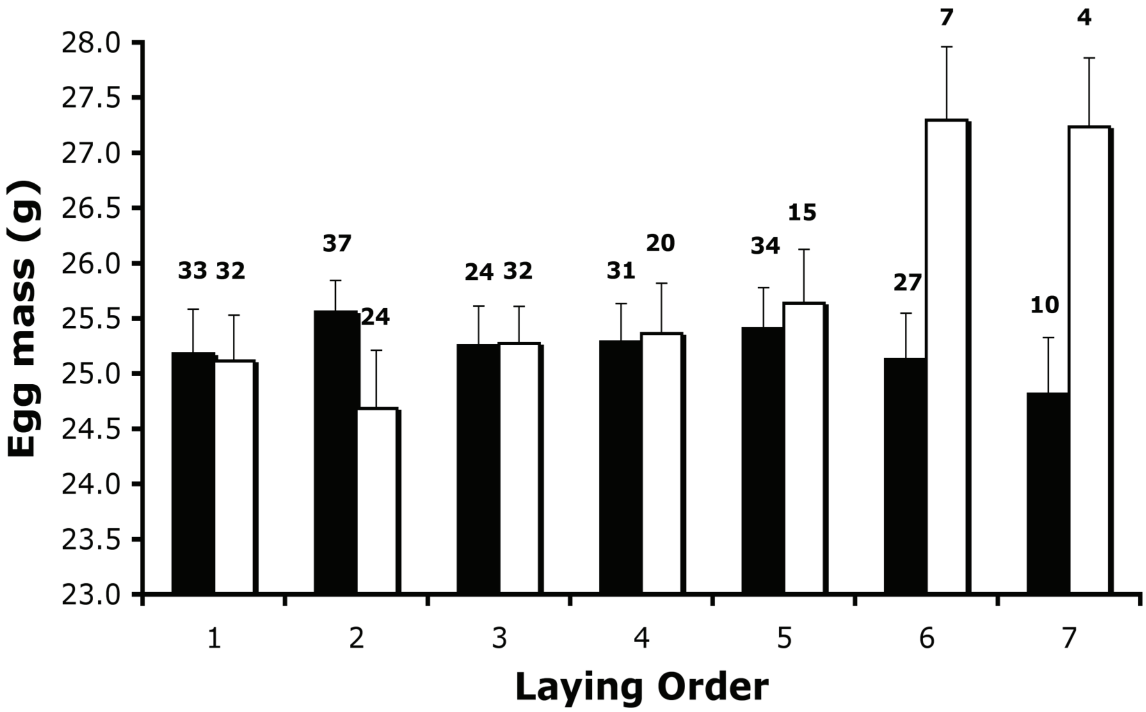

Figure 3.3. Mass of male eggs (closed) tended to remain fairly constant with laying order, whereas the mass of female eggs (open) tended to increase with laying order. The sample size and standard error is shown for each mean.

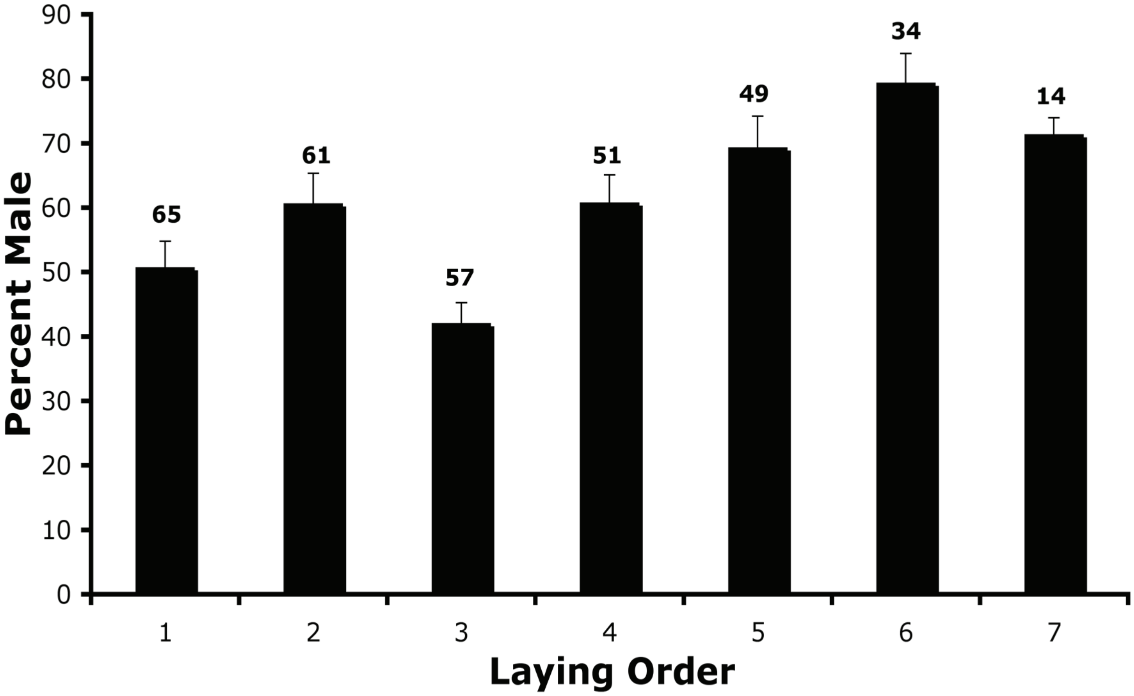

Figure 3.4. Later-laid eggs were more likely to be male than earlier-laid eggs. Sample size and standard error bars shown above each laying order.

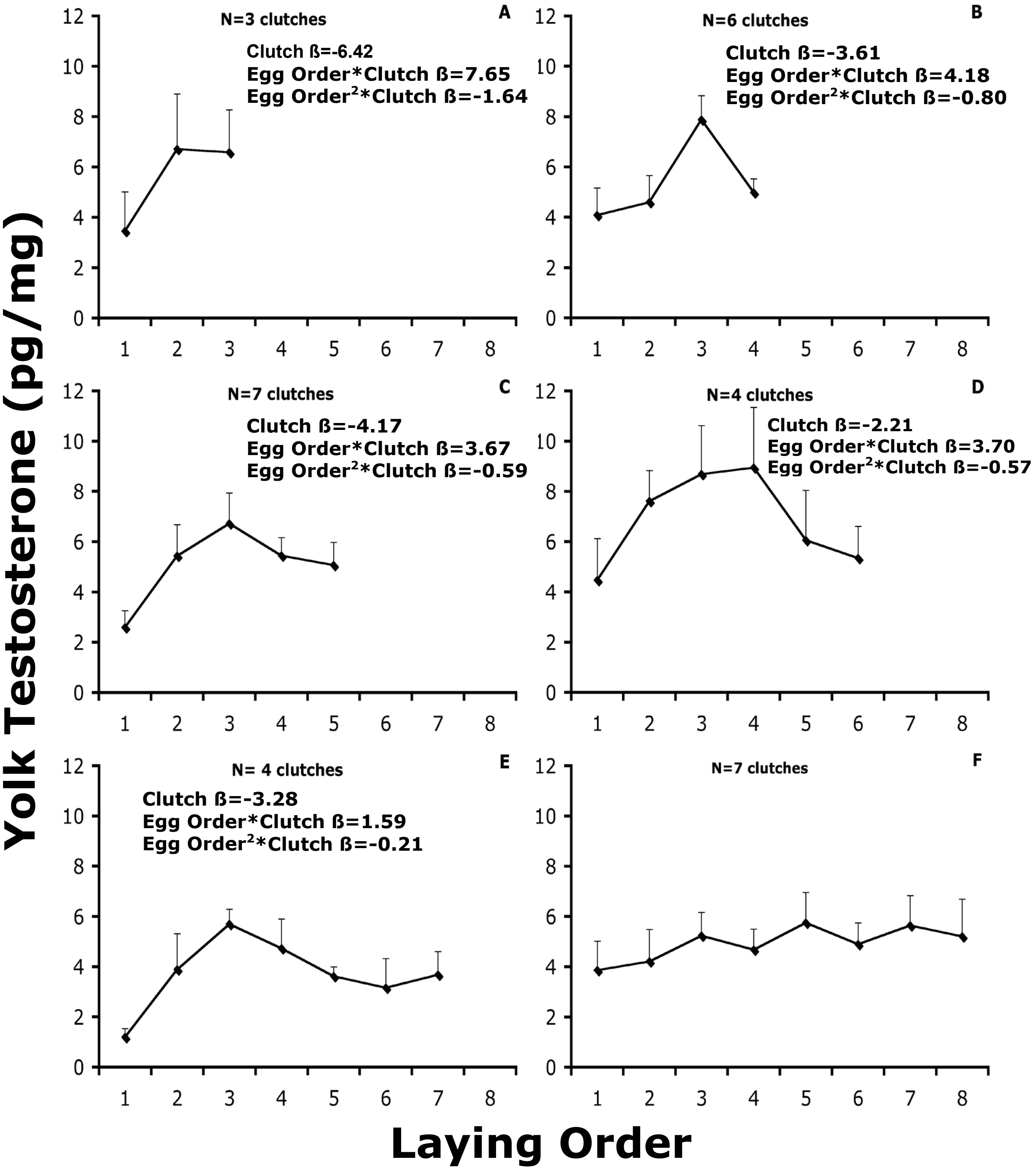

Figure 3.5. Yolk T showed a quadratic relationship with laying order—in general there was an increase in the first 3 to 4 eggs followed by a decrease in later-laid eggs, except for 8-egg clutches that had a slight increase with laying order. Standard error bars, sample size, and β values shown on each graph. A=3-egg clutches, B=4-egg clutches, C=5-egg clutches, D=6-egg clutches, E=7-egg clutches, F=8-egg clutches.

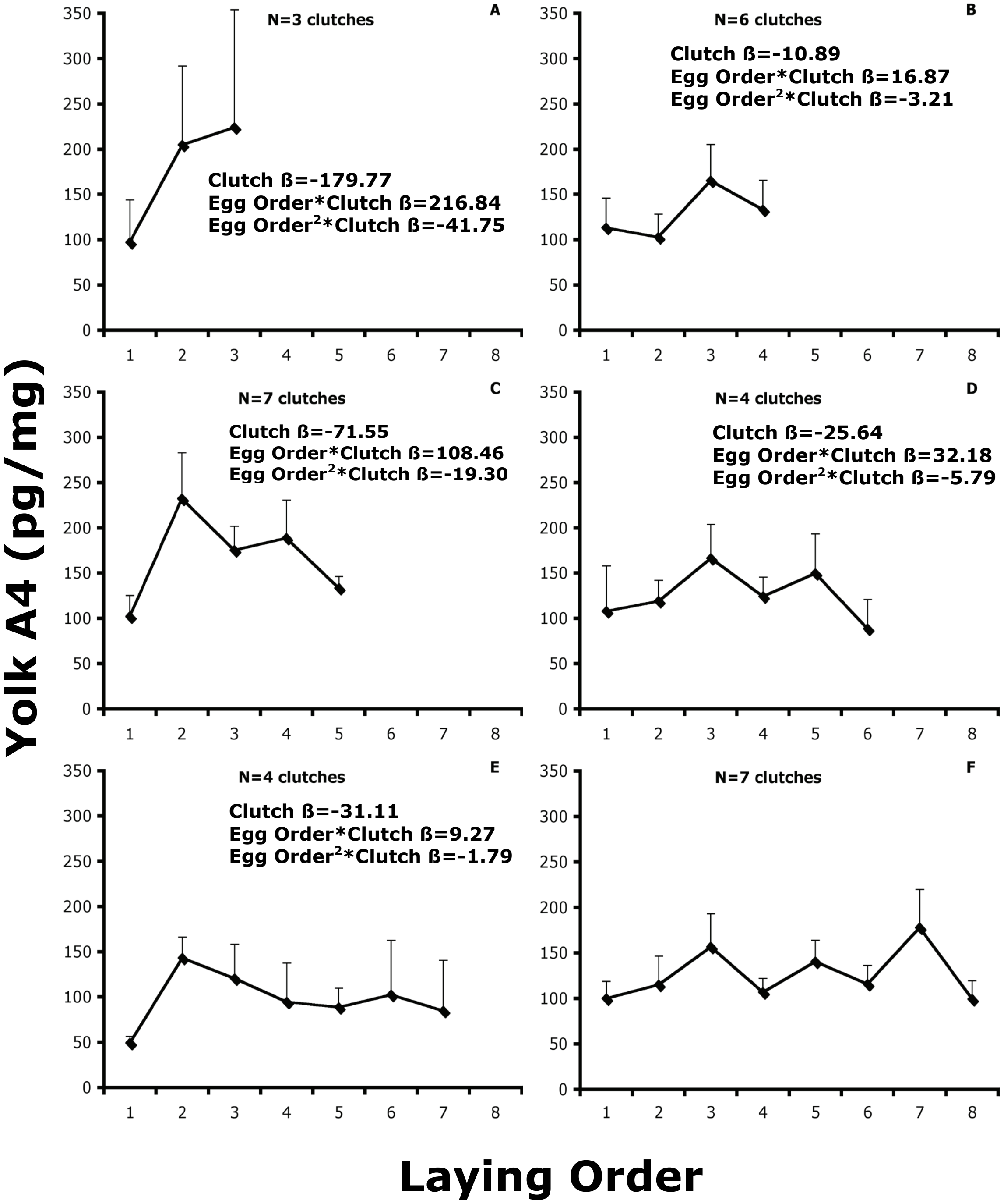

Figure 3.6. Yolk A4 showed a quadratic relationship with laying order—in general there was an increase in the first 2 to 3 eggs followed by a decrease in later-laid eggs, except for 8-egg clutches that have an increase in the first 3 eggs, but do not show a decrease. We show standard error bars, sample size, and β values on each graph. A=3-egg clutches, B=4-egg clutches, C=5-egg clutches, D=6-egg clutches, E=7-egg clutches, F=8-egg clutches.

