EFFECTS OF MATERNAL INVESTMENTS ON EGG METABOLIC RATES, HATCHING SYNCHRONY, AND OFFSPRING PERFORMANCE IN CANADA GEESE

(*Branta canadensis maxima*)

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Todd Allen Boonstra

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Biological Sciences

April 2006

Fargo, North Dakota

APPROVAL BY COMMITTEE

ABSTRACT

Boonstra, Todd Allen, M.S., Department of Biological Sciences, College of Science and Mathematics, North Dakota State University, April 2006. Effects of Maternal Investments on Egg Metabolic Rates, Hatching Synchrony, and Offspring Performance in Canada Geese (*Branta canadensis maxima*). Major Professor: Dr. Mark E. Clark.

Understanding how maternal allocation affects the development and performance of a female's offspring is essential to understanding population growth. In avian development, the optimal incubation duration must be reached prior to the onset of hatching. Premature hatching (relative to the optimal duration) often results in malformed young while delayed hatching may deplete nutrient reserves necessary in the post-hatching environment. Canada geese (*Branta canadensis maxima*) have synchronously hatching offspring, even though the first eggs in a clutch may have been laid ten days prior to the last laid eggs. Among the goslings, alternative developmental strategies are needed for all offspring to hatch within the time frame of females ending incubation and leaving the nest. Alternative developmental strategies may be influenced by the female through resource allocation to eggs or incubation behavior. The major objectives in this study were to determine 1) if maternal investments of yolk steroids are differentially allocated within a female's clutch, 2) if embryonic metabolic rates differ within a clutch, and 3) if alternative developmental rates affect offspring survival. Results indicate that 1) both egg size and yolk testosterone levels decrease throughout the laying sequence, but there is no pattern in variation of yolk estradiol levels; 2) egg metabolic rates increase throughout the laying sequence; and 3) embryonic developmental rates did not affect offspring survival in the two weeks that follow hatching. Hence, within-clutch differences in pre-hatching maternal investments to offspring in Canada geese may facilitate synchronous hatching without compromising short-term offspring performance.

ACKNOWLEDGMENTS

This project would not have been possible without the invaluable support and guidance from many individuals and organizations. I would like to especially thank my major adviser, Dr. Mark Clark, and “co-adviser,” Dr. Wendy Reed, for their guidance, friendship, and generosity they have shared with me during this project. I am grateful to the rest of my committee, Dr. Gary Nuechterlein and Dr. Paul Ode, for their time and professional guidance.

I would like to thank Bryan Safratowich for his field and laboratory assistance during the 2005 season and to all the North Dakota State University biological graduate students for their additional discussion and support in lending an extra hand in the field. A special thanks goes to Ara Anderson and Heath Hagy for their continuous assistance during banding operations and unabating efforts in capturing escaped birds; Jeff Heilveil and Will Clark for editorial comments on numerous drafts; and Rachel Bush, Curt Francis, Susan Spinar, Dan McEwen, Emily Davenport, Jennifer Newbrey, and Dan Frank for their discussions and ideas.

Lastly, I would like to thank my wife, Stacy Boonstra, for her editorial comments, financial support, and encouragement throughout this study. Thank you to my family and friends for always providing encouragement and support to follow my dreams. I am extremely honored and appreciative to have had the support system I have encountered over the course of my education.

Funding was provided through the generosity of numerous individuals and organizations. This project could not have been conducted without funding from the Delta Waterfowl Foundation, American Crystal Sugar Company, Department of Biological Sciences teaching assistant stipends, and Biological Sciences Graduate Student Association.

Research was carried out in accordance with Federal Fish and Wildlife Permit MB814928-1 and Auxiliary Marker Permit 23212, State of Minnesota Department of Natural Resources Special Permit 122242 and 12910, and North Dakota State University Institutional Animal Care and Use Committee (IACUC) #A0431.

TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGMENTS....iv

LIST OF TABLES....ix

LIST OF FIGURES.... x

GENERAL INTRODUCTION....1

Literature Cited 4

CHAPTER 1. ALLOCATION OF MATERNALLY DERIVED STERIODS ACROSS LAYING SEQUENCE IN CANADA GEESE 7

ABSTRACT 8

INTRODUCTION....9

Hormone Allocation to Yolk Layers....9

Variation Among Females....10

Hormone of Interest 10

Acceleration Outcomes of Testosterone 12

Negative Effects of Yolk Testosterone 13

MATERIALS AND METHODS 14

Description of Study Site....14

Nest Monitoring....14

Hormone Analysis and Yolk Sampling 15

Preparation of Stripped Yolk 16

Sample Setup and Extraction of Hormones 18

RESULTS....20

Standard Curves and Corresponding Samples....20

Maternal Testosterone Across Laying Sequence 20

Maternal Estradiol Across Laying Sequence 25

T/E_2 Ratio Across Laying Sequence 28

DISCUSSION 31

LITERATURE CITED 35

CHAPTER 2. EMBRYONIC METABOLIC RATES OF GOSLINGS IN RELATION TO EGG LAYING SEQUENCE OF CANADA GEESE 39

ABSTRACT 40

INTRODUCTION 41

Contributions of Cylindrical Egg Pores to Gas Exchange 41

Oxygen Conductance and Availability 42

Influence of Developmental Strategies on Consumption Rates 43

Implications of Egg Consumption Rates on Hatching Synchrony 44

MATERIALS AND METHODS 46

Nest Monitoring and Metabolic Measuring 46

Collection of Oxygen Samples 47

Determining Oxygen Concentrations 48

Gosling Metabolic Rates 49

Statistical Analysis 50

RESULTS 52

DISCUSSION 64

LITERATURE CITED 68

CHAPTER 3. EFFECTS OF ALTERNATIVE DEVELOPMENT IN SYNCHRONOUS HATCHING ON GOSLING SURVIVAL IN CANADA GEESE 71

ABSTRACT 72

INTRODUCTION 73

MATERIALS AND METHODS 78

Study Site 78

Nest Monitoring and Cross-Foster Design 78

Gosling Survival Rates 79

Statistical Analysis 81

RESULTS 83

DISCUSSION 88

LITERATURE CITED 91

OVERALL SUMMARY 95

LITERATURE CITED 97

APPENDIX A 107

Competitive Binding Radioimmunoassay for T and E_2 107

Statistical Analysis 109

LIST OF TABLES

Table Page

1.1. Regression models describing relative hormone concentrations using position in the laying sequence as the independent variable with sample size (N, based on the number of clutches used), number of parameters (K), change in Akaike's information criterion adjusted for small sample size (Δ AIC_c), Akaike model weights (w_i AIC_c), model likelihood and coefficient of determination (R^2) in Canada geese. 23

2.1. Models describing embryonic oxygen consumption rates as a function of age (i.e., relative incubation length) based on a generalized s-shaped curve from Janoschek (1957) along with sum of squares error (SSE), number of parameters (M), relative Akaike information criteria adjusted for small sample size (Δ AIC_c), Akaike weight (w_i AIC_c), and the coefficient of determination (R^2) 54

2.2. Models describing gosling metabolic rates as a function of sex, egg mass, dry body mass, position in the laying sequence, and time of hatch relative to sample collection along with sum of squares error (SSE), number of parameters (M), relative Akaike information criteria adjusted for small sample size (Δ AIC_c), Akaike weight (w_i AIC_c), and the coefficient of determination (R^2) 56

2.3. Models describing gosling relative percent hematocrit as a function of egg mass, position in the laying sequence, and time of hatch relative to sample collection along with sum of squares error (SSE), number of parameters (M), relative Akaike information criteria adjusted for small sample size (Δ AIC_c), Akaike weight (w_i AIC_c), and the coefficient of determination (R^2) 61

2.4. Models describing fraction of dry yolk relative to hatchling mass as a function of egg mass, position in the laying sequence, and time of hatch relative to sample collection, along with sum of squares error (SSE), number of parameters (M), relative Akaike information criteria adjusted for small sample size (Δ AIC_c), Akaike weight (w_i AIC_c), and the coefficient of determination (R^2) 63

3.1. Relative AIC_c values, AICc weights, and model likelihoods for the candidate models of gosling survival 85

3.2. Parameter estimates based on most parsimonious model {ϕ ()p(A&C, D)} ..86

LIST OF FIGURES

Figure | Page

1.1. A representative standard curve for testosterone and a dilution curve of yolk 21

1.2. A representative standard curve for estradiol and a dilution curve of yolk 22

1.3. Distribution of standardized testosterone concentrations dependent on year collected (2004 and 2005) with results from a t-test comparing the means 24

1.4. Observed relative yolk testosterone concentrations by position in eggs of Canada geese and the models with the highest support (based on AIC_c values) in the candidate set, which both show a decrease in relative testosterone concentrations for later-laid eggs 26

1.5. Mean and 95 percent confidence intervals for observed relative testosterone concentrations in early (positions one through three) and later (positions four through eight) laid eggs in Canada geese along with results from a t-test comparing the means 27

1.6. Observed relative yolk estradiol concentrations by position in eggs of Canada geese and the models with the highest support (based on AIC_c values) in the candidate set, which show no change (Constant model) or a small decrease (Linear model) in relative estradiol concentrations for later laid-eggs 29

1.7. Observed relative yolk testosterone:estradiol ratio by position in eggs of Canada geese and the models with the highest support (based on AIC_c values) in the candidate set, which show no change (Constant model) or a decrease (Linear model) in relative testosterone:estradiol ratio for later-laid eggs 30

2.1. Observed embryonic oxygen consumption rates by relative age (days of incubation) in Canada geese measured from clutches located in the field and lab during 2004 and 2005 (indicated by open circles) and predicted values from the most parsimonious statistical model in which oxygen consumption rates increase with position in the clutch (indicated by the lines, with only three positions shown for clarity) 53

LIST OF FIGURES (Continued)

Figure Page

2.2. Observed values of (A) fresh egg mass, (B) gosling mass, (C) oxygen consumption rates (measured within 24 hours of hatching), and (D) standardized gosling metabolic rates (measured within 24 hours of hatching) by sex in Canada geese 58

2.3. Observed relative hematocrit levels in Canada geese within 24 hours of hatching (open circles) with fresh egg mass and the predicted relationships for goslings hatched from first (solid line), third (dashed line), and sixth (dotted line) laid eggs in a clutch from the most parsimonious model 60

3.1. Diagram representing the design of foster nests to manipulate hatch date..80

3.2. Mean difference in days from predicted hatch dates for eggs from the three foster nest groups with standard errors 84

GENERAL INTRODUCTION

Nelson (2000) described the "parental dilemma" as the tradeoff parents must face when investing in current offspring at the expense of future offspring. Maternal allocation of hormones, resources, and parental care are just a few of the parental investments that may vary among offspring. Often, organisms allocate resources under constraints imposed by their environment and life history. In oviparous species, laying multiple eggs in a reproductive bout may result in strong selection for individuals that distribute resources such that total offspring performance is maximized. Such resources include egg position in the laying sequence (Schwabl 1996, Badzinski et al. 2001), start and duration of the incubation period (Vleck et al. 1985, Arnold et al. 1987, Arnold 1993), egg placement within the nest (Cooper 1978), and maternal hormones and other constituents such as lipids allocated to eggs (Reed and Vleck 2001). Among species with synchronously hatching eggs, the distribution of resources among offspring has large potential to affect offspring performance because the relative age of offspring differs at hatching.

Alternative allocation of hormones to eggs is a possible mechanism females may use to maximize success of current offspring within a clutch. Significant increases of maternally deposited yolk testosterone in eggs in later positions of the laying sequence (i.e., later laid eggs) have been observed in canaries (*Serinus canarius*, Schwabl 1993), dark-eyed juncos (*Junco hyemalis*, Lipar et al. 1999b), American kestrels (*Falco sparverius*, Sockman and Schwabl 2000), red-winged blackbirds (*Agelaius phoeniceus*; Lipar et al. 1999a,1999b; Lipar and Ketterson

2000), and black-headed gulls (*Larus ridibundus*, Eising et al. 2001, Royle et al. 2001, Groothuis and Schwabl 2002). Presumably, these differential levels of yolk testosterone enhance the competitive ability of later hatching, smaller offspring in these species (Schwabl 1993). The unequal distribution of maternal resources among offspring may therefore optimize reproductive success by balancing differences in offspring competitive ability due to position in the laying sequence, egg size, and clutch size (Schwabl 1996).

Previous research on waterfowl has mainly focused on effects of maternal allocation through egg size and clutch size, in part due to the difficulty of identifying the complete laying sequence in free-living populations. For instance, in black brant (*Branta bernicla nigricans*), differential egg sizes appear to regulate development; larger eggs within a clutch had lower metabolic rates than smaller eggs within the clutch (Nicolai et al. 2004). Differential developmental rates among offspring can aid in minimizing negative effects of asynchronous hatching or aid in the synchronization of hatching (Slagsvold 1986). However, differential development among offspring presumably affects early growth and survival after hatching because there is an optimal incubation duration for maximum chick performance (Vleck et al. 1985). When a chick hatches before the optimal incubation duration is reached, an undeveloped chick may emerge; yet if a chick hatches after the optimal incubation duration is reached, fewer yolk reserves (valuable in the initial post-hatching environment) are available (Vleck et al. 1985). Hence, synchronous hatching may compromise performance of individual

offspring, and it is essential that females regulate resources allocated to eggs in conjunction with laying sequence to maximize total offspring success.

A combination of internal resources and external stimuli appear to regulate hatching synchrony. In ducks, geese, and other birds with synchronously hatching young, acoustic stimulation (such as sounds of older offspring hatching) triggers the onset of hatching (Vince 1964, 1966; Vleck et al. 1985). Accelerated hatching (and therefore accelerated embryonic development) is accomplished by an increased metabolic rate in rheas (*Rhea americana*, Vleck et al. 1985) and presumably in other birds. For instance, MacCluskie et al. (1997) found that eggs laid later in the laying sequence exhibited higher metabolic rates than earlier laid eggs in synchronously hatching broods of mallard ducks (*Anas platyrhynchos*). Therefore, metabolic rate may drive accelerated embryonic development that enables later-laid eggs to hatch with earlier laid eggs. Females may regulate metabolic rates of eggs (and consequently development) in part through egg constituents. In American coots (*Fulica americana*), maternally deposited testosterone levels in eggs varied with position in the clutch (Reed and Vleck 2001). While variation in hormone deposition could reflect environmental conditions females experience prior to egg production (Reed and Vleck 2001), the physical condition (another index of pre-natal environment) of female house wrens (*Troglodytes aedon*) at the onset of egg development was not correlated with the degree of hatching synchrony (Ellis et al. 2001). Hormone levels are known to affect metabolic rates in juvenile and adult vertebrates and yolk steroids may play a role in regulating embryonic metabolic rates and ultimately hatching times.

In this project, I quantified relationships among maternally-deposited yolk constituents, egg size, position, and developmental rate as well as the effects of duration of the egg stage on offspring survival in Canada geese (*Branta canadensis maxima*). The study focused on variation in yolk steroid levels and size across the laying sequence, the effect of size and laying position on metabolic rates during the embryo and neonate stages, and the consequence of differential embryonic development on offspring survival in a free-living, breeding population of Canada geese located in the North Dakota-Minnesota region.