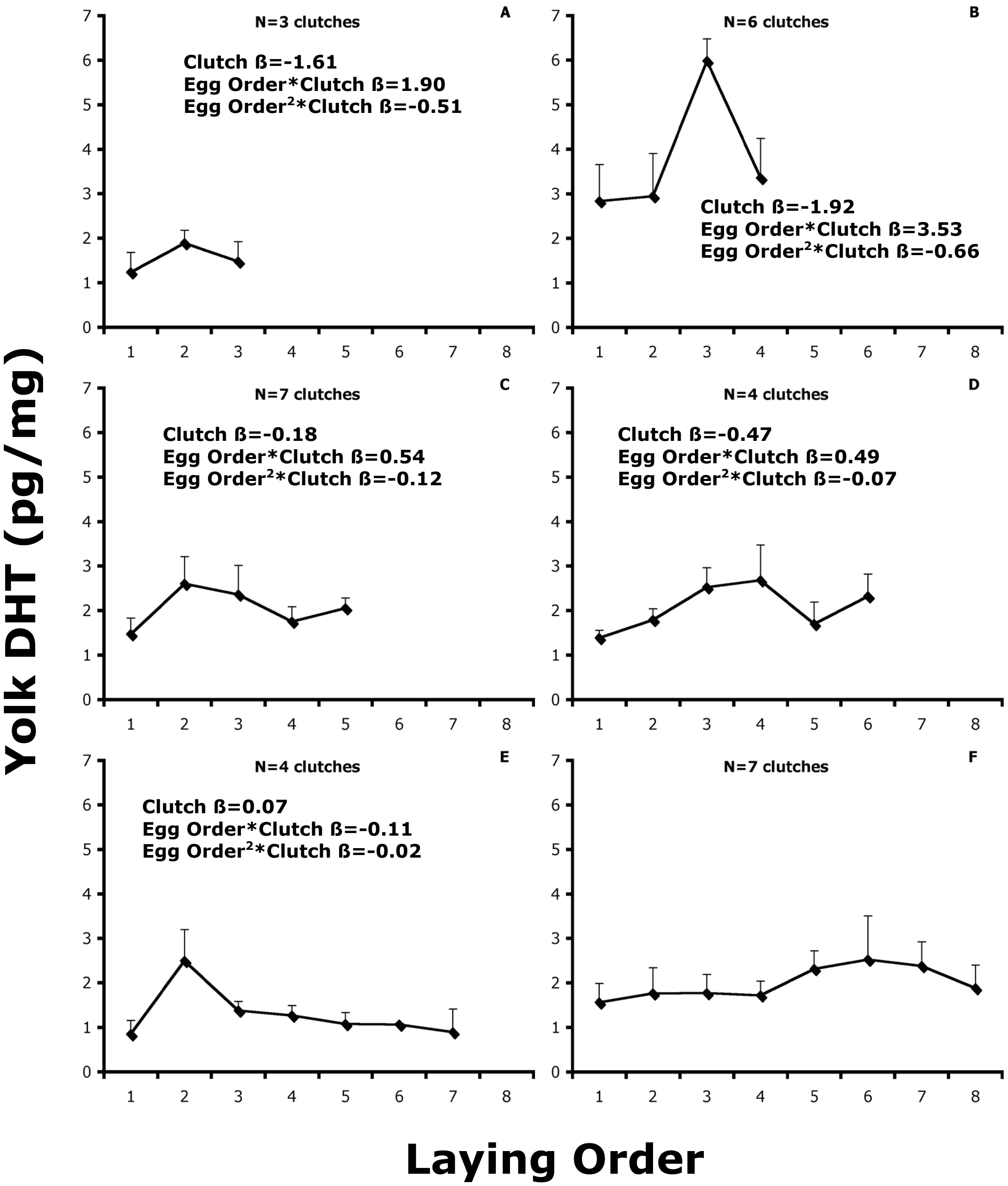

Figure 3.7. Yolk DHT showed a quadratic relationship with laying order—in general there was an increase in the first 2 to 4 eggs followed by a decrease in later-laid eggs, except for 8-egg clutches that show a slight increase in later-laid eggs. Standard error bars, sample size, and β values on each graph. A=3-egg clutches, B=4-egg clutches, C=5-egg clutches, D=6-egg clutches, E=7-egg clutches, F=8-egg clutches.

Table 3.1: Results from a logistic regression analyzed changes in mortality with different variables.

Mortality	*df*	N	ß	X^2	*P*
Laying Order	1	293	-0.419	42.215	<0.001
Sex	1	293	-0.142	39.859	<0.001
Clutch Size	1	293	-0.219	1.459	0.227
Laying Order*Clutch Size	1	293	-0.017	1.971	0.161
Laying Order* Sex	1	293	-0.052	0.138	0.125

Table 3.2: Variables in a general linear mixed model which explain variation in egg mass with laying order. The eggs came from 70 different families.

Egg Mass	*df*	N	ß	*F*	*P*
Laying Order	1	198	0.23	10.46	0.001
Sex	1	198	0.41	0.90	0.346
Laying Order* Sex	1	198	-0.17	3.99	0.047
Julian Date	1	198	-0.02	35.03	<0.001

Table 3.3: Results from a logistic regression that analyzed changes in sex ratio with laying order, clutch size, and Julian date.

Sex Ratio	*df*	N	ß	χ^2	*P*
Laying Order	1	339	0.111	4.239	0.039
Clutch Size	1	339	0.018	0.002	0.968
Julian Date	1	339	0.000	0.044	0.834

Table 3.4: Variables in three general linear mixed models which explain variation in (a) yolk testosterone, (b) yolk DHT, and (c) yolk A4. We used 31 families for each analysis. The ß values for clutch size, egg order*clutch size, and egg order^2*clutch size are found in figure 3.5 for testosterone, 3.6 for A4, and 3.7 for DHT.

Source	*df*	N	ß	*F*	*p*
(a) Testosterone		177			
Egg Order	1	177	0.69	11.44	<0.001
Egg Order^2	1	177	-0.05	5.77	0.018
Clutch Size	5	177	XXX	0.96	0.462
Egg Order*Clutch Size	1	177	XXX	2.69	0.024
Egg Order^2*Clutch Size	1	177	XXX	3.38	0.007
Julian Date	1	177	-0.01	0.16	0.69
(b) DHT					
Egg Order	1	177	0.3551	4.01	0.0472
Egg Order^2	1	177	-0.02865	2.41	0.1228
Clutch Size	5	177	XXX	0.28	0.92
Egg Order*Clutch Size	1	177	XXX	1.40	0.2294
Egg Order^2*Clutch Size	1	177	XXX	1.38	0.2358
(c) A4					
Egg Order	1	177	22.3907	5.92	0.0163
Egg Order^2	1	177	-2.1977	2.92	0.0901
Clutch Size	5	177	XXX	0.38	0.8602
Egg Order*Clutch Size	1	177	XXX	1.47	0.205
Egg Order^2*Clutch Size	1	177	XXX	1.82	0.1138

WORKS CITED

Albrecht DJ, 2000. Sex ratio manipulation within broods of house wrens (*Troglodytes aedon*). Animal Behaviour 59:1227-1234.

Amat JA, Fraga RM, Arroyo GM, 2001. Intraclutch egg-size variation and offspring survival in the kentish plover *Charadrius alexandrinus*. Ibis 134:17-23.

Anderson D, Reeve J, JEM G, Weathers W, Hutson S, Cunningham H, Bird D, 1993. Sexual size dimorphism and food requirements of nestling birds. Canadian Journal of Zoology 71:2541-2545.

Anderson DJ, Reeve J, Bird M, 1997. Sexually dimorphic eggs, nestling growth and sibling competition in American kestrels *Falco sparverius*. Functional Ecology 11:331-335.

Beissinger SR, Waltman JR, 1991. Extraordinary clutch size and hatching asynchrony of a neo tropical parrot. Auk 108:863-871.

Blanco G, Martinez-Padilla J, Serrano D, Davila JA, Vinuela J, 2003. Mass provisioning to different sex eggs within the laying sequence: consequences for adjustment of reproductive effort in a sexually dimorphic bird. Journal of Animal Ecology 72:831.

Blount JD, Metcalfe NB, Arnold KE, Surai PF, Monaghan P, 2006. Effects of neonatal nutrition on adult reproduction in a passerine bird. Ibis 148:509-514.

Bortolotti GR, 1986. Influence of sibling competition on nestling sex ratios of sexually dimorphic birds. American Naturalist 127:495-507.

Carranza J, 2004. Sex allocation within broods: the intrabrood sharing-out hypothesis. Behavioral Ecology 15:223-232.

Charnov EL, 1982. The theory of sex allocation. Princeton, NJ: Princeton University Press.

Christians JK, 2002. Avian egg size: variation within species and inflexibility within individuals. Biological Reviews 77:1-26.

Clark AB, Wilson DS, 1981. Avian breeding adaptations: hatching asynchrony, brood reduction, and nest failure. Quarterly review of biology 56:253-277.

Clutton-Brock TH, 1991. The evolution of parental care. Princeton, NJ: Princeton University press.

Cordero PJ, Griffith SC, Aparicio JM, Parkin DT, 2000. Sexual dimorphism in house sparrow eggs. Behavioral Ecology 48:353-357.

Cordero PJ, Vinuela J, Aparicia JM, Veiga JP, 2001. Seasonal variation in sex ratio and sexual dimorphism favouring daughters in first clutches of the spotless starlings. Journal of Evolutionary Biology 14:829-834.

Cunningham EJ, Russell AF, 2000. Egg investment is influenced by male attractiveness in the mallard. Nature 404:474-77.

de Kogel CH, 1997. Long term effects of brood size manipulations on morphological development and sex-specific mortality of offspring. Journal of Animal Ecology 66:167-178.

Eising CM, Eikenaar C, Schwabl H, Groothuis TGG, 2001. Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development. Proceedings of the Royal Society B-Biological Sciences 268:839-846.

Fisher RA, 1930. The Genetical Theory of Natural Selection. Oxford: Clarendon Press.

Fletcher KL, Hamer KC, 2004. Offspring sex ratio in the common tern *Sterna hirundo*, a species with negligible sexual size dimorphism. Ibis 146:454-460.

Forbes L, Mock D, 1998. Parental optimism and progeny choice: when is screening for offspring quality affordable? Journal of Theoretical Biology 192:3-14.

Forbes LS, Glassey B, Thornton S, Earle L, 2001. The secondary adjustment of clutch size in red-winged blackbirds (*Agelaius phoeniceus*). Behavioural Ecology and Sociobiology 20:37-44.

Forbes LS, Lamey TC, 1996. Insurance, developmental accidents, and the risks of putting all your eggs in one basket. Journal of Theoretical Biology 180:247-256.

Forbes S, Mock DW, 2000. A tale of two strategies: life-history aspects of family strife. Condor 102:23-34.

Frank SA, 1990. Sex allocation theory for birds and mammals. Annual Review of Ecology and Systematics 21:13-55.

Godfray HCJ, Werren JH, 1996. Recent developments in sex ratio studies. Trends in Ecology and Evolution 11:59-63.

Groothuis TGG, Muller W, von Engelhardt N, Carere C, Eising C, 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. Neuroscience and Biobehavioral Reviews 29:329-352.

Haywood S, Perrins CM, 1992. Is clutch size in birds affected by environmental conditions during growth? Proceedings of the Royal Society of London B 1992:195-197.

Howe HF, 1976. Egg size, hatching asynchrony, sex and brood reduction in the common grackle. Ecology 57:1195-1207.

Kahn N, St. John J, Quinn TW, 1998. Chromosome-specific intron size differences in the avian CHD gene provide and efficient method for sex identification in birds. The Auk 115:1074-1078.

Laaksonen T, Fargallo JA, Korpimaki E, Lyytinen S, Valkama J, Poyri V, 2004. Year- and sex-dependent effects of experimental brood sex ratio manipulation on fledging condition of Eurasian kestrels. Journal of Animal Ecology 73:342-352.

Lack D, 1947. The significance of clutch size. Ibis 89:302-352.

Magrath RD, 1990. Hatching asynchrony in altricial birds. Biological Review of the Cambridge Philosophical Society 65:587-622.

Marti CD, Poole AF, Bevier LR, 2005. Barn Owl (*Tyto alba*). In: The birds of North America (Poole A, ed). Ithaca: Cornell Laboratory of Ornithology.

Mead PS, Morton ML, Fish BE, 1987. Sexual dimorphism in egg size and implications regarding facultative manipulation of sex in mountain white-crowned sparrows. Condor 89:798-803.

Mock DW, Parker G, 1998. Siblicide, family conflict and the evolutionary limits of selfishness. Animal Behaviour 56:1-10.

Muller W, Groothuis TGG, Eising CM, Daan S, Dijkstra C, 2005. Within clutch co-variation of egg mass and sex in the black-headed gull. Journal of Evolutionary Biology 18:661-668.

Nager RG, Monaghan P, Houston DC, 2000. Within-clutch trade-offs between the number and quality of eggs: experimental manipulations in gulls. Ecology 81:1339-1350.

Pike TW, Petrie M, 2003. Potential mechanisms of avian sex manipulation. Biological Reviews 78:553-574.

Reidstra B, Dijkstra C, Dann S, 1998. Daily expenditure of male and female Marsh Harrier nestlings. The Auk 115:635-641.

Royle NJ, Surai PF, Hartley IR, 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? Behavioural Ecology 12:381-385.

Rutkowska J, Cichon M, 2005. Egg size, offspring sex and hatching asynchrony in zebra finches (*Taeniopygia guttata*). Journal of Avian Biology 36:12-17.

Saino N, Beracche V, Ferrari RP, Martinelli R, Moller AP, Stradi R, 2002. Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. Proceedings of the Royal Society of London B 269:1729-1733.

Schwabl H, 1993. Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Science USA 90:11446-114450.

Schwabl H, 1996a. Environment modifies the testosterone levels of a female bird and its eggs. The Journal of Experimental Zoology 276:157-163.

Schwabl H, 1996b. Maternal testosterone in the avian egg enhances postnatal growth. Comparative Biochemistry and Physiology 114A:271-276.

Schwabl H, Mock DW, Gieg JA, 1997. A hormonal mechanism for parental favouritism. Nature 386:231.

Slagsvold T, Sandvik J, Rofstad G, Lorentsen O, Husby M, 1984. On the adaptive value of intra-clutch egg size variation in birds. AUK 101:685-697.

Sockman KW, Schwabl H, 2000. Yolk Androgens reduce offspring survival. Proceedings of the Royal Society of London Biology 267:1451-1456.

Stoleson SH, Beissinger SR, 1997. Hatching asynchrony, brood reduction, and food limitation in a neo tropical parrot. Ecological Monographs 67:131-154.

Torres R, Drummond H, 1999. Does large size make daughters of blue-footed booby more expensive than sons? Journal of Animal Ecology 68:1133-1141.

Trivers RL, Willard DE, 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90-92.

Van de Pol M, Bruinzeel LW, Heg D, Van der Jeugd HP, Verhulst S, 2006. A silver spoon for a golden future: long-term effects of natal origin on fitness prospects of oystercatchers (*Haematopus ostralegus*). Journal of Animal Ecology 75:616-626.

Weimerskirch H, Barbraud C, Lys P, 2000. Sex differences in parental investment and chick growth in wandering albatrosses: fitness consequences. Ecology 81:309-318.

Westneat DF, 1990. Genetic parentage in the Indigo Bunting: A study using DNA fingerprinting. Behavioral Ecology and Sociobiology 27:67-76.

Williams TD, 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biological Reviews 68:35-59.

CHAPTER FOUR

PLASMA TESTOSTERONE AND CORTICOSTERONE LEVELS OF DEVOLOPING BARN OWLS (*TYTO ALBA*): RELATIONSHIP WITH SEX, BROOD SIZE, HATCHING POSITION, AND CONDITION

ABSTRACT

Hormones such as sex steroids and glucocorticoids play important roles in the development, differentiation, and growth of all vertebrates. In altricial birds, these hormones have been implicated in sibling competition and begging for parental food. Testosterone levels are thought to increase with high levels of intra-nest competition, but they may also be related to the differentiation of the sexes, thereby influencing nestling mortality rates. Corticosterone, the major avian glucocorticoid and stress hormone, has been proposed to reflect nestling body condition and to induce begging as an honest signal of nestling condition to parents. Studies of these hormones' roles in such functions often measure plasma concentrations during a single time point of development, seriously limiting power due to lack of information about the developmental dynamics of these hormonal systems. We capitalized on the large size and long nestling periods of barn owls, and sampled nestlings every 8 days up to their fledging at about 60 days of age. We related plasma levels of total androgens (testosterone plus 5a-dihydrotestosterone) and corticosterone to age, sex, condition, brood size, and hatching position to study how competition affects stress levels and to get a better understanding of how androgen levels may affect sibling competition. Androgen concentrations were low and tended to decrease during the nestling phase. Male and female nestlings did not differ in androgen levels, indicating that androgens are not responsible for higher male mortality. Androgen levels also did not differ across brood sizes or with hatching position, suggesting that they are not influenced by levels of sibling competition and may not affect nestling growth or survival. Total androgens were also not related to body condition during the time of maximal growth, but were related once nestlings had reached their final tarsus length. Corticosterone levels showed an increase during nestling development. Corticosterone levels were not different between the sexes or among nestlings from different sized broods. However, they were higher in later-hatched nestlings and negatively correlated with body condition during the time of maximal growth and after nestlings had reached their final tarsus length. These results are consistent with the hypothesis that corticosterone maintains honest signaling of nestling need to provisioning parents and may indicate that later-hatched nestlings experience food stress.

INTRODUCTION

Parental reproductive decisions about number and quality of offspring to produce at a given time influence the nest environment that these offspring experience because each nestling’s success and survival is dependent on parental food resources and competition with siblings (Mock and Parker, 1997). Likewise, the sex of an individual offspring might affect its reproductive value and survival (e.g. Griffiths et al., 1998;

Weimerskirch et al., 2000). Though offspring have little control over their environment, they may have mechanisms for coping with some of its variables.

A host of studies have already addressed the evolution of strategies by which parents and offspring cope with the tradeoff between number and quality of offspring. However, the proximate mechanisms by which (1) siblings compete with each other, (2) nestlings signal their needs to parents, and (3) food availability and (4) sex affect nestling growth and quality have only recently been addressed (e.g. Kitaysky et al., 1999; Love et al., 2003b; Goodship and Buchanan, 2006; 2007). The focus of such mechanistic studies has been two hormones. First, androgens, such as testosterone, because of their role in aggression and dominance/subordinate relationships (Wingfield et al., 1990), potentially immunosuppressive actions (reviewed in Owen-Ashley et al., 2004), and effects on growth and begging (Goodship and Buchanan, 2006; 2007; Fargallo et al., 2007). Second, glucocorticoids, such as corticosterone, because of their role in energy regulation and response to stressors such as food shortage (and reviewed by Sapolsky et al., 2000; shown in birds: Kitaysky et al., 2001a; Kitaysky et al., 2001b).

Studies of the roles of these hormones in sibling competition and nestling development usually sample plasma hormones only once, at the most convenient stage of nesting or because of size limitations, and use such single point sampling to test hypotheses (e.g. Williams et al., 1987; Naguib et al., 2004; Goodship and Buchanan, 2006). However, blood hormone concentrations can potentially change rapidly in response to food availability (e.g. Kitaysky et al., 1999) or nestling competition (e.g. Ferree et al., 2004). Because little is known about how nestling hormone levels vary throughout the nesting cycle, in this study we collected multiple plasma hormone samples across nestling development.

Testosterone

Testosterone and nestling mortality: Physiological differences between the sexes that occur during development may also lead to differences in competitive abilities and/or survival. It is thought that males may have decreased survival due to hormones such as androgens that may help regulate sexual differentiation (Olson and Kovacs, 1996; Muller et al., 2003; Muller et al., 2005; Fargallo et al., 2007), however this has only been well documented in mammals. The hormonal components of sexual differentiation during development in birds are not as well understood as they are in mammals (see a review by Carere and Balthazart, 2007). In many species, males are the larger sex, so it is difficult to separate the effects of being male from the costs of being the larger sex. Species with reversed sexual size dimorphism (RSSD), females larger than males, offer a unique opportunity to test the male disadvantage hypothesis because it is possible to examine the effects of male hormones independent from those of a larger body size.

There have been only a few studies of wild bird populations to quantify the differences in circulating hormones between male and female nestlings. In the black coucal, (*Centropus grillii*), male nestlings are the smaller sex and have higher levels of testosterone than female nestlings, but this study did not examine mortality differences (Goymann et al., 2005). In contrast, Cory's shearwaters (*Calonectris diomedea*) and Eurasian kestrels (females are larger than males) (*Falco tinnunculus*), show no sex differences in nestling androgen levels (Fargallo et al., 2007; Quillfeldt et al., 2007).

Thus, evidence for the sex differences in hormone levels during development are conflicting, leading to little support for the male disadvantage hypothesis. In a previous study of the barn owl (*Tyto alba*), a reversed sexually size dimorphic raptor, we found that male nestlings (the smaller sex) experience higher mortality than do female nestlings (Seifert et al., in prep.), leading us to hypothesize that male nestlings will have higher levels of androgens (testosterone) than female nestlings.

Testosterone and sibling competition: Because of testosterone's association with agonistic interactions, aggression, and social dominance in adult birds (e.g. Wingfield et al., 1990), testosterone and other androgens have been proposed to play a role in sibling competition (Naguib et al., 2004) and maintenance of sibling hierarchies (e.g. Goodship and Buchanan, 2006). The only study that has tested this hypothesis found that nestlings from larger broods have higher plasma testosterone levels (this study did not examine sex differences), presumably as a result of increased competition (Naguib et al., 2004). Larger broods are expected to have more sibling competition for food, and testosterone may affect begging (Goodship and Buchanan, 2006; 2007), so we predict that, in the barn owl, nestling androgen levels will be positively correlated with brood size in both sexes.

Because of the resulting age and size hierarchy of brood mates, hatching position also affects sibling interactions and potentially nestling circulating androgen levels. Previous studies have suggested that testosterone may help optimize social interactions between siblings because it can increase begging (Sasvari et al., 1999; Quillfeldt et al., 2006; Goodship and Buchanan, 2006; 2007). However, results of studies on the effects of circulating testosterone on nestling birds are conflicting. It has been proposed to help later hatched nestlings compete with their older, stronger siblings (DelaMora et al., 1996; Nunez-de La Mora et al., 1996; Tarlow et al., 2001; Ferree et al., 2004), to help maintain the dominance of first-hatched nestlings(Sasvari et al., 1999), and also to have a negative effects on begging, growth, and body fat deposits (See Ketterson et al., 1996; Groothuis et al., 2005). For example, Fargallo et al (2007) reported that in the Eurasian kestrel (*Falco tinnunculus*) testosterone implants decreased nestling growth and was detrimental to nestling immune systems. No studies to date have found a direct positive link between testosterone and growth, although increased begging may lead to more food intake and enhance nestling growth, which may lead to higher survival as shown by Goodship and Buchanan (2006). Testosterone treatment has been found to increase a nestling's aggressive behavior toward siblings (e.g. Groothuis and Meeuwissen, 1992; Groothuis and Ros, 2005), which could likewise benefit later-hatched nestlings and may be observed when younger nestlings have a chance to compete with older siblings. Thus, we predict that later-hatched nestling have higher levels of testosterone, increasing their competitive ability and potentially helping them in competitions with older siblings for food. If testosterone is positively related to begging, and begging is a result of nestling need, later-hatched nestlings with greater likelihood of starvation should have higher levels of circulating testosterone.

Corticosterone

The adrenal cortex releases glucocorticoids—in birds, primarily corticosterone (Kitaysky et al., 1999)—which are important regulators of blood glucose concentrations and intermediary metabolism, and typically are acutely secreted during periods of stress (reviewed by Landys et al., 2006). Birds experiencing food shortage, social competition, or potential predation often have increased corticosterone levels (Nunez-de La Mora et al., 1996; Kitaysky et al., 1999; Kitaysky et al., 2001a). Over short periods of time, increased levels of corticosterone may help birds utilize fat stores, increase locomotory behavior, and forage more effectively—traits that are beneficial to survival (Astheimer et al., 1992; Bray, 1993; Smith et al., 1994; Wingfield and Kitaysky, 2002). Over extended periods of time, however, stress-induced high corticosterone levels have detrimental effects on many physiological systems in mammals (reviewed by Sapolsky et al., 2000); studies of birds have demonstrated long term effects on spatial memory (Kitaysky et al., 2003) and song (Spencer et al., 2003). For these reasons, nestling corticosterone levels are relevant for understanding sibling competition and for signaling of nestling need to parents.

In the red-legged kittiwake (*Rissa brevirostris*), nestlings with higher levels of corticosterone beg more than nestlings with lower levels (Kitaysky et al., 2001b). Other studies, including another study of the red-legged kittiwake (CITE?), have found that under poor food conditions circulating corticosterone levels increase in all nestlings, and that food restriction increases nestling corticosterone levels (Nunez-de La Mora et al., 1996; Kitaysky et al., 1999; Kitaysky et al., 2001a). Because of these links between food, body condition, and corticosterone, it has been proposed that corticosterone regulates nestling begging for food and that therefore begging is an honest signal to the parents of nestlings' current condition (Nunez-de La Mora et al., 1996; Kitaysky et al., 1999; Kitaysky et al., 2001a; Kitaysky et al., 2001b; Kitaysky et al., 2003).

We tested predictions of the hypothesis that corticosterone is related to nestling condition in the barn owl. First, male barn owl nestlings are expected to have higher corticosterone levels than female nestlings because the barn owl is an RSSD species in which larger females likely dominate smaller male siblings and limit their access to parentally provided food. Second, nestlings of larger broods, in which all individuals are potentially limited in food supply, should have higher corticosterone levels than nestlings from smaller broods. Third, later-hatched nestlings should have higher corticosterone levels than early-hatched nestlings because they may be out-competed for food by their older and larger siblings. In a previous study we showed that later-hatched nestlings have higher mortality rates than do earlier-hatched nestlings (Seifert et al., in prep.). Finally, because of corticosterone's role in intermediary metabolism and energy regulation its levels should be negatively correlated with nestling body condition.

Hormonal Changes with Age

We have found only one previous study of birds that address how hormone levels change with nestling age, in which no more than four samples were collected per nestling to look at corticosterone levels (Love et al. 2003b). In this study they found that corticosterone increased with age, presumably as the stress access was developing. Previous researchers have predicted that corticosterone levels should increase at the end of the nestling phase to help induce fledging (Heath, 1997; Schwabl, 1999; Kern et al., 2001; Love et al., 2003a). For these reasons, we predict that corticosterone levels will increase over the nestling phase. We believe that corticosterone levels will vary within and among nests with age, due to differences in the food resources each nestling receives.

Because so little work has been done on nestling testosterone we do not have any clear predictions about how nestling testosterone levels might change with age. To study how hormone levels change during the nestling phase we decided to measure hormone levels every by taking multiple samples we can also get a better idea the role of testosterone and corticosterone in sibling rivalry throughout the nestling phase.

Predictions

Testosterone: In summary, 1) we predict that male offspring will have higher testosterone levels than females because testosterone may be necessary for male differentiation. 2) We predict that testosterone levels should increase over the laying order such that later-hatched nestlings have more testosterone than earlier hatched nestlings, which should help them compete with older siblings. 3) We predict that nestlings from larger broods will have higher testosterone levels as a result of increased sibling competition. 4) We have no clear prediction about how testosterone will change during development.

Corticosterone: 1) Males will have higher corticosterone levels because they are smaller than female offspring, which puts them at a competitive disadvantage in obtaining food. 2) We predict that corticosterone levels should increase over the laying order such that later-hatched nestlings have more corticosterone than earlier hatched nestlings because they are smaller and will likely be less successful at obtaining food than their competitively advantage siblings. 3) We predict that nestlings from larger broods will have higher corticosterone levels as a result of increased sibling competition and parental resources spread thinly among many offspring. 4) We predict that corticosterone levels will increase over the nestling phase because higher corticosterone levels are thought to help induce fledging.

METHODS

We studied a nest-box population of barn owls on agricultural ranches in a 20-mile radius of Merced, CA (37.3° N, 120.5° W). In central California barn owls initiate nests from late December until early May and are almost exclusively single brooded (Simmons, personal communication, Seifert, pers. obs.). Clutch sizes range from 3-12 eggs (Seifert, pers. obs.). Only the female incubates the clutch, starting with the first egg, which results in a pronounced hatching asynchrony (eggs hatch at least two days apart; occasionally consecutive eggs hatch as many as 10 days apart; for instance a large brood could take, on average, two to three weeks to completely hatch) and sibling size and age hierarchy. Males feed females as well as their offspring until nestlings are approximately one month old, after which both parents feed nestlings.

We checked nest boxes from the second week in January until the middle of May in 2005 and 2006. We checked boxes every four days until there were signs of nest initiation, (i.e. an adult roosting in the box, and/or a stockpile of rodents in the box), after which we checked boxes every other day for subsequent eggs because barn owls do not lay more than one egg every other day (Marti et al., 2005). On the day each egg was laid we numbered it with a non-toxic permanent marker, weighed it (to the nearest 0.01g), and measured it for length and width (to the nearest 0.1mm).

Once there was more than an 8-day gap between the laying of successive eggs, we checked boxes every 4 days until 26 days had passed since the first egg was laid.

Because the average incubation time is 30 days, but eggs can hatch as early as 28 days, we chose to check nest boxes every other day, starting at 26 days. This allowed us to match hatchlings to their eggs. We then gave each nestling a colored plastic band from the day it was found until we could fit it with an adult-size 6 metal USFWS band—usually around an age of 10 days. We then checked boxes every other day until all of the eggs had hatched or 40 days had passed from the day the last egg was laid. We measured each hatchling's wing length (to the nearest 0.5 mm), body mass, and length of left tarsus (to the nearest 0.5 mm) approximately every 4 days until they were no longer present (because they either fledged or died). There was some variation in the amount of time between measurements, because as nestlings hatched, we were measuring closer to 2 or 3 days apart. Barn owls sleep during the day so we didn't have to worry about promoting premature fledging. In fact, some birds continue to roost in the box during the day even after their technical fledging date.

From each nestling, we collected a blood sample from the brachial wing vein on the first day we found it and stored samples in lysis buffer at 4º C for molecular analysis (below). We also took a minimum of 300ul of blood and as much as 1.5 ml of blood from each bird for plasma hormone analysis on the day it hatched and approximately every 8 days thereafter. We took samples as close together as 5 days, but on average we took them every 7 or 8 days. We then placed samples on ice until the end of the day's fieldwork. When we arrived at our field station, we spun each sample, separated off the plasma and froze it. We brought the samples on dry ice back to our lab at Washington State University, and kept them at -40º C until we were able to analyze them.

Hormone Analysis

We took blood samples at different times of the day, however, we sampled all nestlings at all different times of day. There is no correlation between corticosterone levels and time of day (Pearson correlation= 0.035, $P = 0.716$), or testosterone levels and time of day (Pearson correlation= 0.010, $P = 0.835$). We removed nestlings from their nest box one at a time at random so that the same birds were not always sampled in the same order. We took samples as soon as a bird was removed from its box (within 3 minutes). We found no effect of the order in which the nestlings were sampled on the corticosterone levels (Pearson correlation= 0.076, $P = 0.532$). Therefore, the order in which birds were sampled was not included in statistical analyses. It took between 1 minute and 9 minutes to sample nestlings (the time from opening the box until the bleeding was completed); on average, it took 2.5 minutes to obtain a sample from a nestling. There was a larger range of time to sample entire broods because the brood sizes varied. On average it took 18 minutes to sample an entire brood, however, total time varied from two minutes (a brood of one) to 48 minutes. We returned nestlings to their boxes after we completed all blood samples to minimize disturbance times for each subsequent nestling to be bled.

We measured hormone concentrations using radioimmunoassay. We measured total androgen (testosterone and 5alpha-dihydrotestosterone) concentrations in the plasma using testosterone-antiserum (T 3003, Wien Laboratories; this antibody cross-reacts with both testosterone and 5alpha-dihydrotestosterone). We measured corticosterone concentrations using corticosterone-antiserum (B-3-163, Esoterix Endocrinology). Because there is no cross reactivity of the testosterone-antiserum with corticosterone hormone and coricosterone-antiserum with the testosterone hormone, we analyzed both hormones in extracts of the same samples without separating them via chromatography. We determined corticosterone and testosterone recoveries extraction efficiency in every eighth sample, alternating between testosterone and corticosterone. We used the mean recoveries for testosterone and corticosterone to correct hormone measurements in all samples. We followed the previously described radioimmunoassy procedures for extraction of plasma testosterone and corticosterone following the same methods as Schwabl (1993). The ^{3}H labeled steroids were purchased from Perkin Elmer Life and Analytical Sciences and were as follows: NET 553 for testosterone and NET 399 for corticosterone.