Literature Cited

Arnold, T. W., F. C. Rohwer, and T. Armstrong. 1987. Egg viability, nest predation, and the adaptive significance of clutch size in prairie ducks. American Naturalist 130(5):643-653.

_____, T. W. 1993. Factors affecting egg viability and incubation time in prairie dabbling ducks. Canadian Journal of Zoology 71:1146-1152.

Badzinski, S. S., C. D. Ankeny, J. O. Leafloor, and K. F. Abraham. 2001. Composition of eggs and neonates of Canada geese and lesser snow geese. Auk 118(3):687-697.

Cooper, J. A. 1978. The history and breeding biology of the Canada geese of Marshy Point, Manitoba. Wildlife Monographs 61:1-87.

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

Ellis, L. A., J. D. Styrsky, R. C. Dobbs, and C. F. Thompson. 2001. Female condition: A predictor of hatching synchrony in the house wren? Condor 103:587-591.

Groothuis, T. G. G. and H. Schwabl. 2002. Determinants of within- and among-clutch variation in levels of maternal hormones in black-headed gull eggs. Functional Ecology 16:281-289.

Lipar, J. L., E. D. Ketterson, and V. Nolan Jr. 1999a. Intraclutch variation in testosterone content of red-winged blackbird eggs. Auk 116(1):231-235.

_____, J. L., E. D. Ketterson, V. Nolan Jr., and J. M. Casto 1999b. Egg yolk layers vary in the concentration of steroid hormones in two avian species. General and Comparative Endocrinology 115:220-227.

_____, J. L. and E. D. Ketterson. 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-wing blackbird *Agelaius phoeniceus*. Proceeding of the Royal Society of London B 267:2005-2010.

MacCluskie, M. C., P. L. Flint, and J. S. Sedinger. 1997. Variation in incubation periods and egg metabolism in mallards: Intrinsic mechanisms to promote hatch synchrony. Condor 99:224-228.

Nelson, R. J. 2000. Parental behavior: An introduction to behavioral endocrinology. Sinauer Associates, Inc., Sunderland, Massachusetts.

Nicolai, C. A., J. S. Sedinger, and M. L. Wege. 2004. Regulation of development time and hatch synchronization in black brant (*Branta bernicla nigricans*). Functional Ecology 18:475-482.

Reed, W. L. and C. Vleck. 2001. Functional significance of variation in egg-yolk androgens in the American coot. Oecologia 128:164-171.

Royle, N. J., P. F. Surai, and I. R. Hartley. 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects. Behavioral Ecology 12(4):381-385.

Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Sciences 90:11446-11450.

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

Slagsvold, T. 1986. Asynchronous versus synchronous hatching in birds: Experiments with the pied flycatcher. Journal of Animal Ecology 55:1115-1134.

Sockman, K. W. and H. Schwabl. 2000. Yolk androgens reduce offspring survival. Proceedings of the Royal Society of London B 267:1451-1456.

Vince, M. A. 1964. Potential stimulation produced by avian embryos. Animal Behaviour 14:34-40.

_____, M. A. 1966. Artificial acceleration of hatching in quail embryos. Animal Behaviour 14:389-394.

Vleck, D., C. Vleck, and D. F. Hoyt. 1985. Physiological correlates of synchronous hatching in rhea eggs (*Rhea americana*). Proc. XVIII Int. Ornithol. Cong. 18:864-872.

CHAPTER 1. ALLOCATION OF MATERNALLY DERIVED STERIODS ACROSS LAYING SEQUENCE IN CANADA GEESE

ABSTRACT

In birds, environmental and physiological signals perceived by avian females can trigger mechanisms that initiate the onset of egg laying. These signals are affected by numerous factors, including social interactions, mate quality, photoperiod, weather, and physical condition. In accordance with these signals, it is critical that females optimize investments in offspring such that fitness is maximized. A change in female hormone levels is linked to the onset of egg development and female hormones are also deposited in the yolks of developing eggs and can affect offspring development. Hence, maternal allocation of hormones in the yolk may be a mechanism by which females maximize offspring performance. In this study, maternal allocation of testosterone and estradiol was examined across the laying sequence in giant Canada geese (*Branta canadensis maxima*). Results indicate that testosterone levels decrease across the laying sequence with first laid eggs having relatively higher levels when compared to last laid eggs, yet estradiol concentrations are low and do not change across the laying sequence.

INTRODUCTION

Developmental differences among a female's offspring may exacerbate within-brood competition and reduce overall reproductive output. However, females may compensate for these differences through differential allocation of resources to their offspring. In birds with asynchronously hatching clutches, variable allocation of hormones in the yolk may be a mechanism that reduces the consequence of differences in later hatching offspring such as smaller size (Lipar et al. 1999a, Lipar and Ketterson 2000, Groothuis and Schwabl 2002). However, some species with asynchronously hatching young (cattle egrets, *Bubulcus ibis*, Schwabl et al. 1997; American coots, *Fulica americana*, Reed and Vleck 2001; zebra finch, *Taeniopygia guttata*, Gil et al. 1999, 2004) exhibit patterns in yolk androgens that are opposite of the patterns observed in other asynchronously hatching species, indicating the relationship between hormone allocations to yolk and offspring performance is complex. In birds with synchronously hatching clutches, variability in allocation of hormones may be an underlying mechanism that synchronizes hatching or compensates for developmental differences in offspring at hatching due to differences in the length of the embryonic period.

Hormone Allocation to Yolk Layers

Differential allocation of maternally-derived yolk hormones may occur among as well as within offspring as a result of physiological variation during yolk deposition. Yolk develops in the follicles through a distinct layering process (Grau 1976, Lipar et al. 1999b). Variation in maternal condition (either behavioral or physiological in nature) is typically associated with fluctuations in hormone

concentrations and thus may affect the amount of hormone deposited in yolk layers. Hormone levels are largely a product of ovarian steroidogenesis (Bahr et al. 1983), and therefore change as the follicle matures resulting in differential hormone deposition in the yolk layers. For instance there is a significant difference in progesterone, 17β-estradiol, and testosterone concentrations among yolk layers in dark-eyed juncos (*Junco hyemalis)* and red-winged blackbirds, (*Agelaius phoeniceus*, Lipar et al. 1999b). However, testosterone levels do not vary with yolk layer in American coot eggs (Reed and Vleck 2001).

Variation Among Females

Although there is significant variation in maternally-deposited yolk hormones within females, variation among females accounts for most of the differences among eggs. While differences in hormone allocation to eggs may reflect differences in female quality (Pilz et al. 2003), they may also reflect other factors such as breeding or population density (Winkler 1993, Schwabl 1996a, Reed and Vleck 2001), nesting vegetation (Groothuis and Schwabl 2002), or simply larger eggs with larger yolks capable of greater steroid binding due to relatively more lipoproteins (Pilz et al. 2003). However, such wide variation may exist in populations because selection acts more strongly on relative variation within females rather than among females.

Hormone of Interest

Previous research has identified the effects of maternal androgens, testosterone in particular, on offspring (Schwabl 1993, 1996a; Lipar and Ketterson 2000; Sockman and Schwabl 2000; Eising et al. 2001). Testosterone is a steroid

hormone derived from cholesterol (Bentley 1998). It is largely produced by testes, however small portions may also be produced in the zona reticulosa of the adrenal cortex, the placenta, and the theca and granulosa cells of ovaries. The role of androgens in female reproductive physiology remains elusive (Pilz et al. 2003, references therein), yet, a substantial amount of estrogen in females is produced through the process of androgens being converted to estrogens (Bentley 1998). Testosterone is a well known anabolic steroid that promotes cell growth, particularly muscle and bone tissue. Cell growth results from testosterone's effects on protein synthesis and development of tissues containing androgen receptors. In avian development, androgens are produced in preovulatory follicles (Romanoff and Romanoff 1949) and are hypothesized to play a role in ovulation. In waterschlager canaries *(Serinus canaria*), testosterone is produced in the theca and granulosa cell layers surrounding the ovarian follicles (Schwabl 1996a, references therein). Due to a hierarchy of follicle development typical in oviparious species, testosterone production by the ovaries changes as follicles sequester yolk (Schwabl 1996a, references therein). Because steroids are lipophilic, testosterone diffuses into the yolk lipoprotein during vitellogenesis (Schwabl 1993). Stability of testosterone during diffusion is high because it is bound to proteins (more than 90 percent of testosterone in plasma is bound, Bentley 1998). Schwabl (1993) found that the process of yolk deposition in the follicle occurs during the rapid growth phase five to nine days prior to ovulation of the follicle (depending on species). In chickens, testosterone levels in the circulating blood of females increased eight hours before ovulation, peaked approximately four to five hours before ovulation,

and resulted in an egg being laid 24 hours after ovulation. Within 15 to 75 minutes after an egg has been laid, the next follicle will already have ovulated (Shahabi et al. 1975). This quick ovulation of the next follicle may be correlated to an observed increase in yolk testosterone in the laying sequence of canaries (Schwabl 1993) and red-winged blackbirds (Lipar et al. 1999a). The increase in testosterone may be the summation of elevated follicular contributions by female testosterone. A decrease in testosterone levels may result from follicles producing less testosterone than those produced for first-laid eggs (Lipar et al. 1999b). Increased follicle production of testosterone is correlated with order of follicle maturation in canaries and red-winged blackbirds (Schwabl 1996a, Lipar et al. 1999b).

Acceleration Outcomes of Testosterone

Maternal allocation of testosterone during yolk deposition appears to enhance offspring growth and development. How hormones are deposited during yolk formation determines hormone concentrations available to the offspring at the time of yolk absorption. Embryos and offspring absorb yolk via vascular transfer or phagocytosis (See Lipar et al. 1999b), which simultaneously releases testosterone to the developing bird. Androgens have been observed at targeting neurons as early as day ten in chicken embryos and induce neurite growth in spinal cords of fetal mice (Schwabl 1993, references therein). Muscle development is strongly correlated to elevation in testosterone levels. Testosterone treatments induced anabolic effects on the musculus complexus (which aids in hatching and begging) in red-winged blackbirds and European starlings (*Sturnus vulgaris*, Lipar and Ketterson 2000, Lipar 2001); increased the muscle mass in the forelimb of leopard

frogs (*Rana pipiens*, Sidor and Blackburn 1998); and enhanced postnatal growth of both sexes in canaries (Schwabl 1996a), black-headed gulls (*Larus ridibundus*, Eising et al. 2003b); European starlings (Pilz et al. 2004), and white leghorn chickens (Eising et al. 2003a). A noticeable increase in metabolic rates of adult birds also occurred following testosterone treatments (Schwabl 1996a).

Negative Effects of Yolk Testosterone

Testosterone may not always have positive effects on offspring performance. Elevated testosterone levels delayed embryo growth and reduced survival in offspring of American kestrels (*Falco sparverius*, Sockman and Schwabl 2000). When post-hatching resources are limited, increased testosterone can increase competition and siblicide rates (Schwabl et al. 1997, Bentley 1998).

In synchronously hatching, precocial birds, there is little information on the relationship between maternal allocation of yolk hormones and offspring performance. Canada geese (*Branta canadensis maxima*) have synchronously hatching, precocial offspring that may differ in age by as much as ten days from the first to the last laid egg in the clutch. Differential allocation of resources such as testosterone and estradiol to eggs may account for the differential developmental rates that occur throughout development to result in synchronous hatching. In this study I examine patterns of variation in mass and yolk steroids across the egg laying sequence in a free-living population of Canada geese. Understanding how egg and yolk resources are distributed across to the laying sequence may enhance our understanding of the proximate mechanisms influencing offspring success and recruitment in this species and other waterfowl.

MATERIALS AND METHODS

Description of Study Site

I studied a population of Canada geese breeding in Moorhead, Minnesota on constructed ponds owned by the American Crystal Sugar Company (46° 54.139´ N, 96° 45.020´ W) during the 2004 and 2005 breeding seasons (March through May each year). The property contains 15 constructed wetlands (used primarily for cooling water from sugar beet processing operations) that are enclosed by a fence to restrict human access. The fence reduces both human disturbance and predator activity. I located approximately 260 nesting pairs of Canada geese using the wetlands during the 2004 and 2005 seasons. The Canada geese nested in a variety of cover material, including excavated gravel, construction debris, harvested hay, and natural vegetation found around the ponds.

Nest Monitoring

I located 260 nests as early as possible in the nesting stages and monitored progress through hatching or failure. Geese typically begin nesting at the end of March in this area; therefore, nest searching was initiated each season during the final two weeks of March. I conducted nest searches for the entire study site every two days, concentrating my efforts in areas where pairs exhibited territorial behavior. Intense, daily nest searching occurred in the final weeks of March in areas with many territorial pairs for nests with one to two eggs present. I located nests by observing pair behavior, identifying nest bowl depressions or formations from vegetation, and finding eggs. I then marked locations with pink vinyl flags

staked two to three meters from the nest. By locating nests with one to two eggs present, I was able to identify laying sequence for all eggs in the clutch by subsequent nest visits. Mud and soil on the feet of the adults (and from egg burying when leaving the nest) stains eggs at the site over time, and when a nest with two eggs was located, I was able to distinguish the freshly laid egg by absence of stain. If I could not distinguish the freshly laid egg because of similarity in staining, I did not use these nests for experiments requiring known laying sequence. Nests with unidentifiable laying sequence or found with more than three eggs were monitored for nest survival estimates. I labeled the blunt end of an egg with its position in the laying sequence using a permanent marker (Sharpie®). For eggs of known order, I collected freshly laid egg mass (± 1.0 g) using a Pesola spring scale, and maximum length (long axis, ± 0.1 mm) and breadth (short axis, ± 0.1 mm) using digital calipers. After laying was completed, subsequent nest visits were made every three to five days to monitor progress until hatching or failure was determined. Nests with known laying sequences were randomly assigned to groups for yolk hormone analysis or foster assignment for metabolic measurement and a brood survival study.

Hormone Analysis and Yolk Sampling

Yolks from eggs from nests assigned to the hormone analysis group were sampled to characterize allocation of maternally derived testosterone (T) and estradiol (E_2) in the yolk with the laying sequence. Yolk hormones were determined from 144 yolk samples collected from 26 clutches during the 2004 and 2005 field seasons. Yolk testosterone levels were determined from 131 samples and

estradiol levels from 60 of the 144 yolks sampled, 13 samples were excluded due to uncertainty of position or possible parasitism by other females. I sampled the yolks in conjunction with nest searching and obtained the sample within 24 hours of laying to insure that yolk hormone concentrations were from maternal allocation during yolk deposition and not a byproduct of embryo development. I collected yolk samples by first sterilizing the region of the shell midway along the long axis of the egg with 90% isopropyl alcohol, then used a jeweler's drill to drill a 0.9 mm diameter hole through the shell in the sterilized region, and then inserted a 20 gauge needle (attached to a 10 ml syringe) through the hole and drew a small (approximately 60mg) sample of yolk into the syringe. All yolk sampling occurred with insertion of syringe and needle parallel to the ground to minimize disturbance of the blastodisk region of the yolk. After obtaining the yolk sample, I removed the needle and sealed the extraction hole with Quick Gel® Super Glue (Loctite Ireland) and returned the egg to the nest. All yolk samples remained in the disposable syringes until returned to the lab, at which time they were placed in 1.5 ml Eppendorf tubes and frozen within 12 hours of collection to -20 °C until hormone extraction assays were performed.