The intra-assay variation was as follows: testosterone/DHT = 12.8%, corticosterone = 12.7%. Inter-assay variation was: testosterone/DHT= 9% and corticosterone = 17%. The average recoveries for testosterone/DHT were 81% and 82% for corticosterone. Our detection limit was 2 pg/tube for testosterone and 4 pg/tube for corticosterone.

Molecular Determination of Nestling Sex

Even though nestling barn owls are sexually dimorphic in both size and color, we used a molecular sexing technique to obtain information on the sex ratio before any offspring died or disappeared from the nest. We extracted DNA from blood samples using a standard phenol-cholorform extraction (Westneat, 1990). To determine nestling sex, we followed the protocol of Kahn et al. (1998), using primers 1237L and 1272H. We used adults of known sex to establish the proper temperatures for our PCR, and in each PCR run we had a known adult of each sex to verify our results. PCR reactions consisted of 40 cycles at 94° C for 60 seconds, 55° C for 60 seconds and 72 °C for 45 seconds. The total reaction volume was 10µl and included 1µl of DNA and 1X PCR buffer (Applied Biosystem), 0.15 mM dNTP mix, 0.5 uM of each primer, 2mM of $MgCl_2$ and 2.5U/ul of Taq.

Statistics

To test the effects of brood size, sex, and laying order on circulating testosterone and corticosterone levels we used a Proc Mixed procedure in SAS to do a general linear mixed model with the assumption that the covariance matrix was compound symmetric. In one model our dependent variable was testosterone and in the other it was corticosterone. Independent fixed factor variables in our model were: sex, brood size, hatching position, a brood size*hatching position interaction, condition, age (days from hatching), and age^2. We dropped the brood size*hatching order interaction from the testosterone model because it was highly non-significant. Because we had multiple nestlings from the same brood and we had multiple samples from each nestling, we nested each individual within its nest box and our repeated measure was the individual. The model we used was a conservative model given that we also satisfied the assumptions of a general linear model. We checked all model assumptions and tested for serial correlations; all assumptions of the model were met. We also ran the model with the mean of age re-centered on zero to ensure that there were no problems with co-linearity between age and age^2, but we found that re-centering age did not significantly change our findings, so we used our initial model.

We used all variables as linear predictors because of small sample size (individuals nested within a family and repeated measures of all individuals). Because they were linear predictors we were unable to obtain LSMeans for any of our variables or do any pair-wise tests. In a post-hoc test to examine differences between the different hatching positions and plasma corticosterone we used 95% confidence intervals; we did this with the raw means because it is impossible to get LSMeans from linear predictors. Corticosterone levels were considered to be significantly different if there was no overlap in their 95% confidence intervals.

We analyzed the relationship of relative mass (body condition (mass/tarsus)) to nestling plasma corticosterone and testosterone concentrations using Pearson correlations. We performed these analyses at two nestling stages: first, at the time of maximum growth (between days 16 and 23, (Seifert et al., in prep.)), and second, just after the nestlings had completed their tarsus growth, which is also when they reached their maximum mass (between days 43 and 51, (Seifert et al., in prep.)). At the later stage we could compare maximum mass to final structural size. We used only one hormone and one condition measurement (taken on the same day) from each bird for each correlation to prevent pseudo-replication.

RESULTS

Androgens

Circulating plasma androgen (testosterone plus 5α-DHT) levels did not differ between the sexes (Table 4.1A, Figure 4.1). Androgen levels were not related to brood size (Table 4.1A, Figure 4.2). Although there was considerable variation in androgen levels between individuals over the course of the nestling phase, androgen levels were not related to hatching position (Table 4.1A, Figure 4.3). Androgen levels also were not related to the condition of the nestling when compared throughout the entire nestling phase (Table 4.1A). Androgen levels varied with age (Table 4.1A) and with age^2, meaning that there was a linear decrease in androgen levels with age as well as a quadratic trend in the amount of androgen levels with age (Figure 4.1).

We then examined how androgen levels correlated with condition during two specific times in the nestling phase instead of only examining the effects of condition throughout the entire nestling phase. During the time of maximum growth (days 16-23), there was no relationship between androgens and the relative mass (one measure of condition) of the nestlings (Pearson Correlation= -0.055, $P = 0.647$; Figure 4.4A). However, later in the nestling cycle (days 43-51), after the tarsus had completed its growth, androgen levels were positively related to the relative mass of the nestlings (Pearson Correlation= 0.283, $P = 0.015$; Figure 4.4B).

Corticosterone

Circulating plasma corticosterone levels did not differ between the sexes (Table 4.1B, Figure 4.5). Circulating corticosterone levels were not significantly related to brood size (Table 4.1B, Figure 4.6). However, circulating corticosterone levels were affected by hatching position (Table 4.1B), with later-hatched nestlings tending to show higher plasma corticosterone levels than earlier-hatched nestlings (Figures 4.7 and 4.8). The 95% confidence intervals for corticosterone levels did not overlap between nestlings 1 and nestlings 3, 5, and 6, suggesting that first-hatched nestlings had the lowest corticosterone concentrations. We did find a significant interaction of brood size and hatching order (Table 4.1B), but because it is a linear predictor we could not perform any post-hoc tests to examine the nature of the effect. There was a significant effect of age and age^2, meaning that corticosterone levels have both a linear and non-linear component to how they change during the nestling phase, but it is hard to see any clear pattern with age (Table 4.1B, Figure 4.5).

We found condition (relative mass) was negatively related to corticosterone levels (Table 4.1B). We examined the relationship between condition and corticosterone levels at two different times during the nestling phase. During the time of maximum growth (days 16-23), nestling corticosterone levels showed a significantly negative relationship with relative mass (Pearson Correlation= -0.290, $P = 0.013$, Figure 4.9A). Later in the nestling cycle, after the tarsus had completed its growth and when nestlings reached their maximum mass, corticosterone levels were also negatively, but marginally non-significantly related to relative mass (Pearson Correlation= -0.228, $P = 0.052$, Figure 4.9B).

DISCUSSION

Our study monitored plasma androgen (testosterone and 5α-dihydrotestosterone) and corticosterone concentrations throughout the entire nestling phase in a wild bird population, providing important insight into developmental changes in the levels of these hormones and how they are related to sex, brood size, hatching position, and condition. Both androgen and corticosterone levels varied with age. Corticosterone and androgen levels did not differ between the sexes and also were not significantly related to brood size. Corticosterone, but not androgens, was affected by hatching position, with higher levels in later-hatched chicks, and by condition, with higher levels in nestlings in poorer condition. Androgen concentrations were positively correlated with relative mass (mass/tarsus, a measure of condition) when nestlings had completed much of their growth, but not during the rapid growth phase (Figure 4.4). Corticosterone was negatively correlated with condition during both stages. Below we discuss how these results relate to the hypotheses that have been proposed for the role of these hormones in sibling rivalry and nestling survival.

Testosterone

The male disadvantage hypothesis suggests that hormones secreted in a sex-specific manner, such as androgens, will have detrimental effects on offspring development and lead to high mortality of male nestlings relative to female nestlings (Olson and Kovacs, 1996; Muller et al., 2003; Kalmbach et al., 2005; Muller et al., 2005; Fargallo et al., 2007). However there is no direct evidence in nestling birds that testosterone is used for sexual differentiation. One study found that the female phenotype may develop from high levels of estrogen early in development; it is unclear whether the male phenotype needs high levels of testosterone to develop (Balthazart and Adkins-Regan, 2002). The male phenotype/disadvantage hypothesis, originally designed to explain male mortality in mammals, aims to explain the higher mortality risk of males, and suggests that males are more susceptible to mortality regardless of sexual size dimorphism because they have higher concentrations of circulating testosterone (potentially necessary for sexual differentiation) (Olson and Kovacs, 1996; Muller et al., 2003; Muller et al., 2005; Fargallo et al., 2007). This in turn, may decrease male immune function (Olson and Kovacs, 1996; Muller et al., 2003; Kalmbach et al., 2005; Muller et al., 2005; Fargallo et al., 2007), increase stress hormone (corticosterone) levels (Grossman, 1985; Owens and Short, 1995; Olson and Kovacs, 1996; Muller et al., 2005; Fargallo et al., 2007), and/or decrease growth (e.g. Fargallo et al., 2007). In barn owls, male nestlings have higher rates of mortality than do female nestlings (Seifert et al., in prep.), but in this study we found no differences in circulating levels of androgens between male and female nestlings (Figure 4.1), similar to results found recently in another dimorphic raptor species (Fargallo et al., 2007). Thus, our study does not support the male disadvantage hypothesis, and high rates of male nestling mortality in barn owls are likely due to some other factor(s), such as hatching position and/or stress (Seifert et al., in prep.).

Androgen concentrations also were not related to brood size (Fig. 4.2), a result that does not support a role for androgens in sibling competition. We had predicted that competition for food increases with brood size, which in turn increases androgen levels, as has been reported in studies of other species (Tarlow et al., 2001; Ferree et al., 2004; Goodship and Buchanan, 2006; Fargallo et al., 2007). Our results are somewhat surprising because barn owls can have large broods (we were able to analyze broods of up to 6 nestlings), which should lead to high levels of sibling competition. Possibly we found no difference in androgen levels because not all nestlings actually compete for food. Because there is such a pronounced difference in size between earlier- and later-hatched nestlings, in large broods older siblings could potentially have low testosterone levels, younger siblings might have high testosterone levels, and yet we would observe average amount of testosterone for the entire nest. However, we did not find a hatching order by brood size interaction, indicating that this is probably not the case. There was substantial variation in plasma androgen concentrations within most brood sizes. This variation might reflect hormonal changes resulting from intermittent and transient agonistic interactions between siblings that help establish or maintain the social hierarchy. Support for this idea comes from a study on Nazca boobies (*Sula grantii*), in which nestling testosterone levels are shown to increase only during the time of agnostic interactions with siblings (Ferree et al., 2004). It is also possible that the variation in circulating testosterone levels that we found has no real biological meaning, which should encourage researchers of circulating hormones to take multiple samples throughout the nestling cycle to determine hormonal relevance in their study species.

The finding that androgen concentrations were not related to hatching position (Fig. 4.3) also argues against a role for androgens in sibling competition. Previous studies have suggested that testosterone might help optimize social interactions between siblings by helping older siblings be dominant or younger siblings be competitively competent (Wingfield et al., 1990; Groothuis and Meeuwissen, 1992; Ketterson et al., 1996; Tarlow et al., 2001; Ferree et al., 2004). In the barn owl, we suspect that older nestlings dominated younger nestlings in competition for food because younger nestlings were less likely to survive until fledging (Seifert et al., in prep). Therefore, later-hatched nestlings should face more competitive pressure because they are competing with older, much larger siblings. A number of studies have found that later-hatched/smaller siblings have higher levels of testosterone due to the increased level of competition they face (DelaMora et al., 1996; Nunez-de La Mora et al., 1996; Tarlow et al., 2001; Ferree et al., 2004), however, our results do not support this idea (Figure 4.3). Barn owls may be different because unlike the species tested in earlier studies they are not siblicidal. Therefore increased testosterone levels for competition may be more important in siblicidal species where nestlings that do not fight will die. In the barn owl on the other hand, the female is actively feeding the offspring and thus there may be lower levels of competition for food. Finally, we found no support for the hypothesis that older, earlier-hatched nestlings maintain their dominance by testosterone-regulated aggression.

Androgen levels were not related to relative mass (body condition) during the time of maximum growth. Thus, although some experimental studies have reported that testosterone can negatively impact growth (Groothuis and Ros, 2005; Fargallo et al., 2007), our results suggest that it is unlikely that androgens are causally related to variation in growth among sibling barn owls. However, androgen levels were positively correlated with body condition at the end of the nestling phase, when tarsi had grown to final length and nestlings reached their maximum mass. In barn owls, nestling mass peaks at an age of 40 to 50 days, after which it begins to decrease until fledging (Seifert, unpub. data). Thus it is possible that at this stage of development, androgens have a positive effect on nestlings' relative mass (or mass affects androgen level), which may be mediated through increased begging and, as a result, more food for nestlings, as suggested by Goodship and Buchanan (2006; 2007).

Lastly, androgen levels were significantly related to age and age^2 (Table 4.1, Figure 4.1). Androgen levels showed a general decrease with age in all nestlings, however, there was also a quadratic component, indicating that there is also a nonlinear component to how androgens change with age. High androgen levels may be important for early development in the barn owl. We found a high degree of variation from measurement to measurement of the same bird and also between birds (Seifert unpub. data). This variation may be due to agonistic interactions with siblings, as found in Nazca boobies (*Sula grantii*) (Ferree et al., 2004), or to short-term fluctuations in food resources. The wide variation that we observed illustrates the need for researchers to take multiple hormone samples from each individual when comparing levels in order to accurately identify differences between nestlings.

In summary, our results are not consistent with the predictions of hypotheses suggesting that androgens are important hormonal mediators of sibling competition, begging behavior, or differential rates of development. They also show that the higher male mortality in barn owl nestlings is not related to higher androgen levels in males than females.

Corticosterone

Most of our results are consistent with predictions of the hypothesis that corticosterone is an important regulator of sibling competition that may assure honesty of nestling begging signals (Kitaysky et al., 2001b; Kitaysky et al., 2003). Nestling corticosterone levels tended to be highest during the rapid growth phase (Fig. 4.5), the time when food demands are highest.

While corticosterone levels were not significantly higher in larger broods (Figure 4.6), they were significantly higher in nestlings later in the hatching order (Fig. 4.7). First-hatched nestlings, in particular, had significantly lower corticosterone levels than most subsequently hatched nestlings (Figure 4.8). This result suggests that first-hatched nestlings might receive more food than their siblings, which would help them to further out-compete later-hatched nestlings regardless of brood size. Other researchers have found that nestling corticosterone levels are negatively correlated to the amount of food they receive (e.g. Kitaysky et al., 2001a). One possible explanation for the lack of a relationship between brood size and nestling corticosterone levels is the strong hatching position effect. Early-hatched nestlings have lower corticosterone levels and less variation in those levels than later-hatched nestlings (Figure 4.8), indicating that all first-hatched nestlings are less food stressed than later-hatched nestlings, regardless of brood size. We found an interaction between brood size and hatching position, which may mask the effects of brood size on nestling corticosterone levels. In larger broods it is likely that only the later-hatched nestlings are excessively stressed, because their older siblings can monopolize food resources. Thus, if all first-hatched nestlings are in good condition and later-hatched nestlings are in poor condition, mean corticosterone levels between nestlings of different sized broods can be similar.

There was a negative correlation of corticosterone with condition during the rapid growth phase and after completion of structural growth (i.e. tarsus length, but not wing length). The only one of our predictions about corticosterone not supported was that levels would be higher in males than in females (Figure 4.5). Because the size disparity between older and younger nestlings among the hatching order is more pronounced than the size disparity between male and female offspring, the smaller size of male nestlings may be less of a factor in their ability to obtain food than their hatching position. Overall, these results are consistent with a causal sequence of food supply affecting body condition, which affects corticosterone, which leads to honest nestling begging, which should help nestlings get more food from parents. Nestlings in poor condition have been shown to have increased circulating corticosterone levels, which in turn, has been shown to increase nestling begging (Nunez-de La Mora et al., 1996; Kitaysky et al., 1999; Kitaysky et al., 2001a). An increase in begging in these later-hatched nestlings may help them receive more food from their parents, although in the barn owl population we studied, most of the later-hatched nestlings did not survive.