Preparation of Stripped Yolk

Canada goose yolk stripped of hormones was used as a buffer in standard curves for the competitive binding portion of the radioimmunoassay. Using goose yolk pooled from eggs collected from the field allowed me to account for extra yolk material present in samples by direct assays and not column chromatography. The protocol for stripping hormones from yolk samples was modified from that of

Wingfield et al. (1984) for stripping plasma of steroids. I initiated preparation by placing 180 mg of pooled yolk into five, 15-ml conical tubes. Yolk samples were then dissolved in 1,000 µl of double-distilled water and vortexed until homogenous. The addition of four milliliters of a 30:70 mixture of petroleum ether and diethyl ether was added to each conical tube for hormone extraction, vortexed thoroughly, and followed by resting the samples for 20 minutes. Samples were then snap-frozen in a bath of dry-ice and ethanol and the ether aqueous phase was decanted into 13 x 100-mm test tubes leaving the frozen water phase. Extraction of yolk hormones using 30:70 mixture of petroleum ether: diethyl ether was repeated an additional three times, with decanting of ether phases between all extractions. The hormone ether mixture was then dried (nitrogen gas, 40°C water bath) followed by resuspension in 1,000 µl of 100 percent ethanol and stored at -20°C overnight. The following day, samples were centrifuged at 2,000 rpm for five minutes and I decanted ethanol into other 13 x 100-mm test tubes for drying (nitrogen gas, 40°C water bath) and resuspension in three milliliters of concentrated dextran-coated charcoal in phosphate buffer (PBSg). Samples were incubated for 30 minutes in a 40°C water bath and centrifuged for 45 minutes at 2,500 rpm. Five additional test tubes, each containing three milliliters of concentrated dextran-charcoal, were centrifuged concurrent with stripped yolk samples. I decanted the aqueous phase of samples to pellets in additional test tubes and recentrifuged for an additional 30 minutes at 2,500 rpm. I continued the transferring and centrifuging of samples to new test tubes until no charcoal was present in the tube, after which samples were pooled and stored at 4°C until needed.

Sample Setup and Extraction of Hormones

Separate radioimmunoassays were setup and run in duplicates for testosterone (T) and estradiol (E_2) following the protocol for steroid radioimmunoassay of egg yolks established by Wingfield and Farner (1975) and modified by Schwabl (1993). Samples were prepared for extraction by weighing known amounts of yolk (16.8- 33.8 mg for T, 17.3-29.4 mg for E_2) into 15-ml conical tubes and vortexing until homogenized in 1,000 µl of double-distilled water. Blanks placed in 15-ml conical tubes were the first and last samples and consisted of 1,000 µl double-distilled water. Standard conical tubes were placed in the second and second to last positions and contained 50 µl (250 pg) of cold (non-radioactive) steroids along with 950 µl of double-distilled water; and pooled yolk samples (30.0-35.0 mg) in 1,000 µl of double-distilled water located in the third, central, and third to last position of assay setup. Recovery rates were determined by spiking samples and the standard curve with 20 µl of labeled steroid (2,000 cpm). I extracted T and E_2 hormones with the addition of three milliliters of 30:70 mixture of petroleum ether: diethyl ether to samples followed by resting of samples for 20 minutes. Samples were then snap-frozen in a bath of dry-ice and ethanol and the ether aqueous phase was decanted into 13 x 100-mm test tubes. Extraction of yolk hormones using the 30:70 mixture was repeated an additional three times. I then dried the samples (nitrogen gas, 40°C water bath), reconstituted them in 1,000 µl of 100 percent ethanol, and stored them at -20°C overnight. The following day, samples were centrifuged for five minutes at 2,000 rpm and decanted into 12 x 75-mm test tubes, which were then dried (nitrogen gas, 40°C

water bath). Samples were reconstituted in 550 µl of PBSg buffer and then aliquoted in duplicates of 200 µl (Hamilton syringe) to 12 x 75-mm test tubes. The remainders (approximately 150 µl) of samples were placed in scintillation vials along with 2.5 ml of scintillation fluid (Ultima-Gold, Perkin Elmer, Boston, MA) for recovery rates of initial spiking of samples.

RESULTS

Standard Curves and Corresponding Samples

A total of five assays (maximum of 47 yolk samples per assay) were used to determine testosterone and estradiol yolk concentrations from Canada goose eggs; assays consisted of three testosterone and two estradiol. Of the 144 yolk samples collected, 131 samples were used in determining maternally derived testosterone concentrations. Within each of the three testosterone assays, the majority of estimated testosterone concentrations were detectable with the range of standards and fall within the region of greater power to detect hormonal differences (Figure 1.1). Of the 131 yolk samples used to determine testosterone concentrations, 60 samples also had estradiol concentrations measured or quantified. In both of the estradiol assays, samples fell within a region where power of detection is limiting (Figure 1.2). Some caution is needed in evaluating trends based on estradiol concentrations.

Maternal Testosterone Across Laying Sequence

A total of 75 and 69 egg yolks were sampled for maternal testosterone during the 2004 and 2005 field seasons, respectively. Sample year did not have a detectable effect on standardized testosterone concentrations (Table 1.1 and Figure 1.3), thus I combined samples from both years in further analyses. Maternally derived testosterone concentrations ranged from 0.14 to 17.38 pg/mg of yolk (mean: 3.67 ± 0.50), with inter- and intra-assay variation of 22.42 and 21.34 percent, respectively. See Appendix A for calculations.

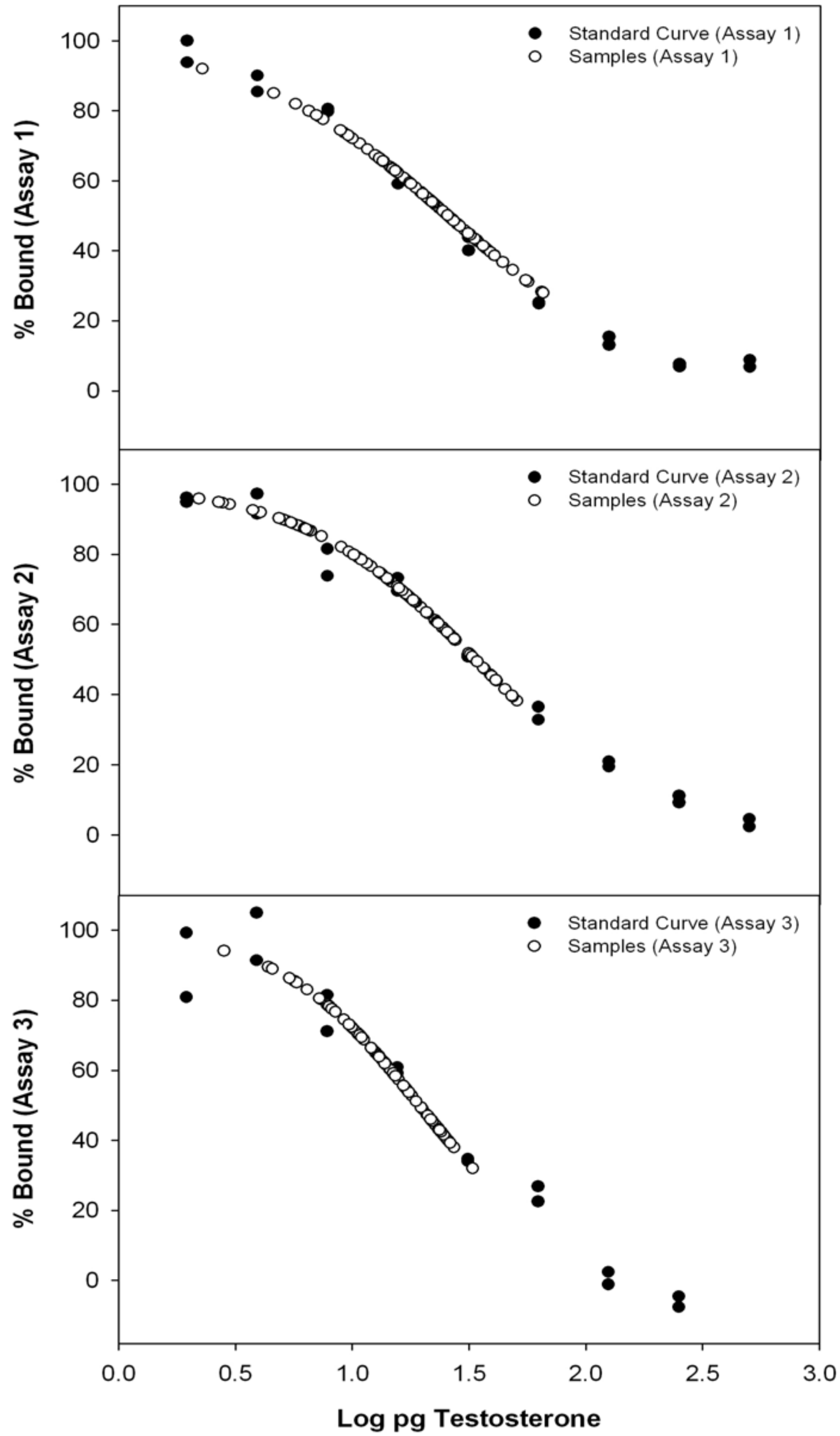


Figure 1.1. A representative standard curve for testosterone and a dilution curve of yolk. Hollow dots represent samples, and solid dots represent standard curve.

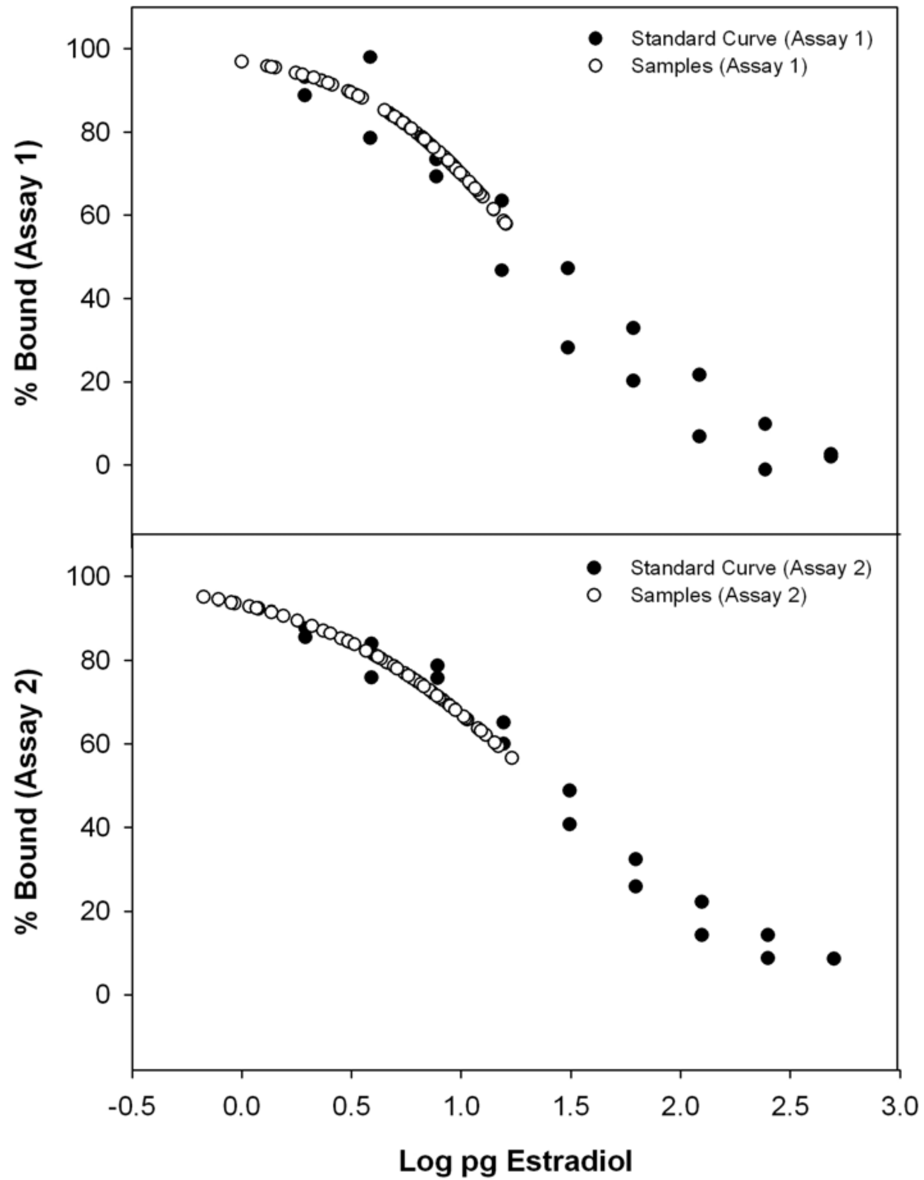


Figure 1.2. A representative standard curve for estradiol and a dilution curve of yolk. Hollow dots represent samples, and solid dots represent standard curve.

Table 1.1. Regression models describing relative hormone concentrations using position in the laying sequence as the independent variable with sample size (N, based on the number of clutches used), number of parameters (K), change in Akaike's information criterion adjusted for small sample size (Δ AIC_c), Akaike model weights (wi AIC_c), model likelihood, and coefficient of determination (R^2) in Canada geese.