Because barn owl nestling condition varies widely within nests, a plausible explanation for the variation in corticosterone levels we observed is that not all nestlings received equal amounts of food. In later-hatched nestlings, corticosterone may induce begging as a signal to parents of nestling need and parents may respond by provisioning more food if they are able to (see Kitaysky et al., 2001b). However, in most cases we observed smaller body size (wing and mass), when compared to same age earlier hatched nestlings) and higher mortality in later-hatched barn owl nestlings, implying that parents (fathers) may not be able to provide enough food to support maximal growth of all nestlings and keep all nestlings alive (Seifert et al., in prep.). The higher corticosterone levels of later-hatched nestlings and those in poor condition might be compounded by sub-optimal food supply to result in greater susceptibility to disease and mortality. Indeed, the relative mass of nestlings (mass/tarsus, a measure of condition) was negatively related to corticosterone levels during maximum growth and when nestlings reached their greatest mass (Figure 4.5).

The corticosterone levels of later-hatched nestlings were rather high (Figure 4.7), as were those of the nestlings in poorest condition (smallest relative mass) (Figure 4.9). These levels tended to be higher than basal levels in most nestling birds, being representative of stress-induced secretion (Landys et al., 2006). Moreover, such elevated corticosterone levels occurred throughout the 60 day nestling period in later-hatched nestlings. We propose that this may represent chronic stress—elevated corticosterone levels over a prolonged period of time—which may result in adverse effects on physiological systems, including immune function (reviewed by Sapolsky et al., 2000) and cognitive abilities (Kitaysky et al., 2003). This might explain the higher mortality of later-hatched nestlings; it might also affect their long-term health and survival. It is well documented that stress-elevated corticosterone levels during development can have effects that carry over into adult life (reviewed by Sapolsky et al., 2000; e.g. Kitaysky et al., 2003). Experimental manipulations of corticosterone are now required to establish the causal relationships between corticosterone, body condition, and hatching position that are suggested by our correlative study.

Researchers have observed that corticosterone levels increase at the end of the nestling phase and proposed that it induces fledging (Heath, 1997; Schwabl, 1999; Kern et al., 2001; Love et al., 2003a). Love et al. (2003b) took 4 plasma samples during the nestling phase of American kestrel chicks (*Falco sparverius*) and found that corticosterone levels increased over the duration of the nestling phase. We did find a highly significant effect of age and age^2 on corticosterone levels, however, there does not seem to be a clear increase of corticosterone towards fledging, corticosterone had a non-linear component to its variation with age, but it is hard to discern a clear pattern. Thus, our data do not support a role for corticosterone levels to induce fledging. However, results may be masked by the fact that barn owls, like other raptors, do not have an easily-defined fledging date (Marti et al., 2005). Some nestlings briefly leave the nest at night and return to the nest for a couple of weeks after their first flight to continue to supplement their own hunting with parental feeding. Therefore, there may be variation in nestling corticosterone levels between species that, at fledging, are leaving the nest for good and those that return to the nest and are still being fed by parents.

Conclusions

We found no support for the male disadvantage hypothesis nor for a role for androgens in sibling rivalry. However, our results do support many of the predictions of a role for corticosterone in competition for food and sibling rivalry. These results open up the possibility for future experimental research on the interactions of body condition, corticosterone, and nestling growth in sibships, as well as for studies examining the role of corticosterone in nestling begging. Now that a complete developmental profile for corticosterone is available for barn owls, we can potentially manipulate corticosterone levels and examine begging responses, body condition, and growth to further test the honest signaling hypothesis. Alternatively, food supply to nests could be manipulated to test the consequences for condition and for corticosterone levels.

Our study shows that researchers should be cautious about using nestling hormone measurements collected at a single convenient time during the nestling phase to study the roles of hormones in sibling competition. We saw so much variation from day to day without a clear pattern that if researchers take only one sample from nestlings they may pick up spurious variation and find a trend in their data that may not have actual biological meaning. Therefore, we encourage researchers addressing the functions of hormones to first determine hormone profiles covering the entire nestling phase before deciding on the timing of measurement. Multiple measures of hormone levels may be necessary if levels vary stochastically across the nestling cycle, as we found in our study. Had we measured corticosterone only one set age, we would have found no relationship of corticosterone with hatching position. Yet, overall corticosterone concentrations are higher in later-hatched nestlings than in earlier-hatched ones. We found that androgen levels are not consistently related to condition because they were not correlated to condition during maximum growth, but they were positively related to condition after the tarsus was done growing. There are different relationships during maximum growth and after most growth has occurred. Hormones may have different effects early and late in the nestling phase to help regulate growth and to allow nestlings to effectively compete with siblings.

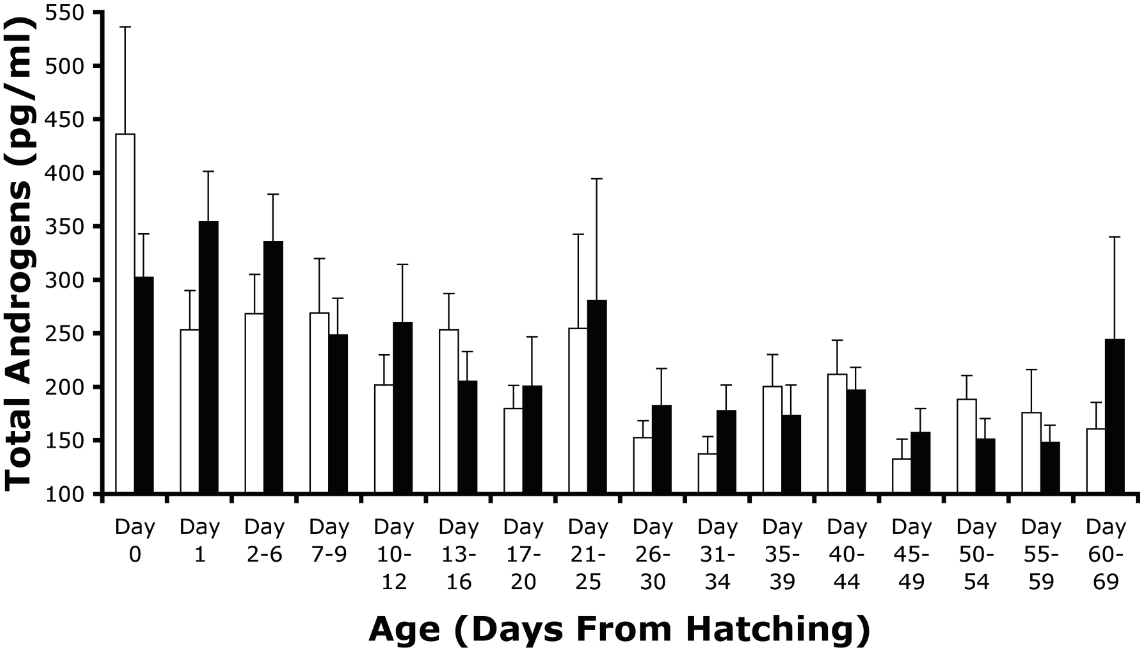

Figure 4.1. Mean nestling plasma androgens by age with standard error bars in relation to sex. Circulating androgen levels were relatively low throughout the nestling cycle and did not differ between male (closed) and female (open) nestlings. Sample includes a total of 91 individuals (47 males and 44 females) from 21 different nests.

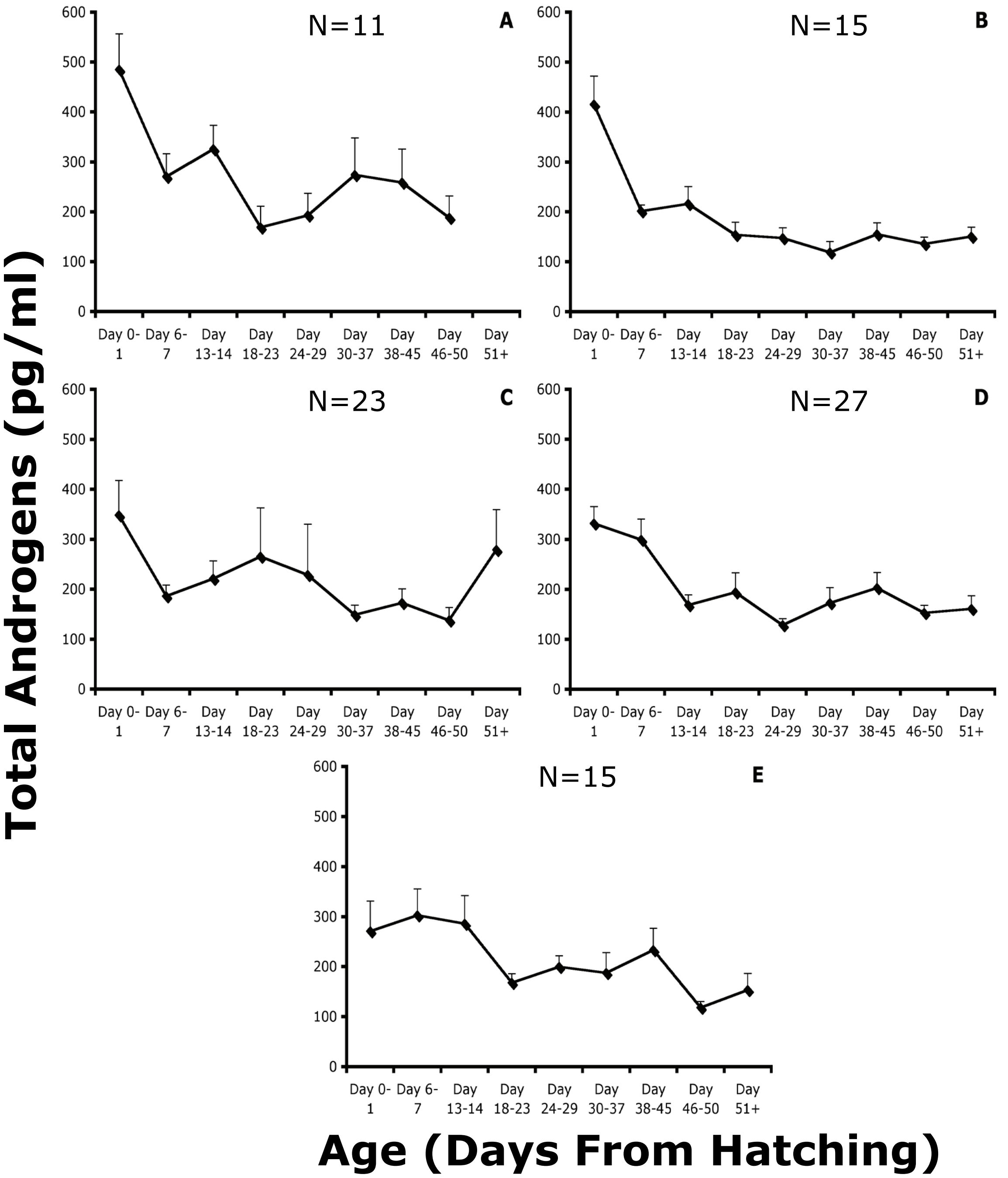

Figure 4.2. Circulating androgen levels of nestlings are not related to brood size during the nestling phase. Brood sizes 1 and 2 are shown in A and brood sizes 3-6 in B-E respectively; error bars show one standard error of the mean. The number of nestlings sampled from each brood size is shown on each graph (total of 91 individuals from 21 nests).

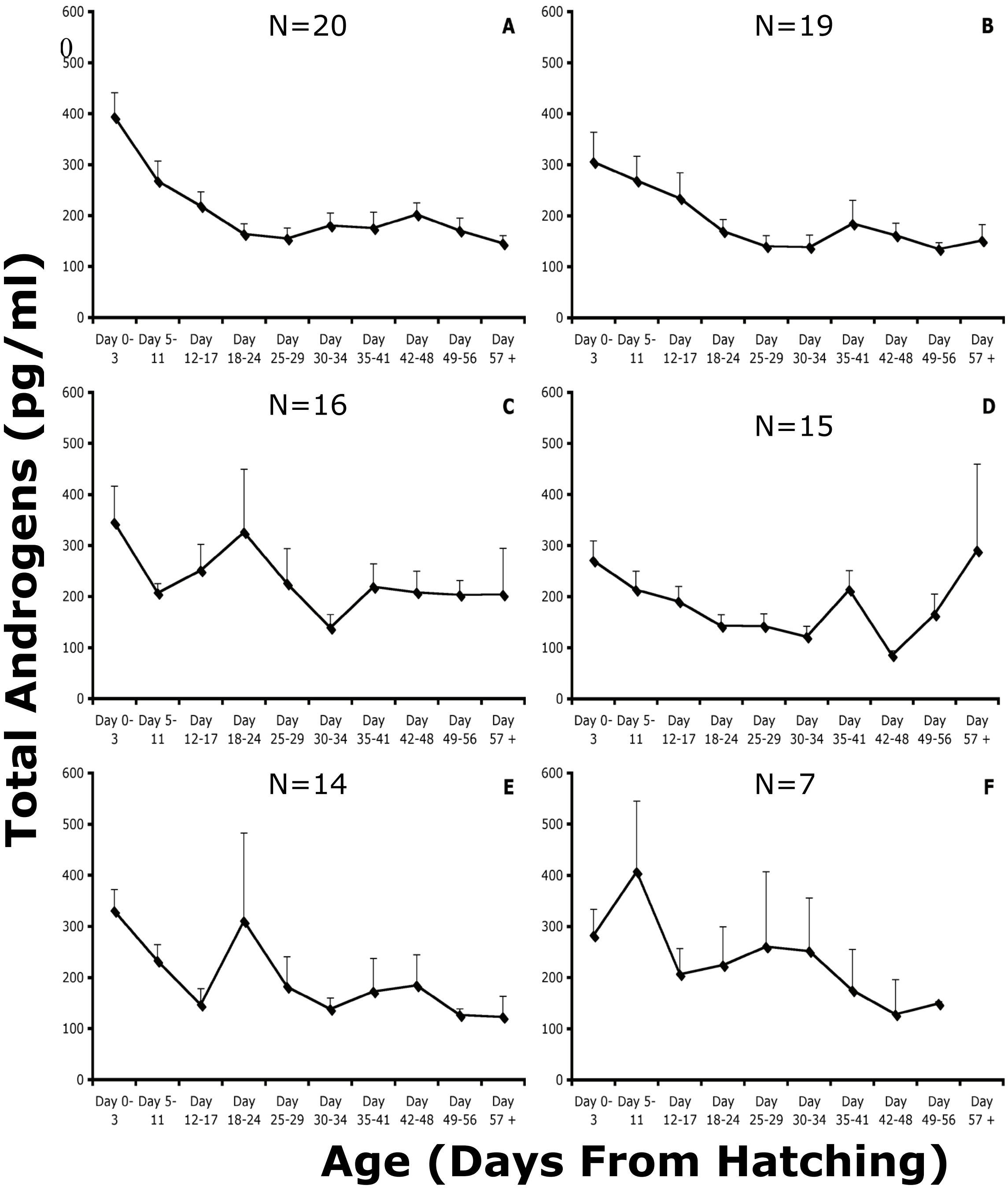

Figure 4.3. Circulating androgen levels were not related to hatching position. Error bars show one standard error. Hatching positions 1-6 are shown in A-F respectively (91 individuals from 21 nests). The number of nestlings sampled from each hatching position is shown in each panel.