Hormone	Model	N	SSE	K	AIC	AIC_c	Δ AIC_c	w_i AIC_c	Model Likelihood	R^2
Testosterone	Quadratic	26	94.04	2	39.426	40.517	0.000	0.371	1.0000	0.14342
	Linear	26	95.20	2	39.746	40.837	0.320	0.316	0.8523	0.13283
	Constant	26	109.79	1	41.451	41.973	1.456	0.179	0.4829	0.00000
	Quadratic*Year	26	94.03	3	41.423	43.328	2.811	0.091	0.2453	0.14353
	Log Normal	26	92.81	4	43.083	46.083	5.566	0.023	0.0619	0.15466
	Quadratic *Assay	26	93.69	4	43.330	46.330	5.813	0.020	0.0547	0.14659
Estradiol	Constant	12	48.00	1	20.636	21.969	0.000	0.758	1.0000	0.00000
	Linear	12	47.99	2	22.635	25.635	3.666	0.121	0.1599	0.00005
	Quadratic	12	48.00	2	22.636	25.636	3.667	0.121	0.1599	0.00000
T/E_2 ratio	Constant	12	48.00	1	20.636	21.969	0.000	0.664	1.0000	0.00000
	Linear	12	39.67	2	20.348	23.348	1.379	0.333	0.5017	0.17354
	Linear combination	12	39.54	4	24.309	34.309	12.34	0.001	0.0021	0.17625
	Log Normal	12	39.62	4	24.333	34.333	12.36	0.001	0.0021	0.17458

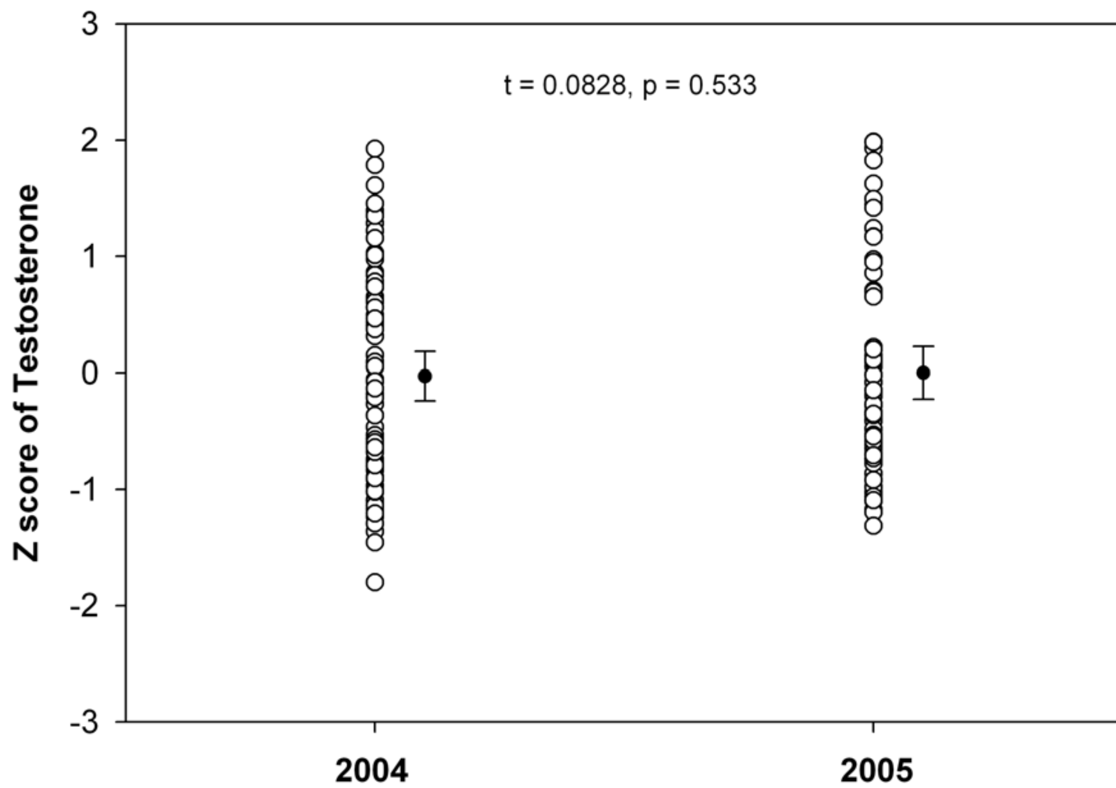


Figure 1.3. Distribution of standardized testosterone concentrations dependent on year collected (2004 and 2005) with results from a t-test comparing the means. Mean values and 95 percent confidence intervals are indicated by the filled circles and lines collected during that year.

Information criteria indicate yolk testosterone concentrations decrease across the laying sequence in this population of Canada geese. According to AIC_c, quadratic and linear models in which relative yolk testosterone concentrations were highest in eggs from positions one through three and lowest in eggs from positions five through seven (Table 1.1 and Figure 1.4) had the most support, together accounting for over 68 percent of the weight among candidate models (Table 1.1). Some support existed for a model in which concentrations were constant across the sequence, but the quadratic model was more than twice as likely (given the data) as the constant model (Table 1.1) and the linear model was more than 1.75 times as likely as the constant model. Furthermore, there was a detectable difference in relative yolk testosterone concentrations from eggs in positions one through three compared to concentrations from eggs in positions four through eight ($t = 3.67$, $p < 0.0004$, $df = 127$) (Figure 1.5).

Maternal Estradiol Across Laying Sequence

Of the 131 samples, 60 samples collected during the 2005 field season were used to determine estradiol concentrations. Absolute estradiol concentrations ranged from 0.22 to 2.78 pg/mg of yolk (mean: 1.11 ± 0.13) with an inter- and intra-assay variation of 32.09 and 28.97 percent respectively. Estradiol concentrations were not determined for samples from 2004 because the quantity of yolk sampled in the 2004 field season precluded measuring multiple hormone concentrations. Unlike relative testosterone concentrations, standardized estradiol concentrations were low and did not vary across the laying sequence.

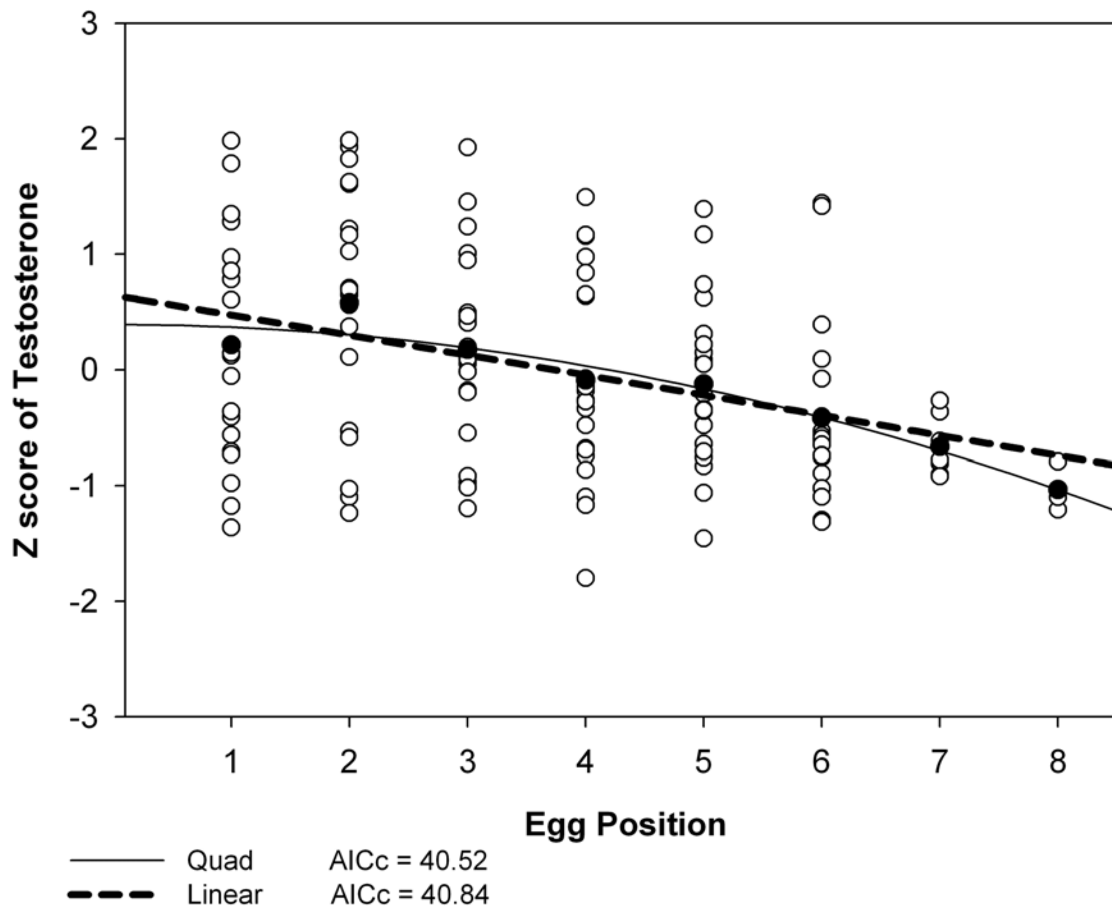


Figure 1.4. Observed relative yolk testosterone concentrations by position in eggs of Canada geese and the models with the highest support (based on AIC_c values) in the candidate set, which both show a decrease in relative testosterone concentrations for later-laid eggs.

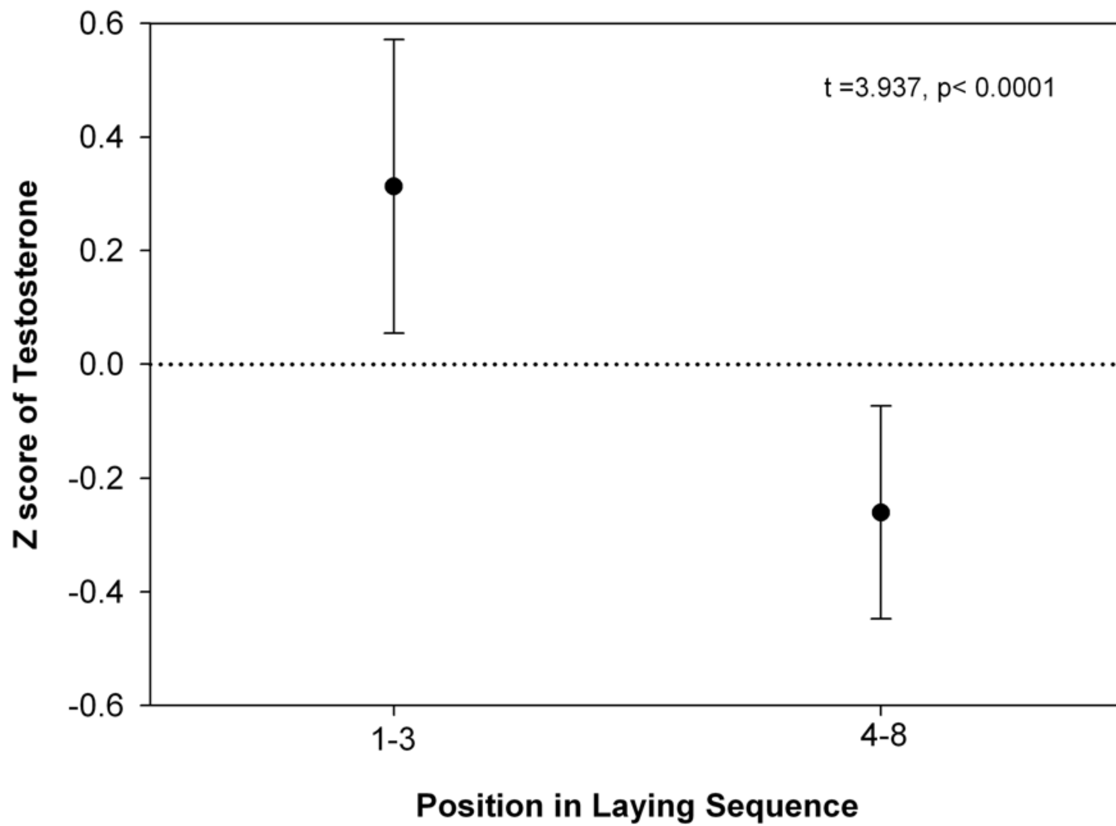


Figure 1.5. Mean and 95 percent confidence intervals for observed relative testosterone concentrations in early (positions 1-3) and later (positions 4-8) laid eggs in Canada geese along with results from a t-test comparing the means. The dashed line indicates the mean across all positions.

Information criteria indicated that the best model describing relative yolk estradiol concentrations was a simple constant for all positions (Table 1.1 and Figure 1.6). The constant relative concentration model had over 75 percent of the weight in the candidate model suite (Table 1.1) and was more than six times as likely (given the data) than any other model compared (Table 1.1). Furthermore, the best fitting linear model had a slope very close to zero (-0.0035), indicating little effect due to position (Figure 1.6). A similar result holds for the quadratic model.

T/E_2 Ratio Across Laying Sequence

Analysis of models of the relative ratio of yolk testosterone to yolk estradiol concentrations indicated marginal evidence for a decrease in T/E_2 concentrations in the laying sequence. A model with constant T/E_2 ratio for all positions had the most support (Table 1.1), although there was limited evidence for a linear model in which T/E_2 ratio declined with position in the laying sequence (Table 1.1 and Figure 1.7). The small sample size may limit power in support of models with more parameters.

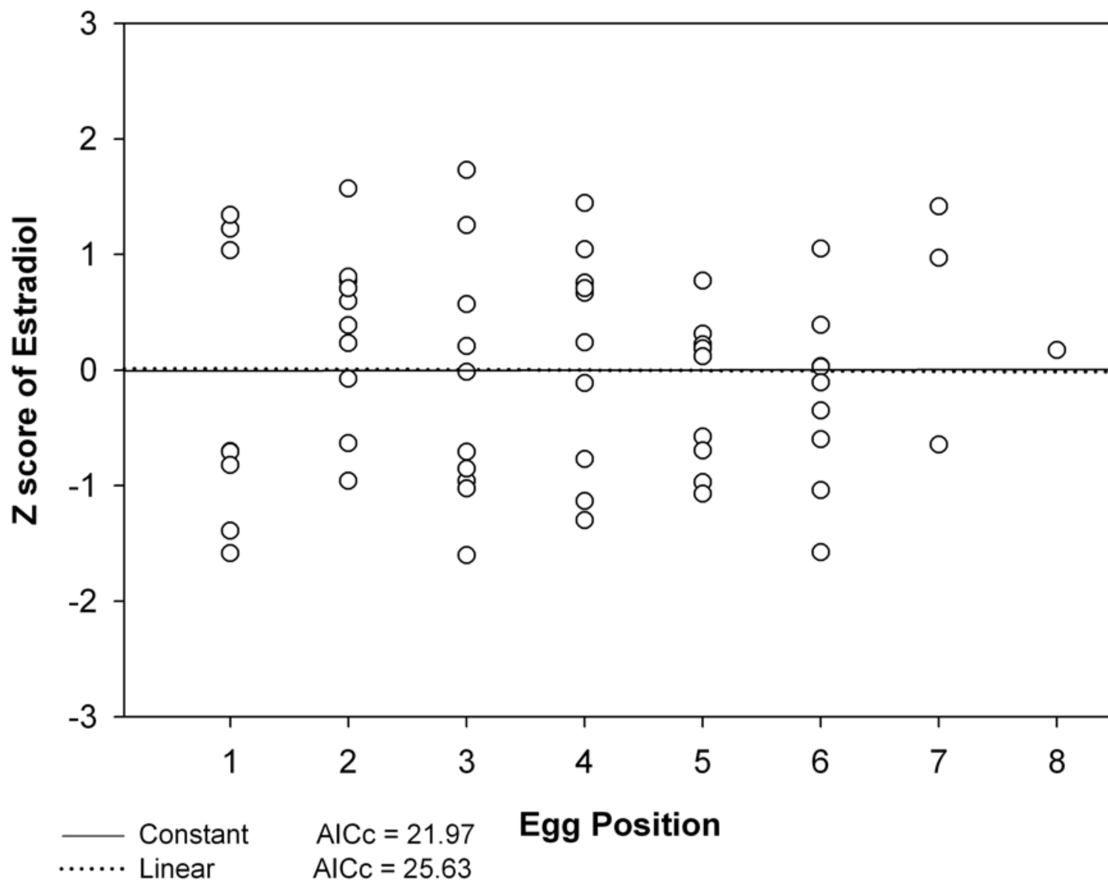


Figure 1.6. Observed relative yolk estradiol concentrations by position in eggs of Canada geese and the models with the highest support (based on AIC_c values) in the candidate set, which show no change (Constant model) or a small decrease (Linear model) in relative estradiol concentrations for later-laid eggs.

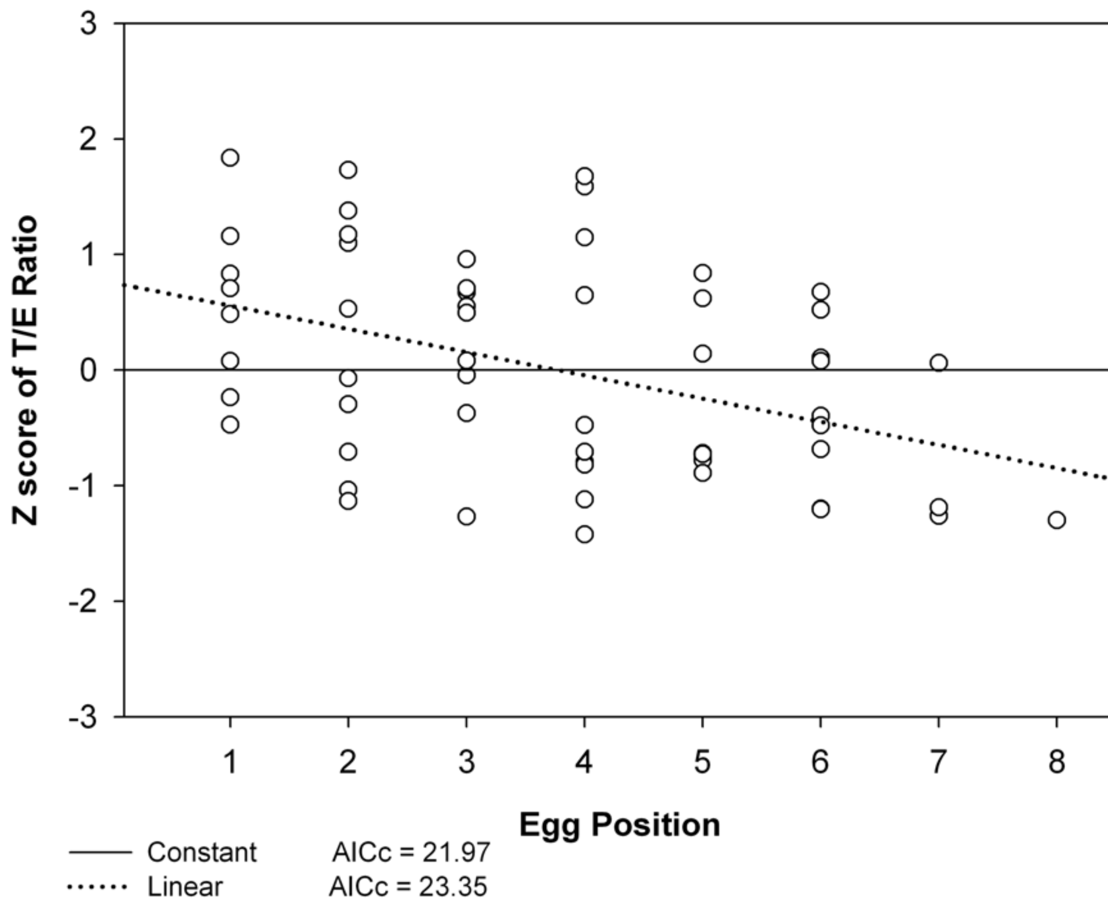


Figure 1.7. Observed relative yolk testosterone:estradiol ratio by position in eggs of Canada geese and the models with the highest support (based on AIC_c values) in the candidate set, which show no change (Constant model) or a decrease (Linear model) in relative testosterone:estradiol ratio for later-laid eggs.

DISCUSSION

In Canada geese, yolk testosterone levels decreased across the laying sequence. Maternally derived yolk testosterone concentrations decreased through the laying sequence, with eggs in positions one through three having the highest concentrations followed by a decline with each additional egg laid (Figures 1.4 and 1.5). Yolk testosterone concentrations have not been reported previously for Canada geese. Yolk testosterone levels have been observed to both increase (canaries, Schwabl 1993; dark-eyed juncos, Lipar et al. 1999b; American kestrels, Sockman and Schwabl 2000; red-winged blackbirds, Lipar and Ketterson 2000, Lipar et al. 1999a; and black-headed gulls, Eising et al. 2001, Royle et al. 2001, Groothuis and Schwabl 2002) and decrease (cattle egrets, Schwabl et al. 1997; American coots, Reed and Vleck 2001; and zebra finch, Gil et al. 1999, 2004) across the laying sequence in birds. If yolk testosterone levels reflect circulating levels in females, my results would indicate that testosterone levels in females are decreasing throughout the laying period, but they may also indicate that females allocate less testosterone to their later-laid eggs.

Social interactions, photoperiod, population densities, food availability, and weather can influence fluctuations in female hormone levels (Schwabl 1996a, Hirschenhauser et al. 1999, Reed and Vleck 2001, Groothuis and Schwabl 2002, Whittingham and Schwabl 2002, Mazuc et al. 2003, Verboven et al. 2003, Michl et al. 2004, Groothuis et al. 2005). Annual fluctuations in plasma hormones are evident in many avian species (Hirschenhauser et al. 1999). Cyclic and episodic release of hormones may occur annually, daily, and even hourly (Bentley 1998).

Circulating testosterone in females is particularly influenced by the time of year and is often regulated by photoperiod (Schwabl 1996b, Bentley 1998). Circulating levels of hormones can have varied and far-reaching effects on behavior, physiology, and morphology of individuals. In migratory species, testosterone is linked to premigration activities, migration, and breeding activities (Bentley 1998).

During the breeding season, circulating hormones affect territorial defense, initiation of follicle development, onset of incubation, and levels of parental care in avian females (Johnson and v. Tienhoven 1980, Winkler 1993, Bentley 1998, Sockman and Schwabl 1999, Groothuis and Schwabl 2002, Eising et al. 2003a, Williams et al. 2004). Moreover, hormone levels present in breeding females may be passed directly to offspring. For instance, yolk hormone levels are correlated with circulating plasma estradiol and androgen levels in female European starlings during follicle development (Williams et al. 2004). Thus, maternal yolk hormone concentrations appear to result from the physiological and environmental stimuli affecting females during the formation of egg constituents.

Interestingly, the pattern I observed in Canada geese is similar to the pattern observed in another wetland bird that lays large clutches, the American coot (Reed and Vleck 2001), although this species hatches asynchronously. Almost no previous information on yolk testosterone concentrations is available for other birds with moderate to large clutches (six or more eggs) that hatch synchronously, and none for other anseriform species. What consequence the lower allocation of testosterone to later-laid eggs has for embryonic development and offspring performance is not known.

In contrast with testosterone levels, I found that maternally derived yolk estradiol concentrations were low and did not exhibit detectable changes with position of egg-laying (although there was low power in the analysis) (Figure 1.5). The absolute estradiol concentrations I observed in this population (0.22 - 2.78 pg E_2 / mg yolk) are lower than circulating concentrations reported in a similar species, the greylag goose (*Anser anser*, Hirschenhauser et al. 1999). Female estradiol levels typically increase at the onset of ovulation then rapidly decrease (Hirschenhauser et al. 1999, Sockman and Schwabl 1999, Williams et al. 2004), which has been used to explain low yolk estradiol concentrations observed in species such as European starlings and canaries. Results found here for Canada geese are consistent with this hypothesis.

Patterns in the relative T/E_2 ratio within the clutch were driven by patterns in yolk testosterone, but are occluded by low power. Because yolk testosterone concentrations decreased while yolk estradiol concentrations remained constant across the laying sequence, the ratio of T/E_2 declined, although there was also support for a model with constant T/E_2 ratio (Table 1.1 and Figure 1.7).

My results suggest that allocation of pre-hatching resources varies among a female's offspring in Canada geese. In particular, more testosterone is deposited in the yolks of the first few eggs laid in the clutch, but similar levels of estradiol are deposited in the yolks of all eggs. Canada geese lay a moderate number of eggs that hatch at the same time despite a noticeable difference in the age of the embryos, similar to other waterfowl species. Incubation typically begins after the third egg is laid, the point at which yolk testosterone levels are also observed to

decline (Figure 1.4). More work is needed to determine if yolk testosterone concentrations facilitate alternative developmental rates such that hatching is synchronized in Canada geese and other waterfowl species.

LITERATURE CITED

Bahr, J. M., S-C. Wang, M. Y. Huang, and F. O. Calvo. 1983. Steroid concentrations in isolated theca and granulose layers of preovulatory follicles during the ovulatory cycle of the domestic hen. Biology of Reproduction 29:26-334.

Bentley, P. J. 1998. Comparative Vertebrate Endocrinology. Cambridge University Press, United Kingdom

Burnham, K. P. and D. R. Anderson. 2002. Model selection and multi-model inference: a practical information-theoretic approach. Second edition. Springer Verlag, New York.

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

_____, C. M., W. Müller, C. Dijkstra, and T. G. G. Groothuis. 2003a. Maternal androgens in egg yolks: relation with sex, incubation time and embryonic growth. General and Comparative Endocrinology 132:241-247.

_____, C. M., G. H. Visser, W. Müller, and T. G. G. Groothuis. 2003b. Steroids for free? No metabolic costs of elevated maternal androgen levels in the black-headed gull. Journal of Experimental Biology 206:3211-3218.

Gil, D., J. A. Graves, N. Hazon, and A. Wells. 1999. Male attractiveness and differential testosterone investment in zebra finch eggs. Science 286:126-128.

_____, D., C. Heim, E. Bulmer, M. Rocha, M. Puerta, and M. Naguib. 2004. Negative effects of early development stress on yolk testosterone levels in a passerine bird. Journal of Experimental Biology 207:2215-2220.

Grau, C. R. 1976. Ring structure of avian egg yolk. Poultry Science 55:1418-1422.

Groothuis, T. G. G. and H. Schwabl. 2002. Determinants of within- and among-clutch variation in levels of maternal hormones in Black-headed Gull eggs. Functional Ecology 16:281-289.

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

Hirschenhauser, K., E. Möstl, and K. Kotrschal. 1999. Seasonal patterns of sex steroids determined from feces of different social categories of greylag geese, *Anser anser*. General Comparative Endocrinology 114:67-79.

Johnson, A. L. and A. van Tienhoven. 1980. Plasma concentrations of six steroids and LH during the ovulatory cycle of the hen, *Gallus domesticus*. Biology of Reproduction 23:386-393.

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

_____, J. L. and E. D. Ketterson. 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-wing blackbird *Agelaius phoeniceus*. Proceedings of the Royal Society of London B 267:2005-2010.

_____, J. L., E. D. Ketterson, V. Nolan Jr. 1999a. Intraclutch variation in testosterone content of red-winged blackbird eggs. Auk 116(1):231-235.

_____, J. L., E. D. Ketterson, V. Nolan Jr., and J. M. Casto. 1999b. Egg yolk layers vary in the concentration of steroid hormones in two avian species. General and Comparative Endocrinology 115:220-227.

Mazuc, J., C. Bonneaud, O. Chastel, and G. Sorci. 2003. Social environment affects female and egg testosterone levels in the house sparrow (*Passer domesticus*). Ecology Letters 6:1084-1090.

Michl, G., J. Török, P. Péczely, L. Z. Garamszegi, and H. Schwabl. 2004. Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. Behavioral Ecology 16(2):383-388.

Pilz, K. M., H. G. Smith, M. I. Sandell, and H. Schwabl. 2003. Interfemale variation in egg yolk androgen allocation in the European starling: Do high-quality females invest more? Animal Behaviour 65:841-850.

_____, K. M., M. Quiroga, H. Schwabl, and E. Adkins-Regan. 2004. European starling chicks benefit from high yolk testosterone levels during a drought year. Hormones and Behavior 46:179-192.

Reed, W. L. and C. Vleck. 2001. Functional significance of variation in egg-yolk androgens in the American coot. Oecologia 128:164-171.

Romanoff, A. L. and A. J. Romanoff. 1949. The Avian Egg. Academic Press. New York.

Royle, N. J., P. F. Surai, and I. R. Hartley. 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects. Behavioral Ecology 12(4):381-385.

Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Science 90:11446-11450.

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

_____, H. 1996b. Maternal testosterone in the avian egg enhances postnatal growth. Comparative Biochemistry and Physiology 114A(3):271-276.

_____, H., D. W. Mock, and J. A. Gieg. 1997. A hormonal mechanism for parental favouritism. Nature 386:231.

Shahabi, N.A., H. W. Norton, and A. V. Nalbandov. 1975. Steroid levels in follicles and the plasma of hens during the ovulatory cycle. Endocrinology 96:962-968.

Sidor, C. A. and D.G. Blackburn. 1998. Effects of testosterone administration and castration on the forelimb musculature of male leopard frog (*Rana pipiens*). Journal of Experimental Zoology 280:28-37.

Sockman, K. W. and H. Schwabl. 1999. Daily estradiol and progesterone levels relative to laying and onset of Incubation in canaries. General and Comparative Endocrinology 114:257-268.

_____, K. W., and H. Schwabl. 2000. Yolk androgens reduce offspring survival. Proceedings of the Royal Society of London B 267:1451-1456.

Verboven, N., P. Monaghan, D. M. Evans, H. Schwabl, N. Evans, C. Whitelaw, and R. G. Nager. 2003. Maternal condition, yolk androgens and offspring performance: a supplemental feeding experiment in the lesser black-backed gull (*Larus fuscus*). Proceedings of the Royal Society of London B. 270:2223-2232.

Whittingham, L. A. and H. Schwabl. 2002. Maternal testosterone in tree swallow eggs varies with female aggression. Animal Behaviour 63:63-67.

Williams, T. D., A. S. Kitaysky, and F. Vézina. 2004. Individual variation in plasma estradiol-17β and androgen levels during egg formation in the European starling *Sturnus vulgaris*: implications for regulation of yolk steroids. General and Comparative Endocrinology 136:346-352.

Wingfield, J. C. and D. S. Farner. 1975. The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. Steroids 26:311-327.

_____, J. C., K. S. Matt, and D. S. Farner. 1984. Physiologic properties of steroid hormone-binding proteins in avian blood. General and Comparative Endocrinology 53:281-292.

Winkler, D. W. 1993. Testosterone in egg yolks: An ornithologist's perspective. Proceedings of the National Academy of Science USA 90:11439-11441.