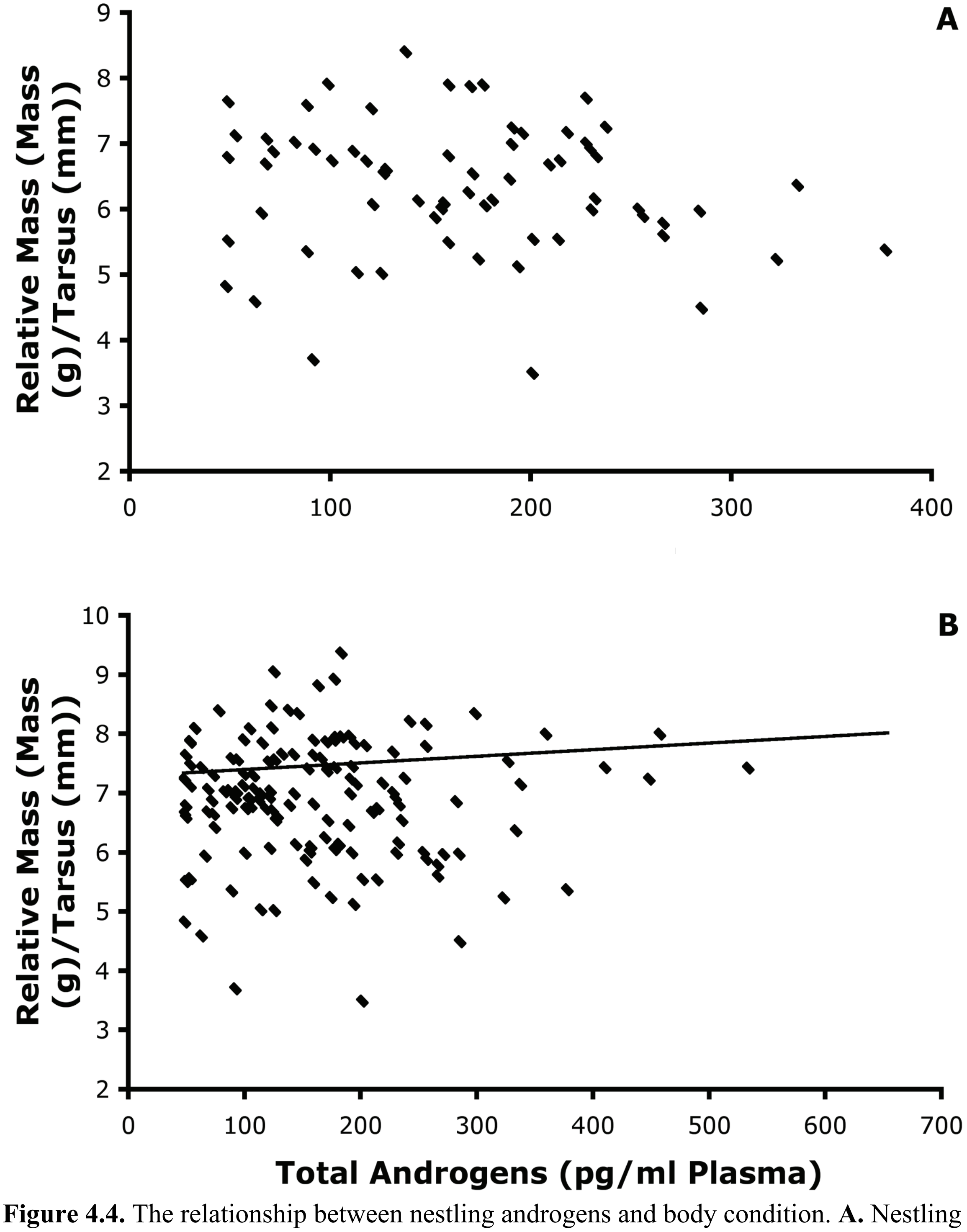

Figure 4.4. The relationship between nestling androgens and body condition. **A.** Nestling androgens were not related to condition (mass/tarsus) in nestlings during maximum growth (days 16-23) (N=81 birds, from 21 broods). **B.** Nestling androgens were positively related to nestling condition towards the end of development when the tarsus has stopped growing and nestlings were reaching their maximum mass (days 43-51) (N=78 birds, from 21 broods).

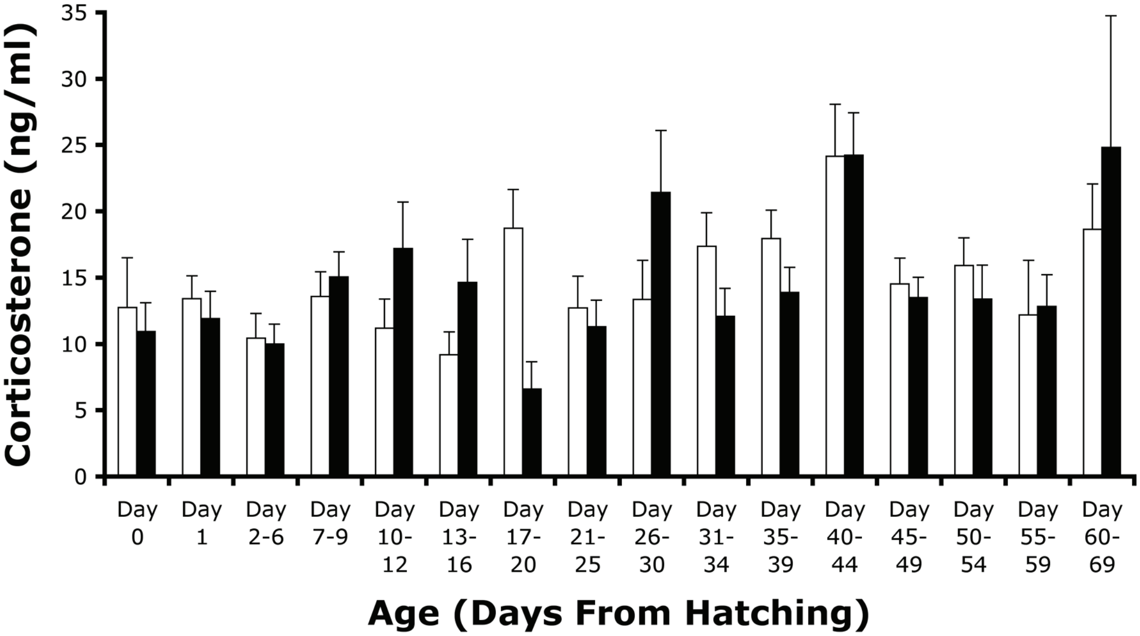

Figure 4.5. Mean nestling plasma corticosterone versus age in relation to sex. Circulating corticosterone levels did not differ between males (closed) and females (open), but they increase over the nestling phase. Error bars show one standard error of the means. Samples include a total of 91 individuals (47 males and 44 females) from 21 different nests.

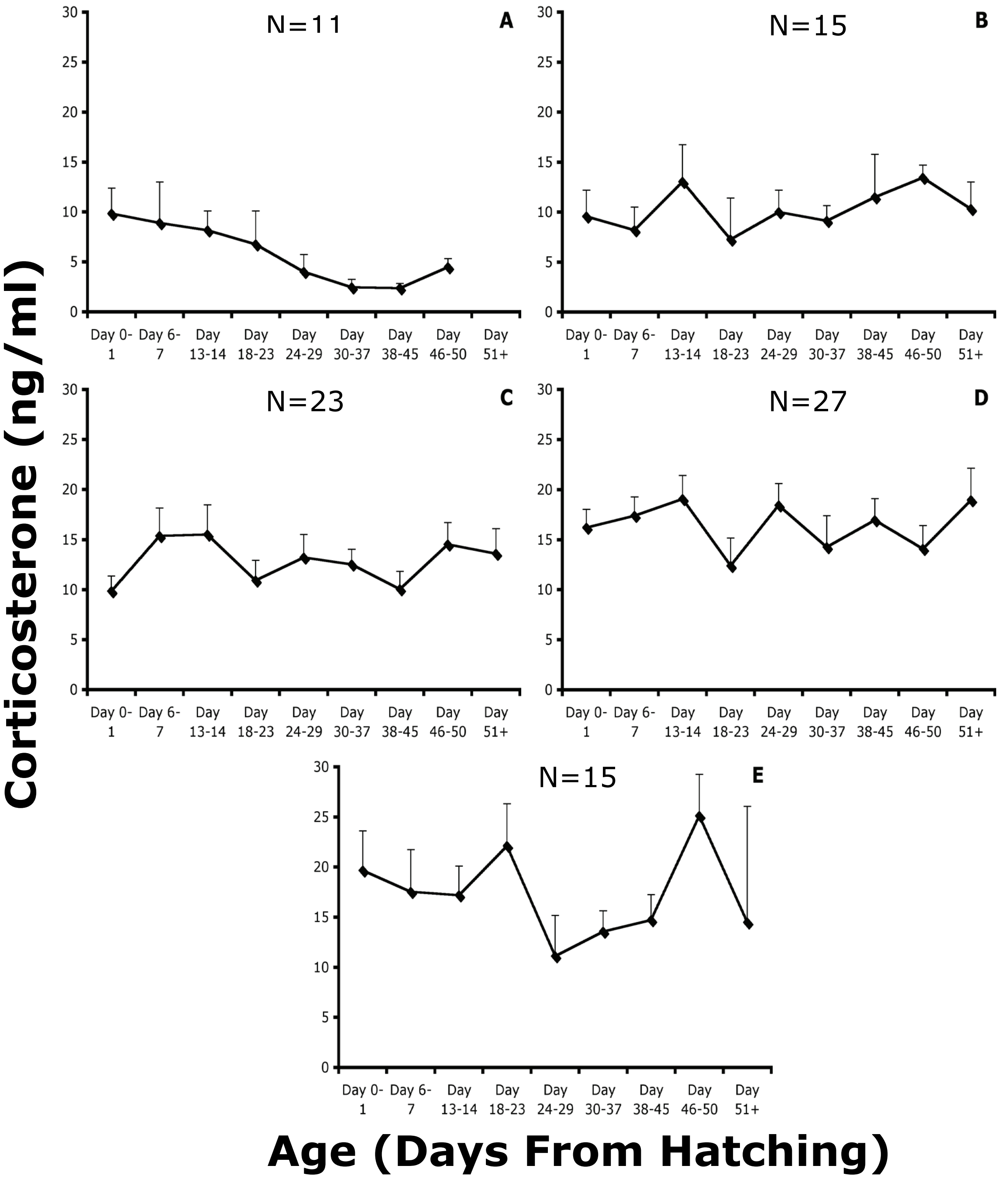

Figure 4.6. Circulating corticosterone levels of nestlings are not related to brood size during the nestling phase. Brood sizes 1 and 2 are shown in A and brood sizes 3-6 in B-E respectively; error bars show one standard error of the mean. The number of nestlings sampled from each brood size is shown on each graph (total of 91 individuals from 21 nests).

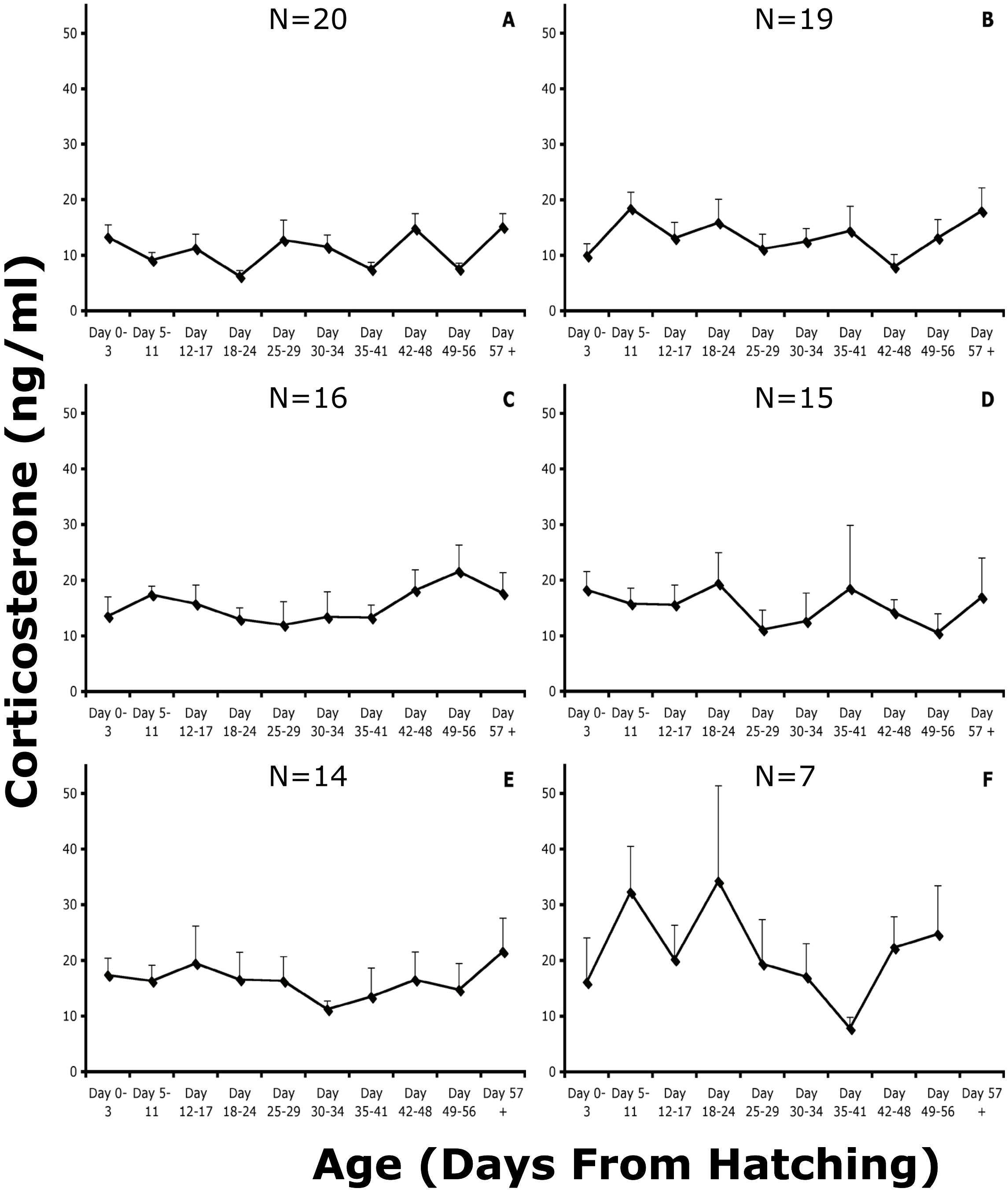

Figure 4.7. Circulating corticosterone levels were related to hatching position. Earlier-hatched nestlings had lower levels of corticosterone than those that hatch later. Error bars show one standard error. Hatching positions 1-6 are shown in A-F respectively (91 individuals from 21 nests). The number of nestlings sampled from each hatching position is shown in each panel.