CHAPTER 2. EMBRYONIC METABOLIC RATES OF GOSLINGS IN RELATION TO EGG LAYING SEQUENCE OF CANADA GEESE

ABSTRACT

Waterfowl species lay large clutches of eggs over many days, yet the offspring hatch synchronously which indicates mechanisms regulating embryonic development minimize developmental differences among offspring. In this study, I examined relationships among egg size, position in the laying sequence, embryonic oxygen consumption rates, gosling oxygen consumption rates, gosling body composition, and hematocrit levels in Canada geese (*Branta canadensis maxima*) to assess the regulatory role of embryonic metabolism in hatching synchrony. Results from this study indicate that embryonic oxygen consumption first increases exponentially with age then plateau to a constant rate approximately 85 percent through the 28-day incubation period, which is consistent with patterns found in other precocial birds. Moreover, laying position best explained the observed patterns in embryonic metabolic rates, with embryos from eggs laid later in the laying sequence having higher metabolic rates than those from eggs laid earlier in the sequence. Upon hatching, gosling metabolic rates and body composition were not related to position or embryonic metabolic rates, however gosling hematocrit levels were higher in goslings hatched from eggs laid later in the sequence. Based on my results, embryonic metabolic rates appear to facilitate within-clutch hatching synchrony of goslings, as embryonic developmental rates are higher in eggs laid later in the sequence. Greater oxygen carrying capacity may be the mechanism by which higher metabolic rates are sustained in later laid offspring, but there does not appear to be a post-hatching affect on condition.

INTRODUCTION

External development of avian embryos has both advantages and disadvantages. While separation from developing embryos may be advantageous for adult survival, it may be disadvantageous for maintaining a consistent environment for the developing embryos. The external environment can affect embryonic development. Hence, conditions and mechanisms regulating embryonic development are critical to understanding avian reproductive success, especially in species in which all embryonic development must be completed within a relatively narrow time frame (such as species with synchronously hatching offspring).

While the egg shell provides some consistency between the external environment and the embryo, it also limits gas transfer between the embryo and the environment. Gas transfer across the shell is passive, with water vapor conductance across the shell regulated by partial pressure gradients described by Fick's Law (Ar and Rahn 1985, Rahn et al. 1987) and affected by environmental conditions such as nest humidity and temperature. However, egg porosity (determined by the number, size, and length of pores) ultimately determines the rate of vapor conductance between the embryo and the environment (Rahn and Paganelli 1990, Booth and Sotherland 1991).

Contributions of Cylindrical Egg Pores to Gas Exchange

Cylindrical egg pores provide a direct corridor of gas exchange between the embryo and the external environment (Tyler 1969, Hoyt et al. 1979). Pores have a simple morphology, essentially straight cylindrical tubes with dimensions that vary

with egg size and shell thickness (Hoyt et al. 1979). Shell thickness appears to ultimately determine gas transfer, with rates of vapor conductance inversely proportional to shell thickness (i.e., pore length) in most species, including anseriform species (Ar and Rahn 1985), but an ultimate threshold in shell thickness may limit effective diffusion because, in larger species (e.g., rhea, *Rhea americana,* and ostrich, *Struthio camelus*), more complex pore morphology is observed (Rahn et al. 1979).

Oxygen Conductance and Availability

Oxygen uptake occurs by diffusion in the avian embryo and is equivalent to metabolic consumption. Although other membranes surround the embryo, diffusion across the shell remains the limiting factor in gas transfer (especially oxygen) between the external environment and the embryo. External material blocking pores (which may provide filtering of microorganisms detrimental to the embryo, see Rahn and Paganelli 1990) may also contribute to limiting air movement. Although the inner and outer shell membranes are located between the shell and embryo, they are infused with air-filled fibers (Rahn et al. 1974, Paganelli et al. 1978) that are not limiting to oxygen diffusion. Oxygen uptake, even if diffusion limited, is equivalent to the rate of oxygen consumption of the developing embryo (Vleck et al. 1979, 1980a), and as the developmental rate increases, consumption rate also increases, therefore driving a greater pressure gradient between the air cell and the surrounding environment (Vleck et al. 1979). In species with altricial young, the rates of embryonic oxygen consumption do not exceed maximum diffusion rates possible across the shell prior to internal pipping

(breaking the inner shell membrane), however, in species with precocial young, embryonic oxygen consumption rates are limited by diffusion rates in the final days of development (Rahn et al. 1974, Hoyt et al. 1979, Vleck et al. 1979).

Influence of Developmental Strategies on Consumption Rates

Oxygen consumption and gas exchange are driven by embryonic physiology. Three primary physiological activities (generation of new tissue, maintenance and regeneration of existing tissue, and an increase in muscular activity at hatching) occur during embryonic development that, in combination, result in increasing gas exchange and diminishing yolk reserves over the course of incubation.

The rate of oxygen consumption is related to body size and the degree of offspring development at hatching. Throughout development, embryonic metabolic rates exhibit a negative allometric relationship to embryo mass (Vleck et al. 1979, 1980a). Oxygen consumption rates are also correlated to developmental patterns present at hatching (Vleck et al. 1979, 1980a, 1980b). Nice (1962) classified the pattern of hatchling development as a gradient of altricial to precocial offspring. Altricial offspring hatch naked, with eyes closed and are incapable of thermoregulation and locomotion. Precocial offspring hatch covered with down, with eyes open, and are capable of limited thermoregulation and locomotion. Waterfowl, including Canada geese (*Branta canadensis maxima*), are considered precocial species. In altricial offspring, embryonic metabolic rates increase exponentially throughout incubation (Vleck et al. 1979, 1980a). Precocial offspring also exhibit an exponential increase in oxygen consumption rates for

approximately 80 percent of the initial incubation period, but after which oxygen consumption rates plateau to an approximate constant level until internal pipping occurs (Rahn et al. 1974; Hoyt et al. 1978; Vleck et al. 1979,1980b). Species with offspring between the altricial and precocial endpoints (e.g., semi-altricial) have oxygen consumption rates that are likewise intermediate (Hamilton 1985). It is also notable that precocial offspring reach their hatchling mass approximately 80 percent of the way through incubation (Vleck et al. 1980b).

The plateau in embryo oxygen consumption rates provides cues for hatching in precocial species. The final period of incubation (when metabolic rates plateau) is marked by a shift in energy allocation to tissue maintenance, maturation of nervous and developmental systems, and an increase in synthesis efficiency (Vleck et al. 1980a, Dietz et al. 1998). During this period, air cell gas tensions reach the maximum threshold of vapor conductance (approximately 104 torr for oxygen and 37 torr for carbon dioxide) of avian egg shells (Rahn et al. 1974, Hoyt et al. 1979). Oxygen pressures below 104 torr induces hypoxia, while carbon dioxide pressures above 37 torr results in acidosis; together hypoxia and acidosis appear to trigger the onset of hatching (Rahn et al. 1974, Vleck et al. 1980a). Altering these pressure gradients may be a mechanism by which the duration of the incubation is reduced to facilitate synchronous hatching of later laid eggs in precocial species.

Implications of Egg Consumption Rates on Hatching Synchrony

In this study, I examined relationships among egg size, position in the laying sequence, embryonic oxygen consumption rates, gosling oxygen

consumption rates, gosling body composition, and hematocrit levels in Canada geese to assess the regulatory role of embryonic metabolism in hatching synchrony. Canada geese lay approximately six eggs in a clutch (Cooper 1978), with each egg laid approximately every 36 hours. Goslings hatch within a 24-hour period, typical for synchronously hatching waterfowl, even though the age difference between the first and the last laid egg in a clutch can be as great as ten days (Cooper 1978). Understanding how embryo metabolic rates vary across the laying sequence may provide insight into intrinsic mechanisms (e.g., relative embryo size) regulating hatching synchrony compared to extrinsic mechanisms (e.g., female incubation behavior). Understanding the mechanisms underlying hatching synchrony is necessary to understand recruitment because females depart the nest site to brood-rearing areas shortly after goslings hatch and factors that delay embryonic development could reduce reproductive success because later laid eggs may be abandoned prior to hatching.

MATERIALS AND METHODS

I measured embryo metabolic rates from eggs collected from a population of Canada geese breeding in Moorhead, Minnesota (46° 54.139´ N, 96° 45.020´ W) during the 2004 and 2005 breeding seasons. The breeding population nests on artificially constructed wetlands at the American Crystal Sugar Plant in Clay County, Minnesota and is approximately 17 km from laboratories at North Dakota State University.

Nest Monitoring and Metabolic Measuring

I searched for and located nests at onset of breeding (approximately mid-March) and subsequently monitored nests located at the start of laying such that the position of all eggs in the clutch was known. Nest searching and monitoring is fully described in Chapter 1. Nests for which the complete laying sequence could not be determined were not used to measure embryo metabolic rates.

I measured embryo metabolic rates every two to three days across the incubation period from eggs incubated artificially in the laboratory, as well as from eggs incubated naturally by geese in the field. To obtain embryonic metabolic measurements under standardized incubation conditions, I brought whole clutches of eggs into the lab two days following the end of laying and placed them in an incubator (GQF Manufacturing Co., Model 1527) held at approximately 37°C and 60 percent relative humidity with auto egg rotation on the long axis every three hours. I also obtained embryonic metabolic measurements from clutches incubated naturally in the field. Metabolic rates were measured approximately 10 to 14 days after the first egg of the clutch was laid and were measured

subsequently every two days in the lab and every three days in the field. Egg viability was checked regularly via candling and unfertilized eggs, or those containing dead embryos, were excluded from further metabolic measurements and statistical analyses were conservatively limited to those at least two periods prior to the last known date of viability. Similarly, I excluded eggs when there was evidence of internal pipping (e.g., vocalizations).

Collection of Oxygen Samples

I measured metabolic rates using closed system respirometry modified from Vleck (1987). The respirometer chamber consisted of airtight containers (1.1 L Rubbermaid®) fitted with two tubes (0.3 cm I.D.) serving as incurrent and excurrent pathways for gas flow. Two-way polyethylene stopcocks were attached to the tubing to control air transfer between the chamber and the environment. I measured embryonic oxygen consumption over time for all eggs in a clutch simultaneously by removing eggs from the incubator (or from a nest in the field) and immediately sealing them into individual respirometry chambers. In the field, false eggs were placed in the nest while oxygen consumption was measured to prevent female abandonment of the nest. Chambers were submerged in a water bath (stabilized at 37°C) fashioned from a circulating heater and large cooler for portability to the field. Submerged chambers were continuously flushed with air at 100 percent relative humidity until internal chamber temperatures reached equilibrium with the water bath (approximately 15 minutes later). Internal chamber temperatures were monitored with digital meters attached to copper thermocouples secured through lids. After the chambers stabilized to the

temperature of the bath, I collected initial air samples in 60-cc disposable syringes fitted with two-way polyethylene stopcocks that were attached to the excurrent tubes. I then closed the stopcocks attached to the incurrent tubes on the chambers, restricting inflow of saturated air, and finally closed the excurrent tubes (approximately 30 seconds later, which allowed all chambers to equilibrate internal air pressure prior to sealing) and recorded the time. Eggs remained sealed in the chambers for five to 20 minutes, time duration dependent on the developmental stage of the embryo, after which a final air sample was extracted from the chambers using additional 60-cc disposable syringes fitted with two-way polyethylene stopcocks. Chambers were opened following the collection of the final air sample and eggs were immediately returned to the incubator or to their nest. Air samples in the syringes were maintained under pressure to minimize atmospheric oxygen uptake until the samples were analyzed (which occurred within ten minutes from collection in the lab and within four hours from collection in the field).

Determining Oxygen Concentrations

Oxygen concentrations were determined using a FC-1B O_2 Analyzer (Sable Systems, Las Vegas, NV). Initial and final air samples were dispensed through the FC-1B Analyzer at a constant rate of 25.5 ml/min via a syringe pump (New Era Pump Systems INC.) Water vapor and carbon dioxide were removed from the air samples by passing the air stream through disposable 5-cc syringes packed with Drierite (upstream) and Ascarite (downstream) prior to entering the analyzer. Analyzer readouts were graphed and oxygen content determined based on

asymptotic values. Oxygen consumption rate (ml O_2/hr) was calculated from the difference in oxygen content between initial (Fi) and final (Fe) air samples divided by change in time (Δt, hours), and corrected to standard pressure (760 torr) and temperature (273 K) following Vleck (1987):

$$\dot{V}O_2 = \frac{\left\{1135.56 - \left(M_e / 1.11\right)\right\} * \left(Fi - Fe / 1 - Fe\right) * \left(273K / T_c\right) * \left(P_{atm} / 760torr\right)}{\Delta t}$$

where 1135.56 ml is the volume of the metabolic chamber, 1.11 is egg density (used to convert egg mass, M_e to egg volume, in ml), T_c is the average temperature of respirometry chamber, and P_{atm} is the observed atmospheric pressure.

Gosling Metabolic Rates

In 2005, I measured gosling metabolic rates within 24 hours of hatching using flow through respiratory. The chamber consisted of a sealed plastic container (2016 ml) with fittings for inflow and outflow of air through tubing that was darkened by covering with a box. Flow of air into the chamber was regulated by a flowmeter (Sable systems Mass flow Controller V1.1) at 250 ml/min and was saturated with water vapor by bubbling through a water chamber prior to entering the chamber. Outflow air was subsampled from a manifold (inverted syringe) on the outflow tubing using a subsampler (Sable systems Gas Analyzer Sub-sampler V2.0) at rates from 60-105 ml/min (typical individual average of 83 ml/min) before carbon dioxide and water vapor (Ascarite and Drierite, respectively) were removed

prior to entering the FC-1B O_2 analyzer (Sable Systems, Inc.) to determine oxygen content. Inflow air was subsampled and passed through the analyzer at the start and end of the measurement period to determine oxygen content of inflow air. The measurement period lasted approximately ten minutes.

After gosling oxygen consumption rates were measured, body size measurements and body composition samples were obtained. Gosling body mass was obtained at hatching. Following metabolic measurements, gosling mass was recorded again, then goslings were euthanized, blood samples were obtained, and sex determined by dissection. Blood samples were centrifuged (7,000 rpm; six minutes) and I measured hematocrit levels from the height of erythrocytes to total blood sample height in capillary tubes. I then dissected gosling carcasses to separate the yolk sack from the carcass. After dissection, yolk sacks and yolk-free carcasses were dried (100° C) for one week. After recording masses for the yolks and carcasses, I dried the samples again (100° C) for an additional week and recorded masses at the end of the second week.

Statistical Analysis

I used a combination of regression analyses, student's t-tests, and chi-square tests to analyze embryo and gosling measurements. I modeled embryonic oxygen consumption rates as a function of age and a number of other variables (e.g., initial egg mass, egg position) along with a random effect of clutch (i.e., female) because eggs within a clutch are not independent. I based embryo age on the date the third egg was laid because waterfowl generally initiate incubation at this point (Loos and Rohwer 2004). Metabolic rates of precocial embryos are

known to follow an S-shaped pattern with age (Vleck et al. 1979) and I used a generalized curve developed by Janoschek (1957) (referenced in Gille et al. 1999). This curve is determined by four parameters: A_0, the initial value (or lower asymptote); A_f, the upper asymptote; K, a parameter affecting the slope and inflection point; and p, another parameter that affects both slope and inflection point. I compared models for embryonic oxygen consumption using Akaike information criterion adjusted for small sample size (AIC_c) for least squares regression (Burham and Anderson 2002). AIC_c ranks models based on parsimony by simultaneously accounting for explained variance as well as uncertainty (i.e., variance) in model parameter estimates, and models with the lowest AIC_c value represent the most parsimonious model in the suite of models compared. Model goodness-of-fit was assessed using the coefficient of determination (R^2). I also developed linear regression models to analyze relationships among gosling metabolic rates, hematocrit levels, sex, body size, and yolk size. Both absolute and within-clutch relative (based on the z-score of the variable using mean and standard deviations from a clutch) values were used in these regression models, and AIC_c was again used to compare models. I also used a t-test to compare gosling oxygen consumption with gosling sex. Finally, I used a chi-square test to compare primary sex ratios of goslings.

RESULTS

I obtained metabolic measurements and other data from 41 complete clutches (11 from 2004 and 30 from 2005). I collected the eggs of 21 complete clutches for incubation in the lab and used eggs from 20 naturally incubated complete clutches for field measurements of oxygen consumption. Of the 189 eggs from the 41 complete clutches, 141 eggs were used for further analysis; 48 were infertile, had embryos that died shortly after collection, or lacked fresh mass measurements.

Position in the laying sequence best explained patterns of embryonic oxygen consumption over the incubation period. Embryonic metabolic rates increased exponentially for approximately 85 percent of the incubation period after which they reached a plateau of approximately 66 ml O_2 / h (Figure 2.1). Models incorporating a position effect were the most parsimonious according to AIC_c (Table 2.1). Models incorporating a linear effect of position on the growth curve slope/inflection parameter, p (i.e., $p = p_0 + p_1 \bullet \text{Position}$), accounted for over 98 percent of the evidence given the data (from sum of AIC_c weights, Table 2.1). In these models, slope of the oxygen consumption rate curve increases for embryos from eggs laid later in the clutch such that embryos from later laid eggs have higher metabolic rates at a given age (Figure 2.1). There was weak support (approximately 1.4 percent of the evidence given the data, Table 2.1) for a model in which position had a linear effect only on the upper asymptote (i.e., $A_f = A_{f0} + A_{f1} \bullet \text{Position}$), such that embryos from later laid eggs have a higher metabolic plateau, but otherwise rates are similar to those of embryos from earlier laid eggs.

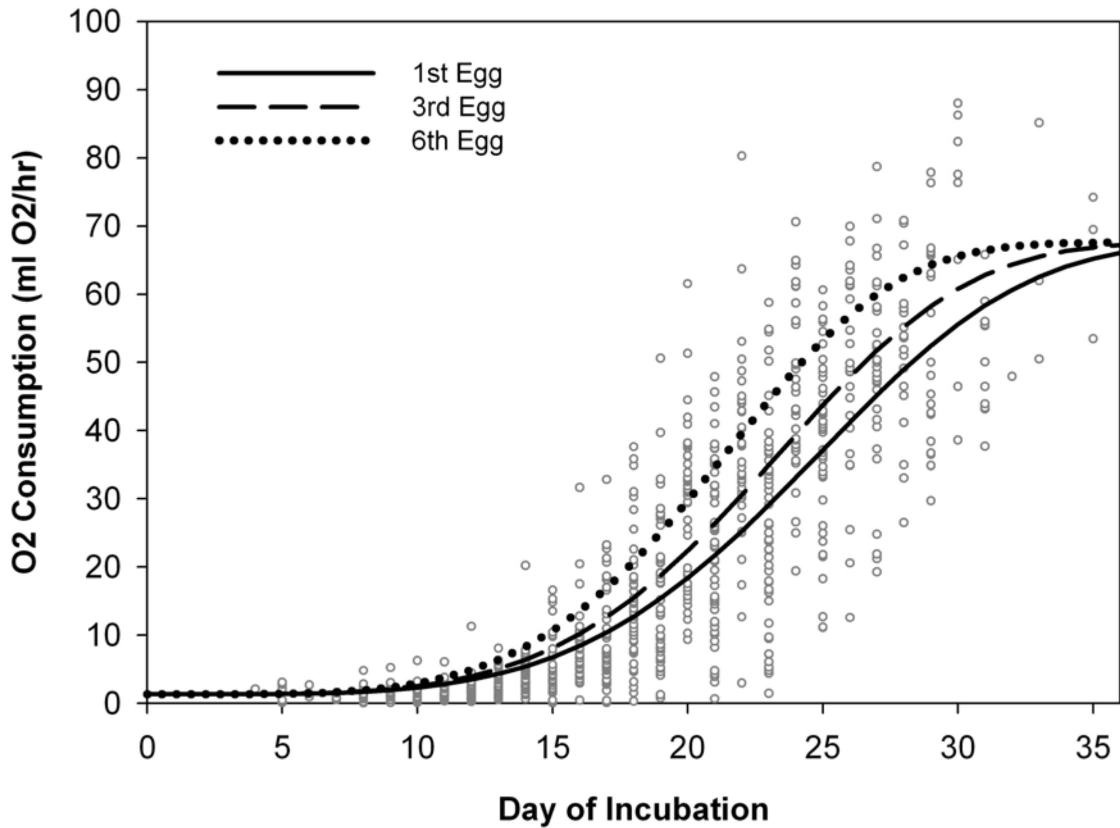


Figure 2.1. Observed embryonic oxygen consumption rates by relative age (days of incubation) in Canada geese measured from clutches located in the field and lab during 2004 and 2005 (indicated by open circles) and predicted values from the most parsimonious statistical model in which oxygen consumption rates increase with position in the clutch (indicated by the lines, with only three positions shown for clarity).

Table 2.1. Models describing embryonic oxygen consumption rates as a function of age (i.e., relative incubation length) based on a generalized s-shaped curve from Janoschek (1957) along with sum of squares error (SSE), number of parameters (M), relative Akaike information criteria adjusted for small sample size (Δ AIC_c), Akaike weight (w_i AIC_c), and the coefficient of determination (R^2). The model name indicates which of the four curve parameters (A_0, A_f, P, and K) had a linear effect (indicated by *) included. For instance, P*position indicates parameter $P = P_0 + P_1 \bullet position$ (a linear function of position, but the other three parameters are constant). Year refers to the year the sample was collected, and location refers to field- versus lab-incubated eggs. The all constant model did not include any linear effects in parameters. All models assumed a random effect due to clutch. Models excluding a sex effect had a sample size of n = 141 eggs, and models including a sex effect (representing only eggs with goslings of known sex) had a sample size of n = 43 eggs.

Model	SSE	M	Δ AIC_c	w_i AIC_c	R^2
P*position	73956.30	5	0.000	0.51614	0.1245
(A_0&P)*position	73171.05	6	0.710	0.36188	0.1338
(A_0,A_f,K,P)*position	72084.93	8	3.133	0.10773	0.1467
A_0*position	77836.61	5	7.210	0.01403	0.0786
Constant	84477.89	4	16.573	0.00013	0.0000
P*mass	84417.13	5	18.654	0.00005	0.0007
P*year	84205.22	6	20.515	0.00002	0.0032
P*location	84210.49	6	20.523	0.00002	0.0032
Includes Sex Effect					
A_0*position	38374.72	5	0.000	0.46845	0.1044
Constant	42845.76	4	1.762	0.19409	0.0000
(A_0&P)*position	37759.73	6	2.261	0.15125	0.1187
(P*Sex)*position	39682.88	6	4.298	0.05463	0.0738
P*position	42733.33	5	4.411	0.05163	0.0026
P*mass	42823.97	5	4.498	0.04943	0.0005
A_0*sex	42660.90	6	7.265	0.01239	0.0043
P*sex	42733.33	6	7.334	0.01197	0.0026
(A_0,A_f,K,P)*position	37751.31	8	8.664	0.00615	0.1189

Models that did not incorporate an effect of position had Δ AIC_c values above 16 (Table 2.1), which indicates no support given the data (Burnham and Anderson 2002). These models included those in which year or sampling location (i.e., lab versus field) effects were included, indicating metabolic rates do not appear to vary with year or location based on the data.

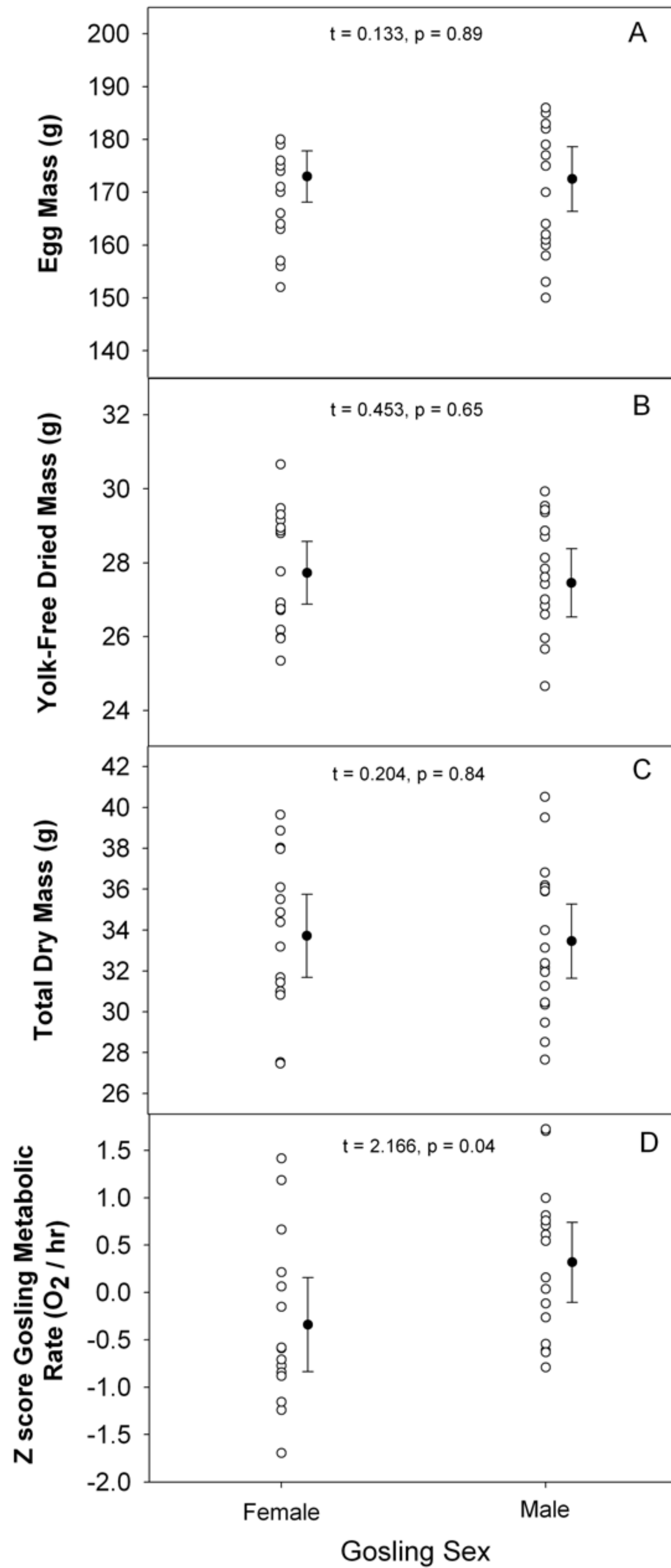
Gosling metabolic rates were not related to position in the clutch or size. I standardized gosling oxygen consumption rates within clutch using a z-score because of variation in the observed levels among clutches. The most parsimonious statistical model (based on AIC_c) of relative gosling oxygen consumption assumed a sex effect on the rate of oxygen consumption for goslings <24 hours old (Table 2.2). While there was some support for a model assuming a quadratic relationship between gosling oxygen consumption rates with position in the laying sequence and egg mass (Δ AIC_c = 1.844, Table 2.2). A model that incorporates the effect of dry body mass on gosling metabolic rates did have stronger support (Δ AIC_c = 0.835, Table 2.2). Male hatchings tended to have slightly higher gosling metabolic rates relative to females ($t = 2.166$, $p = 0.04$, $df = 1$) (Figure 2.2), with individuals of greater body mass representing minimally higher metabolic rates with a slope parameter of (0.100).

Primary sex ratios were not different from 50:50, and gosling sex was not related to egg size, size at hatching, gosling metabolic rates, or position in the clutch. I was able to determine sex in 34 of 43 goslings that were incubated in the lab and hatched in 2005, the remaining 9 goslings were excluded due to uncertainty in origin of gosling upon hatching (in 2004, goslings were euthanized

Table 2.2. Models describing gosling metabolic rates as a function of sex, egg mass, dry body mass, position in the laying sequence, and time of hatch relative to sample collection along with sum of squares error (SSE), number of parameters (M), relative Akaike information criteria adjusted for small sample size (Δ AIC_c), Akaike weight (w_i AIC_c), and the coefficient of determination (R^2). The constant model assumed no effects, and sample size was n = 31 goslings.

Model	SSE	M	Δ AIC_c	w_i AIC_c	R^2
Linear (Sex)	20.25	3	0.000	0.2706	0.2233
Linear (Dry Body Mass)	22.66	2	0.835	0.1782	0.1309
Quadratic (Position + Egg Mass)	23.41	2	1.844	0.1076	0.1022
Quadratic (Position)	23.98	2	2.590	0.0741	0.0803
Linear (Time of Hatch)	23.99	2	2.608	0.0735	0.0798
Quadratic (Time of Hatch)	24.00	2	2.616	0.0732	0.0795
Constant	26.07	1	2.725	0.0693	0.0000
Quad (Position + Dry body Mass)	22.53	3	3.302	0.0519	0.1360
Linear (Position + Egg Mass)	23.56	3	4.692	0.0259	0.0964
Linear (Egg Mass)	25.93	2	5.009	0.0221	0.0057
Linear (Position)	26.07	2	5.180	0.0203	0.0002
Quadratic (Position + Time of Hatch)	23.97	3	5.224	0.0199	0.0808
Quadratic (Position + Egg Mass + Time of Hatch)	23.35	4	7.274	0.0071	0.1045
Linear (Position + Egg Mass + Time of Hatch)	23.52	4	7.495	0.0064	0.0981

Figure 2.2. Observed values of (A) fresh egg mass, (B) gosling mass, (C) oxygen consumption rates (measured within 24 hours of hatching), and (D) standardized gosling metabolic rates (measured within 24 hours of hatching) by sex in Canada geese. Mean values and 95 percent confidence intervals are indicated by the filled circles and lines.



t = 0.133, p = 0.89
A
Egg Mass (g)
200
190
180
170
160
150
140
t = 0.453, p = 0.65
B
Yolk-Free Dried Mass (g)
32
30
28
26
24
t = 0.204, p = 0.84
C
Total Dry Mass (g)
42
40
38
36
34
32
30
28
26
t = 2.166, p = 0.04
D
Z score Gosling Metabolic Rate (O_2 / hr)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Female
Male
Gosling Sex

at pipping or immediately after hatching and I did not dissect the carcasses for sex determination). Of the 34 goslings for which I determined sex, 16 were females, and 18 were males, which is not detectably different from 50:50 (χ^2 = 0.118, p = 0.73, df = 1). Similarly, egg size, gosling total dry mass and yolk-free dry mass at hatching, and gosling oxygen consumption rates did not differ between males and females (Figure 2.2). Finally, logistic regression of sex by position in the laying sequence indicated sex ratios did not differ with position (χ^2 = 1.156, p = 0.28, df = 1).