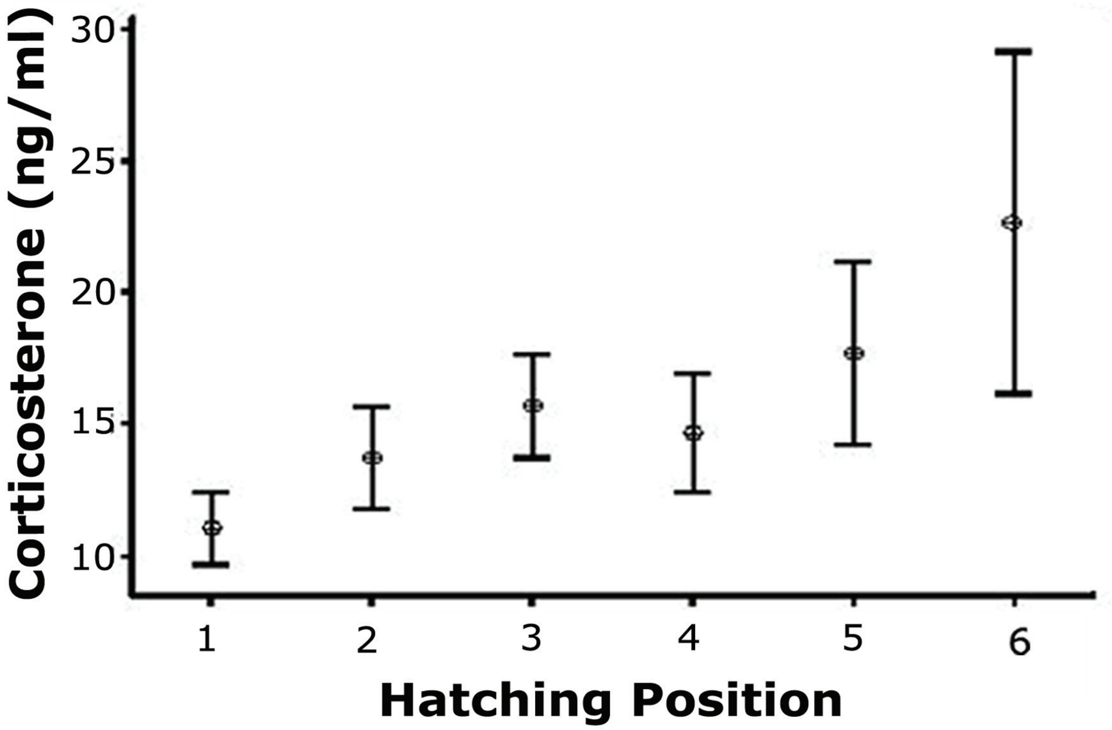

Figure 4.8. The mean plasma corticosterone (not controlling for any other factors or for repeated measures of the same nestlings), for each hatching position with 95% confidence intervals for each hatching position. There was an increase in corticosterone with hatching position.

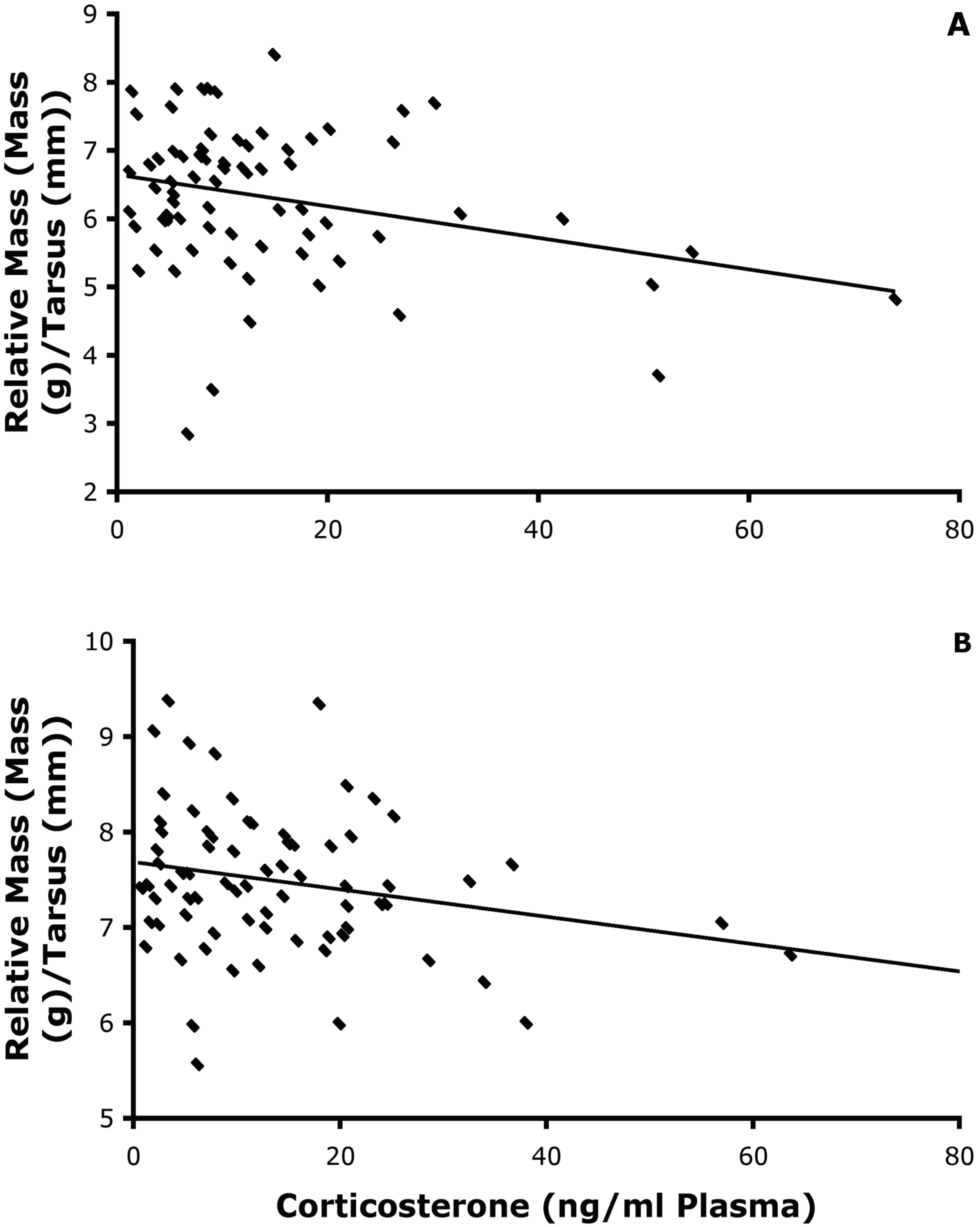

Figure 4.9. The relationship between nestling plasma corticosterone and body condition. **A.** Nestling corticosterone is negatively related to body condition (mass/tarsus) in nestlings during maximum growth (days 16-23), (N=81 nestlings in 20 broods). **B.** Nestling corticosterone is negatively related to nestling body condition in nestlings after the tarsus has finished growing and mass is at is maximum (43-51 days), (N=78 nestlings from 20 broods).

Table 4.1. Variables in two general linear mixed models which explain variation in (**a**) nestling androgen, and (**b**) nestling corticosterone plasma concentrations. For our androgen and corticosterone models we had 561 plasma samples from 81 birds in 21 different nests. Brood sizes ranged from 1-6. The condition variable that was used incorporates condition measurements taken throughout the entire nestling phase instead of at certain points in time.

Source	*df*	β	N	*F*	*p*
(a) Circulating Androgens			561		
Family	20		20	1.73	0.053
Sex	1	-7.29	81	0.13	0.724
Brood Size	1	52.84	81	0.69	0.406
Hatch Order	1	-3.59	81	0.64	0.422
Condition	1	-3.59	81	0.17	0.679
Age	1	-6.79	81	5.77	0.017
Age^2	1	0.07	81	4.61	0.032
(b) Circulating Corticosterone			561		
Family	20		20	3.01	<0.001
Sex	1	-9.41	81	0.00	0.994
Brood Size	1	5548.31	81	1.71	0.191
Hatch Order	1	4774.88	81	7.97	0.005
Hatch Order*Brood Size	1	1153.67	81	11.62	<0.001
Condition	1	-3194.16	81	29.99	<0.001
Age	1	783.01	81	17	<0.001
Age^2	1	-8.73	81	12.21	<0.001

WORKS CITED

Astheimer LB, Buttemer WA, Wingfield JC, 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scandanvis 23:355-365.

Balthazart J, Adkins-Regan, E. 2002. Sexual differentiation of brain and behavior in birds. Hormones, Brain and Behavior. 6: 233-301.

Bray MM, 1993. Effect of ACTH and glucocorticoids on lipid metabolism in the Japanese quail, *Coturnix coturnix japonica*. Comparative Physiology and Biochemistry A 105:689-696.

Carere C, Balthazart J, 2007. Sexual versus individual differentiation: the controversial role of avian maternal hormones. Trends in Endocrinology and Metabolism 18:73-80.

DelaMora AN, Drummond H, Wingfield JC, 1996. Hormonal corrleates of dominance and starvation-induced aggression in chicks of the blue-footed booby. Ethology 102:748-761.

Fargallo JA, Martinez-Padilla J, Toledano-Diaz A, Santiago-Moreno J, Davila JA, 2007. Sex and testosterone effects on growth, immunity, and melanin coloration of nestling Eurasian kestrels. Journal of Animal Ecology 76:201-209.

Ferree ED, Wilkelski MC, Anderson DJ, 2004. Hormonal correlates of siblicide in Nazca boobies: support for the challenge hypothesis. Hormones and Behavior 46:655-662.

Goodship NM, Buchanan KL, 2006. Nestling testosterone is associated with begging behaviour and fledging success in the pied flycatcher, *Ficedula hypoleuca*. The Proceedings of the Royal Society B 273:71-76.

Goodship NM, Buchanan KL, 2007. Nestling testosterone controls begging behavior in the pied flycatcher, *Ficedula hypoleuca*. Hormones and Behavior In press.

Goymann W, Kempenaers B, Wingfield JC, 2005. Breeding biology, sexually dimorphic development and nestling testosterone concentrations of the classically polyandrous African black coucal, *Centropus grillii*. Journal of Ornithology 146:314-324.

Griffiths R, Double MC, Orr K, Dawson RJG, 1998. Sex-biased mortality in the lesser black-headed gull Larus fuscus during the nestling stage. Ibis 134:237-244.

Groothuis TGG, Meeuwissen G, 1992. The influence of testosterone on the development and fixation of the form of displays in two age classes of young black-headed gulls. Animal Behaviour 43:189-208.

Groothuis TGG, Muller W, von Engelhardt N, Carere C, Eising C, 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. Neuroscience and Biobehavioral Reviews 29:329-352.

Groothuis TGG, Ros AFH, 2005. The hormonal control of begging and early aggressive behavior: experiments in black-headed gull chicks. Hormones and Behavior 48:207-215.

Grossman CJ, 1985. Interactions between the gonadal steroids and the immune system. Science 227:257-261.

Heath J, 1997. Corticosterone levels during nest departure of juvenile American kestrels. Condor 99:806-811.

Kahn N, St. John J, Quinn TW, 1998. Chromosome-specific intron size differences in the avian CHD gene provide and efficient method for sex identification in birds. The Auk 115:1074-1078.

Kalmbach E, Furness RW, Griffiths R, 2005. Sex-biased environmental sensitivity in: natural and experimental evidence from a species with larger females. Behavioral Ecology 16:442-449.

Kern M, Bacon W, Long D, Cowie RJ, 2001. Possible roles for corticosterone and critical size in the fledging of nestling pied flycatchers. Physiological Biochemical Zoology 74:651-659.

Ketterson ED, Nolan V, Cawthorn MJ, Parker PG, Ziegenfus C, 1996. Phenotypic engineering: Using hormones to explore the mechanistic and functional basis for phenotypic variation in nature. Ibis 138:70-86.

Kitaysky AS, Kitaiskaia EV, Piatt JF, Wingfield JC, 2003. Benefits and costs of increased levels of corticosterone in seabird chicks. Hormones and Behavior 43:140-149.

Kitaysky AS, Kitaiskaia EV, Wingfield JC, Piatt JF, 2001a. Dietary restriction causes chronic elevation of corticosterone and enhances the stress response in red-legged kittiwake chicks. Journal of Comparative Physiology B: Biochemistry Systematics and Environmental Physiology 171:701-709.

Kitaysky AS, Piatt JF, Wingfield JC, Romano M, 1999. The adrenocortical stress-response of black-legged Kittiwake chicks in relation to dietary restrictions. Journal of Comparative Physiology B: Biochemistry Systematics and Environmental Physiology 169:303-310.

Kitaysky AS, Wingfield JC, Piatt JF, 2001b. Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. Behavioral Ecology 12:619-625.

Landys MM, Ramenofsky M, Wingfield JC, 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. General and Comparative Endocrinology 148:132-149.

Love OP, Bird DM, Shutt LJ, 2003a. Corticosterone levels during post-natal development in captive American kestrels (Falco sparverius). General and Comparative Endocrinology 130:135-141.

Love OP, Bird DM, Shutt LJ, 2003b. Plasma corticosterone in American Kestrel siblings: effects of age, hatching order, and hatching asynchrony. Hormones and Behavior 43:480-488.

Marti CD, Poole AF, Bevier LR, 2005. Barn Owl (*Tyto alba*). In: The birds of North America (Poole A, ed). Ithaca: Cornell Laboratory of Ornithology.

Mock DW, Parker GA, 1997. The evolution of sibling rivalry. Oxford: Oxford University Press.

Muller W, Dijkstra C, Groothuis TGG, 2003. Intersexual differences in T-cell mediated immunity of blackheaded gull chicks (Larus ridibundus) depend on the hatching order. Behavioral Ecology and Sociobiology 55:80-86.

Muller W, Groothuis TGG, Eising C, Dijkstra C, 2005. An experimental study on the causes of sex-biased mortality in the black-headed gull- the possible role of testosterone. Journal of Animal Ecology 74:735-741.

Naguib M, Riebel K, Marzal A, Gil D, 2004. Nestling immunocompetance and testosterone co-vary with brood size in a songbird. Proceedings of the Royal Society B 271:833-838.

Nunez-de La Mora A, Drummond H, Wingfield JC, 1996. Hormonal correlates of dominance and starvation-induced aggression in chicks of the blue-footed booby. Ethology 102:748-761.

Olson NJ, Kovacs WJ, 1996. Gonadal steroids and immunity. Endocrinology Reviews 17:369-384.

Owen-Ashley NI, Hasselquist D, Wingfield JC, 2004. Androgens and the immunocompetence handicap hypothesis: unraveling direct and indirect pathways of immunosuppression in song sparrows. American Naturalist 164:490-505.

Owens IPF, Short RV, 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories on sexual selection. Trends in Ecology and Evolution 10:44-47.

Quillfeldt P, Massello JF, Strange IJ, Buchanan KL, 2006. Begging and provisioning of thin-billed prions, *Pachyptila belcheri*, are related to testosterone and corticosterone. Animal Behavior 71:1359-1369.

Quillfeldt P, Trager I, Griffiths K, Buchanan KL, Massello JF, 2007. Is sex-specific mass gain in Cory's shearwaters *Calonectris diomedea* related to begging and steroid hormone expression? Behavioral Ecology and Sociobiology 61:793-800.

Sapolsky R, Romero M, Munck A, 2000. How do glucocorticoids influence stress responses? Integrating permissive, supressive, stimulatory, and preparative actions. Endocrinology Reviews 21:55-89.

Sasvari L, Hegyi Z, Peczely P, 1999. Brood reduction in white storks mediated through asymmetries in plasma testosterone concentrations in chicks. Ethology 105:569-582.

Schwabl H, 1993. Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Science USA 90:11446-114450.

Schwabl H, 1999. Developmental changes and among-sibling variation of corticosterone levels in an altricial avian species. General and Comparative Endocrinology 116:403-408.

Smith GT, Wingfield JC, Veit RR, 1994. Adrenocortical response to stress in the common diving petrel *Pelecanoides urinatrix*. Physiological Zoology 67:526-537.

Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK, 2003. Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). Hormones and Behavior 44:132-139.

Tarlow EM, Wilkelski MC, Anderson DJ, 2001. Hormonal correlates of siblicide in Galapagos Nazca boobies. Hormones and Behavior 40:14-20.

Weimerskirch H, Barbraud C, Lys P, 2000. Sex differences in parental investment and chick growth in the wandering albatrosses: fitness consquences. Ecology 81:309-318.

Westneat DF, 1990. Genetic parentage in the Indigo Bunting: A study using DNA fingerprinting. Behavioral Ecology and Sociobiology 27:67-76.

Williams T, Dawson A, Nicholls T, Goldsmith A, 1987. Reproductive endocrinology of free-living nestling and juvenille starlings, *Sturnus vulgaris*; an altricial species. Journal of Zoology London 212:619-628.

Wingfield J, Kitaysky A, 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integrative and Comparitive Biology 42:600-609.

Wingfield JC, Hegner RE, Dufty AM, Ball GF, 1990. The challenge hypothesis: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. American Naturalist 136:829-846.

CHAPTER FIVE

CONCLUSION

SUMMARY OF FINDINGS

Barn owls differentially allocate androgens to the eggs within a clutch. Females seem to optimize resources to each egg such that, in general there is an increasing amount of androgens in the first 3 or four eggs, and then a decreasing amount in eggs 4 through the end of the laying order (however 8-egg clutches do not follow this pattern). Females may minimize hatching asynchrony among advantaged nestlings (as defined by Mock and Forbes, 1995) by investing more hormones in second- and third-laid eggs than in first eggs. Nestlings laid later in the laying order receive fewer hormones and are much smaller because of barn owls' asynchronous hatching and, as a result, are less likely to survive. Within the later-hatched nestlings, androgen patterns suggest a parental strategy that increases the effects of hatching asynchrony, potentially in order to hasten mortality of these "insurance" offspring. The sex ratio of the later-laid eggs is also male biased. Males are the smaller sex, which is another indication to us that these later eggs may be insurance against earlier-laid eggs not hatching.

We found that the sex ratio in the population was not related to food resources, however the number of eggs laid was positively related to the food resources the female was supplied. We found that the size of a clutch did not dramatically affect the circulating corticosterone or testosterone levels of nestling birds. The large hatching asynchrony however, has implications for both nestlings stress hormone levels and nestling survival. We found that later hatched nestlings had increased mortality. These later-hatched nestlings had higher levels of corticosterone and it and negatively correlated with body condition during the time of maximal growth and after nestlings had reached their final tarsus length. However, we found no significant differences in nestling testosterone levels in relation to hatching order.

Chapter Two

The primary sex ratio at hatching for this population of barn owls was significantly male biased, but the sex ratio at fledging did not differ from parity. These results imply that even though females are more costly to raise (Seifert et al., in prep.), male barn owl nestlings suffered higher mortality than females, which leads us to reject Myers' hypothesis. Nor do our results support the equal allocation hypothesis, though the hatching sex ratio biased toward the less costly sex (males) would have led us to faulty assumptions about parental investment if we had not also quantified sex ratio at fledging. Females seem to reach equal investment in the sexes by initially producing more male eggs and continuing to invest in male nestlings that ultimately die. There appears to be no relation between current environmental conditions (supply of food by the male) and sex ratio—neither at laying, nor fledging. However, females mated to males that provide more food to their nest are able to lay more eggs and fledge more offspring than those mated to low-delivering males.

Chapter Three

Females seem to allocate egg mass and hormones differentially to individual eggs, which could have adaptive implications for certain nestlings. Female eggs tend to increase in size over the laying order, however, male eggs stay fairly stable in size and male nestlings tend to experience increased mortality, although later-hatched males are no more likely to die before fledging than later-hatched females. We found that yolk androgens follow a different pattern through the laying order in barn owl eggs than has been previously reported. Yolk androgens increase in the first 3 eggs and then decrease. We believe that, in barn owls, the first 3 eggs receive increasing hormone levels in their yolk because mothers are trying to mitigate the effects of hatching asynchrony among their advantaged offspring. Nestlings later in the hatching order, in contrast, are insurance offspring that receive less hormones, potentially as a means to hasten their eventual reduction. It seems that female barn owls follow a previously undescribed "hybrid" strategy that includes making insurance offspring that are set up to die if all early-hatched advantaged nestlings survive.

Chapter Four

Nestling plasma corticosterone and testosterone levels do not differ between male and female offspring. We originally predicted that male nestlings would have higher levels of testosterone because it has been proposed that it is necessary for sexual differentiation during the nestling phase (Owens and Short, 1995; Olson and Kovacs, 1996). However there is good evidence for high male testosterone levels only for mammals (Olson and Kovacs, 1996), but not for birds (Balthazart and Adkins-Regan, 2002). Increased levels of testosterone may be detrimental to male offspring, causing a compromised immune system and increased mortality (e.g. Goymann et al., 2005; Fargallo et al., 2007). High male testosterone levels in male barn owls do not explain the higher levels of male mortality because there are no sex differences in circulating testosterone levels. Testosterone levels among the nestlings from broods of different sizes and nestlings from different hatching positions were also similar. This does not fit the hypothesis that testosterone regulates sibling competition. Although nestling corticosterone levels did not increase with brood size, they were elevated in nestlings from later hatching positions, indicating that these nestlings experience more stress than earlier-hatched nestlings. Nestlings from later hatching positions may be more stressed than those from earlier hatching positions because they are less capable of competing with siblings for food. Therefore, parental allocation via early incubation affects nestling stress hormone levels, which may impact their growth, health, and survival.

SYNTHESIS

There are two competing ideas about how mothers might allocate resources in species with hatching asynchrony. First, they may try to minimize the effects of hatching asynchrony by allocating more resources to later-laid eggs to try to compensate for the competitive advantages of first-hatched nestlings (review in: Magrath, 1990; reviewed in Stoleson and Beissinger, 1999; a potential hormonal mechanism: Schwabl et al., 1997). This hypothesis assumes that hatching asynchrony is not adaptive, and that it is adaptive for females to reduce the size difference between nestlings, by other mechanisms such as hormones and egg size, caused by hatching asynchrony. The second hypothesis predicts that hatching asynchrony is adaptive, in which case females enhance the size difference between nestlings by allocating the most resources to early-hatched nestlings (Forbes and Mock, 1998). This hypothesis is based on the assumption that parents produce more offspring than they can successfully raise- an insurance against hatching failure and offspring mortality. In this case, parents should minimize the amount of resources that they allocate to later-hatched insurance nestlings. In a situation where there is no unexpected mortality of early-hatched nestlings, this allocation strategy should reduce the time required for the favored offspring to out-compete their later-hatched siblings. Thus, parents minimize the resources in insurance offspring that offer no/little future reproductive potential unless some earlier-hatched nestlings die. In barn owls, unlike bird species studied in the past, we see both strategies within the same nests. The first 3 eggs are equally likely to be male or female, and have increasing yolk androgens (which have been shown to positively affect mass and begging behavior in nestlings of some species) with each successive egg. Nestlings that hatch from these eggs have much higher survival rates than nestlings hatching from later-laid eggs; they are larger at fledging and they have lower circulating plasma corticosterone levels than later-laid nestlings. On the other hand, eggs from the second half of the laying order are more likely to be male than female. Male offspring are smaller than female offspring and, additionally, are smaller than the nestlings that hatch ahead of them, making them doubly disadvantaged. The eggs from which they hatch also have decreasing yolk androgen levels. The combination of these variables may lead to severely handicapped nestlings hatching in the later half of a clutch. This combination seems to indicate that barn owls employ a “hybrid” strategy, mitigating the effects of hatching asynchrony in early-hatched nestlings and amplifying the effects in later-hatched nestlings. We found that later-hatched nestlings have much higher mortality than earlier-hatched nestlings, and that they have increased levels of circulating plasma corticosterone levels, indicating that they are under more stress than early-hatched nestlings.

We found support for the idea that hatching asynchrony and differential allocation of androgens may have an effect on nestling growth, survival and plasma stress hormone levels. Sex ratio biases are not related to food resources, but rather parents seem to invest equally in males and females. Male nestlings are less costly to raise than females (Seifert et al., in prep.), and as a result, they may be preferentially laid into later hatching positions, possibly because smaller male nestling may be better at surviving when there are insufficient resources for all nestlings. Additionally, later-hatched male nestlings have minimal competitive impact on favored early-hatched nestlings, which must compete with them for food. In environments abundant in food resources, parents may be able to raise these extra, predominantly male nestlings more easily than extra females because they are less expensive.

We found that male nestlings have higher mortality than female nestlings, which was unexpected given that, in times of food stress, the larger sex is expected to experience higher mortality (Lack, 1968; Clutton-Brock et al., 1985; Teather and Weatherhead, 1989; 1994). Although this hypothesis has been criticized because it does not separate the costs of being large from the costs of being male, studies of reversed sexually size dimorphic species, like the barn owl, can decouple them. The hypothesis that the larger sex has increased mortality is usually tested in species where males are larger. The problem is that males are physiologically different from females and may possess characteristics that make them less likely to survive, such as increased testosterone levels, which suppress the immune system. The male disadvantage hypothesis predicts that increased mortality of males is related to their higher circulating testosterone levels (Grossman, 1985; Owens and Short, 1995; Olson and Kovacs, 1996; Muller et al., 2005; Fargallo et al., 2007). We found no support for this hypothesis because male and female nestlings have very similar levels of testosterone throughout the nestling phase. An alternative to the male disadvantage hypothesis in barn owls is that males have increased mortality because they are being out-competed by female siblings. However, increased male mortality is not exclusively a result of competition from larger females siblings because males in early hatching positions are just as likely to survive as female nestlings. Likewise, male and female nestlings in late hatching positions are equally likely to survive. We propose that males have higher mortality rates primarily because the second half of the clutch is male biased. As a result of extreme hatching asynchrony and RSSD, these later hatched nestlings are so small that they can't successfully compete with their older, larger siblings for food, and thus suffer elevated mortality.

FUTURE DIRECTIONS

Now that our background studies are complete, the next step is to manipulate barn owls' external environment by changing food at the nest and manipulating egg content so that we can experimentally study how the environment affects differential allocation and its implications for the nestlings.

We found that hatching asynchrony has a profound effect on nestling growth, survival, and circulating stress hormone levels. Maternal egg yolk androgen deposition seems intended to minimize hatching asynchrony's effects in eggs laid in the first half of the laying order, and to amplify its effects in eggs later in the laying order, leading us to the conclusion that the hormone pattern may be adaptive, if not in any way that's been previously reported. To truly understand the adaptive function of hatching asynchrony and how and why parents differentially allocate hormones, we should manipulate some of the variables that we have been studying. We could create synchronous broods and compare nestling growth, survival and stress hormone levels to nestlings in asynchronous broods. This would allow us to determine whether hatching asynchrony allows some nestlings to grow at the expense of their siblings, and whether maternal hormones in early-laid eggs gives the resulting nestlings significant growth advantages. We could also compare the number of nestlings that fledge from each group to see how hatching asynchrony affects brood survival. Such an experiment would help to determine whether hatching asynchrony is adaptive for parents, and what effects it has on nestlings. In addition, it would be interesting to look at egg microbes in relation to the onset of incubation. In tropical parrots with large, asynchronous broods, nestlings do better in artificially created synchronous broods, but if females delay incubation they experience an increase in hatching failure due to egg microbes. Even though hatching asynchrony isn't beneficial to the parents or offspring in terms of the consequences for nestling success, immediate incubation initiation reduces the likelihood of egg microbes, which decrease the chances of successful hatching. Because egg microbe work has been done mainly in asynchronously-hatching species in the tropics, where microbes have favorable living conditions (warm and wet), it would be interesting to determine whether egg microbes play a role in the incubation strategies of temperate species, such as the barn owl, as well. In fact, in the barn owl (because of its cosmopolitan distribution), temperate and tropical eggs could be compared to conclude whether egg microbes are differentially prevalent.

Manipulation of barn owls' food supply at the nest boxes before and during egg laying could be very useful for answering questions about how variation in food supply by the male affects female allocation to eggs and changes sex ratio. We would be able to examine female investment in terms of the number and size of eggs laid, as well as the sex ratio of the nestlings in relation to current resources. We could also manipulate the food supply during the nestling phase to determine whether parents are able to fledge more offspring given increased food resources and how this affects nestling hormone levels. We predict that if later-hatched nestlings are indeed dying as a result of inadequate food resources, then food supplementation should decrease nestling mortality. If later-hatched nestlings nevertheless show high mortality we would conclude that they are only an insurance policy against the early mortality of older nestlings or hatching failure in early-laid eggs. We could also test if higher corticosterone levels of later-hatched nestlings are the result of food shortage for the entire brood.

Such manipulations will allow us to more precisely test whether and how parents optimize their clutches in relation to current environmental conditions. It will also allow us to experimentally test the adaptive significance of hatching asynchrony to see whether it is part of the parental strategy to help fledge as many offspring as possible, or the byproduct of external parental constraint (such as egg microbes). Lastly, by manipulating egg resources we can determine whether females manipulate egg content how this may affect nestling success.

WORKS CITED

Balthazart J, Adkins-Regan, E. 2002. Sexual differentiation of brain and behavior in birds. Hormones, Brain and Behavior. 6: 233-301.

Clutton-Brock TH, Albon S, Guinness PE, 1985. Parental investment and sex differences in juvenile birds and mammals. Nature 313:131-133.

Fargallo JA, Martinez-Padilla J, Toledano-Diaz A, Santiago-Moreno J, Davila JA, 2007. Sex and testosterone effects on growth, immunity, and melanin coloration of nestling Eurasian kestrels. Journal of Animal Ecology 76:201-209.

Forbes L, Mock D, 1998. Parental optimism and progeny choice: when is screening for offspring quality affordable? Journal of Theoretical Biology 192:3-14.

Goymann W, Kempenaers B, Wingfield JC, 2005. Breeding biology, sexually dimorphic development and nestling testosterone concentrations of the classically polyandrous African black coucal, *Centropus grillii*. Journal of Ornithology 146:314-324.

Grossman CJ, 1985. Interactions between the gonadal steroids and the immune system. Science 227:257-261.

Lack D, 1968. Ecological adaptations for breeding in birds. London: Methuen.

Magrath RD, 1990. Hatching Asynchrony in altricial birds. Biological Reviews 65:587-622.

Mock DW, Forbes L, 1995. The evolution of parental optimism. Trends in Ecology and Evolution 10:130-134.

Muller W, Groothuis TGG, Eising C, Dijkstra C, 2005. An experimental study on the causes of sex-biased mortality in the black-headed gull- the possible role of testosterone. Journal of Animal Ecology 74:735-741.

Olson NJ, Kovacs WJ, 1996. Gonadal steroids and immunity. Endocrinology Reviews 17:369-384.

Owens IPF, Short RV, 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories on sexual selection. Trends in Ecology and Evolution 10:44-47.

Schwabl H, Mock DW, Gieg JA, 1997. A hormonal mechanism for parental favouritism. Nature 386:231.

Stoleson SH, Beissinger SR, 1999. Egg viability as a constraint on hatching asynchrony at high ambient temperatures. Journal of Animal Ecology 68:951-962.

Teather KL, Weatherhead PJ, 1989. Sex-specific mortality in nestling great-tailed grackles. Ecology 70:1485-1493.

Teather KL, Weatherhead PJ, 1994. Allometry, adaptation, and the growth and development of sexually dimorphic birds. Oikos 71:515-525.