Hematocrit levels varied by position and size in newly hatched goslings. In 2005, I measured hematocrit levels from 43 goslings within 24 hours of hatching in the lab. I standardized measures of hematocrit within a clutch using the z-score because there was considerable variation in levels among clutches and developed a suite of linear regression models to compare the effects of position, size, and time of hatch relative to sample collected on relative hematocrit levels. Of the 43 goslings with hematocrit levels determined, 8 were eliminated due to uncertainty in the time of hatch relative to collection of samples. The most parsimonious model (according to AIC_c) included positive linear effects of position in the laying sequence and fresh egg mass on the relative proportion of hematocrit in goslings (Figure 2.3 and Table 2.3), including a negative association of time hatch relative to collection of hematocrit levels (slope= -0.56). Because sample size was small, I also used a t-test to compare relative hematocrit levels of goslings hatched from eggs in positions one and six, and found a detectable difference

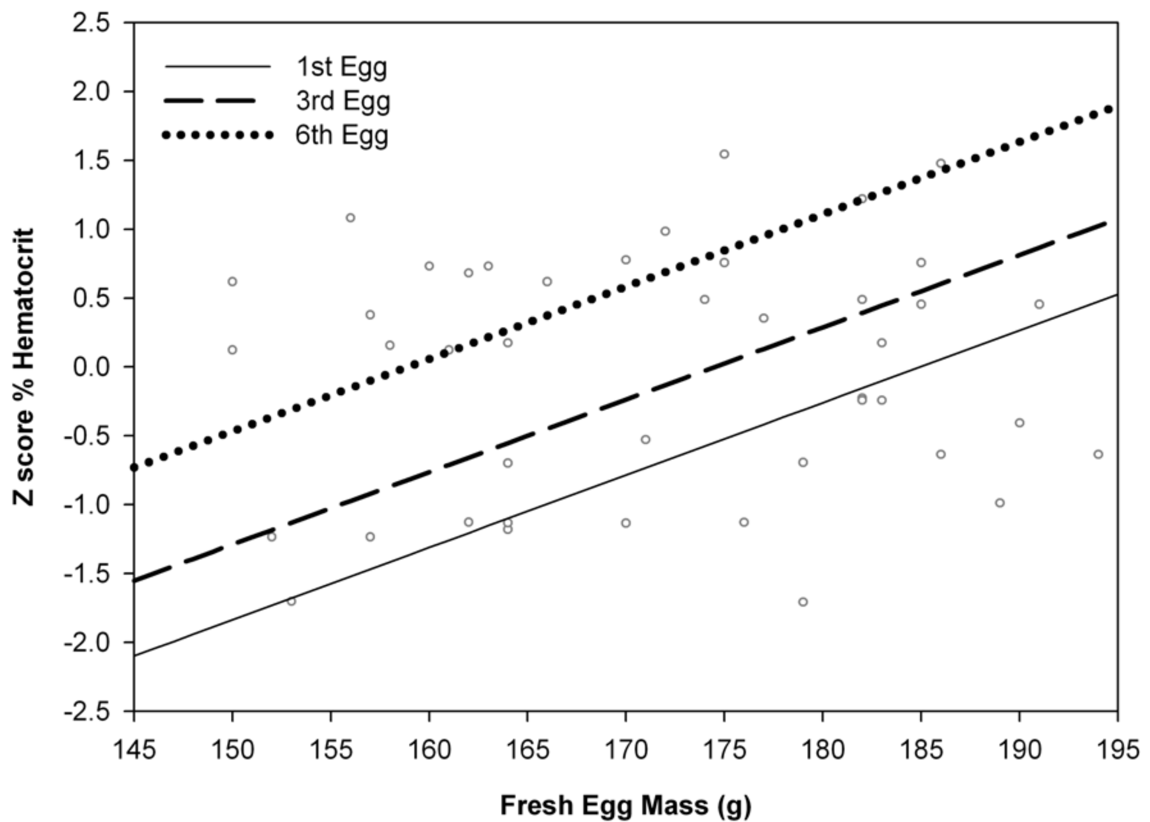


Figure 2.3. Observed relative hematocrit levels in Canada geese within 24 hours of hatching (open circles) with fresh egg mass and the predicted relationships for goslings hatched from first (solid line), third (dashed line), and sixth (dotted line) laid eggs in a clutch from the most parsimonious model.

Table 2.3. Models describing gosling relative percent hematocrit as a function of egg mass, position in the laying sequence, and time of hatch relative to sample collection along with sum of squares error (SSE), number of parameters (M), relative Akaike information criteria adjusted for small sample size (ΔAIC_c), Akaike weight ($w_i AIC_c$), and the coefficient of determination (R^2). The constant model assumed no effects, and sample size was n = 34 goslings.

Model	SSE	M	ΔAIC_c	$w_i AIC_c$	R^2
Linear (Egg Mass + Position +Time of Hatch)	19.08	4	0.000	0.8489	1.0000
Linear (Time of Hatch)	25.34	2	4.636	0.0836	0.0985
Linear (Egg Mass + Position)	24.04	3	5.352	0.0584	0.0688
Linear (Position)	29.59	2	10.068	0.0055	0.0065
Linear (Egg Mass)	31.39	2	12.130	0.0020	0.0023
Constant	34.00	1	12.526	0.0016	0.0019

in the relative hematocrit levels of goslings hatched from eggs in these positions ($t = 1.25$, $p = 0.036$).

Yolk reserves in goslings were not related to position in the laying sequence. I measured the relative dried yolk mass in 34 of the 43 goslings within 24 hours of hatching in 2005; 9 goslings were eliminated due to uncertainty in origin of the gosling upon hatching. Goslings typically hatch overnight (Cooper 1978), thus I estimated the time from hatching to euthanization to account for metabolic losses of yolk in the period following hatching. I then developed regression models to assess effects of time from hatching and position on relative yolk reserves (i.e., fraction of dry yolk mass to total dry mass). The most parsimonious statistical model (based on AIC_c) indicated that the relative yolk reserves of goslings decreased as time from hatching to euthanization increased with position accounting for 90 percent of the evidence given the data position was included (from sum of AIC_c weights, Table 2.4).

Table 2.4. Models describing fraction of dry yolk relative to hatchling mass as a function of egg mass, position in the laying sequence, and time of hatch relative to sample collection, along with sum of squares error (SSE), number of parameters (M), relative Akaike information criteria adjusted for small sample size (Δ AIC_c), Akaike weight (w_i AIC_c), and the coefficient of determination (R^2). The constant model assumed no effects, and sample size was n = 34 goslings.

Model	SSE	M	Δ AIC_c	w_i AIC_c	R^2
Linear (Position + Time of Hatch)	0.0200	3	0.000	0.707792	0.8387
Linear (Mass + Position + Time of Hatch)	0.0199	4	2.593	0.193560	0.8395
Linear (Time of Hatch)	0.0246	2	4.459	0.076139	0.8016
Linear (Mass + Time of Hatch)	0.0245	3	6.900	0.022469	0.8024
Linear (Mass)	0.0397	2	20.731	0.000020	0.6798
Linear (Mass + Position)	0.0390	3	22.706	0.000008	0.6855
Linear (Position)	0.0428	2	23.288	0.000006	0.6548
Linear (Sex)	0.0428	3	25.867	0.000002	0.6548
Constant	0.1240	1	57.042	2.90E-13	0.0000

DISCUSSION

Mechanisms regulating embryonic development in birds that lay synchronously hatching broods are poorly understood. In this study, I found that embryonic oxygen consumption rates increase to a plateau as the embryo ages, but rates also increase with position in the laying sequence in Canada geese. The general pattern of embryonic metabolic rates increasing to a plateau, which I observed, has also been seen in other precocial species (Vleck et al. 1979, Vleck et al. 1980b, Booth and Sotherland 1991). In addition, MacCluskie et al. (1997) found embryonic metabolic rates were higher just prior to hatching in later laid eggs of mallard ducks (*Anas platyrhynchos*). However, MacCluskie et al. (1997) measured oxygen consumption rates at only three points in the incubation stage and could not determine if the observed differences were due to intrinsic factors (such as position or size), or external stimuli (such as movement of embryos in other eggs in the nest; Vleck et al. 1979). I measured oxygen consumption rates at two to three day intervals throughout the incubation period both in the field and in the lab under standardized incubation conditions without egg-to-egg contact to develop specific nonlinear statistical models of the s-shaped oxygen consumption curve (Figure 2.1) that incorporated effects of several variables on the parameters underlying the consumption curve. Analysis of these models indicated that models incorporating effects of laying position (an intrinsic factor) best described embryonic oxygen consumption rates in Canada geese, but models including other intrinsic factors (e.g., egg size) were not supported (Table 2.1). Moreover, effects of laying position on metabolic rates were evident prior to the plateau phase, and

the inclusion of effects of incubation (natural versus lab, an extrinsic factor) was not supported by the data. The differences predicted by models incorporating laying position effects indicate that oxygen consumption rates of embryos from eggs located in position eight at the 20th day of incubation would be equivalent to oxygen consumption rates from eggs located in position one at the 25th day of incubation. Presumably, this difference indicates the embryo from the eighth egg is developmentally similar to the embryo from the first egg in the days prior to hatching (approximately the 28th day of incubation) because embryonic metabolic rates reflect the degree of development (Vleck et al. 1979, 1980a, 1980b). Hence, based on predicted rates of oxygen consumption, embryos in later laid eggs appear to be developmentally ready to hatch when the earliest laid eggs begin hatching in the clutch of a Canada goose.

Relationships between egg size and embryonic metabolism are confounded with position in waterfowl. Egg size can affect embryonic metabolic rates (Vleck and Vleck 1987, Nicolai et al. 2004), perhaps due to larger surface to volume ratios and, therefore, higher rates of water conductance (Rahn and Ar 1974), oxygen conductance (Rahn et al. 1974), and thermal capacity (Turner 2002). Because waterfowl egg size is typically nonlinear (increasing in the first laid eggs then decreasing with later laid eggs; Cooper 1978, LeBlanc 1987, Flint and Sedinger 1992), it is logical to assume that metabolic rates would follow a similar pattern across the laying sequence. Indeed, Nicolai et al. (2004) hypothesized that the mechanism driving increasing consumption rates across the sequence observed in black brant (*Branta bernicla nigricans*) was due to egg size rather than position.

Egg size follows the bell-shaped pattern in Canada geese (Cooper 1978, LeBlanc 1987, and see Chapter 1 in this study), however, egg position was a better predictor of embryonic metabolic rates of Canada geese than egg size (Table 2.1 and Figure 2.1) indicating that the relationship between egg size and embryonic metabolism may not be a general pattern for all birds. If shell conductance varies across the laying sequence such that later laid eggs have higher conductance (and therefore capable of supporting higher metabolic rates, Seymour and Visschedijk 1988), this could provide a mechanism by which the patterns in embryonic oxygen consumption observed in this study arose. However, studies of variation in shell conductance with laying position have not been performed for waterfowl.

Patterns in embryonic metabolic rates did not extend beyond hatching. I found that gosling oxygen consumption rates were not related to laying position, initial egg mass, or yolk-free dry body mass. The lack of connection between embryonic and gosling metabolic rates may be a mechanism for conserving limited energy reserves following hatching (Moe et al. 2005).

Although embryonic and gosling metabolic rates were not related, there was evidence of carryover of embryonic physiological characteristics into the post-hatching period. I found that hematocrit levels of newly hatched goslings were related to fresh egg mass and position in the laying sequence, which corresponds with part of the variation in embryonic oxygen consumption rates. Thus, some connections between embryonic physiology is apparent in neonates. However, I did not assess the duration of the elevated hematocrit levels associated with larger

and later laid eggs and further evaluation is needed to assess the long-term implications of patterns of variation in hematocrit levels.

Evidence from this study indicates that elevated embryonic metabolic rates in later laid eggs is a mechanism by which synchronization of hatching is accomplished in Canada geese. An acceleration in embryonic development (by several days to a week) is required for all eggs to hatch within 24 hours in waterfowl species because incubation begins prior to completion of the clutch. I found that position, rather than size or external stimuli, explained observed patterns in embryonic oxygen consumption. Physical egg characteristics that vary with position may provide clues to the proximate factors regulating metabolic rates across the laying sequence in waterfowl. The implications of embryonic oxygen consumption rates on recently hatched goslings were not apparent, however, offspring from smaller, later laid eggs have a reduced probability of survival in other waterfowl species (Dawson and Clark 1996), indicating potential long-term consequences of patterns in embryonic metabolism for recruitment in waterfowl populations.

LITERATURE CITED

Ar, A. and H. Rahn. 1985. Pores in avian eggshells: Gas conductance and embryonic growth rate. Respiration Physiology 61:1-20.

Booth, D. T. and P. R. Sotherland. 1991. Oxygen consumption, air-cell gas tensions, and incubation parameters of mute swan eggs. Physiological Zoology 64(2):473-484.

Burnham, K. P. and D. R. Anderson. 2002. Model selection and multi-model inference: A practical information-theoretic approach. Second edition. Springer Verlag, New York.

Cooper, J. A. 1978. The history and breeding biology of the Canada geese of Marshy Point, Manitoba. Wildlife Monographs 61:1-87.

Dawson, R. D. and R. G. Clark. 1996. Effects of variation in egg size and hatching date on survival of lesser scaup (*Aythya affinis*) ducklings. Ibis 138(4):693-699.

Dietz, M. W., M. Kampen,M. J. M. van Griensven, and S. van Mourik. 1998. Daily energy budgets of avian embryos: The paradox of the plateau phase in egg metabolic rate. Physiological Zoology 71(2):147-156.

Flint, P. L. and J. S. Sedinger. 1992. Reproductive implications of egg-size variation in the black brant. Auk 109(4):896-903.

Gille, U., F.V. Salomon, and J. Ronnert. 1999. Growth of the digestive organs in ducks with considerations on their growth in birds in general. British Poultry Science 40(2):194-202.

Hamilton, K. L. 1985. Metabolism of barn owl eggs. American Midland Naturalist. 114(2):209-215.

Hoyt, D. F., D. Vleck, and C. M. Vleck. 1978. Metabolism of avian embryos: Ontogeny and temperature effects in the ostrich. Condor 80:265-271.

_____, D. F., R. G. Board, H. Rahn, and C. V. Paganelli. 1979. The eggs of the anatidae: Conductance, pore structure, and metabolism. Physiological Zoology. 52:438-450.

Janoschek, A. 1957. Das reaktionskinetische Grundgesetz und seine Beziehungen zum Wachstums- und Ertragsgesetz. Stat. Vjschr. 10:25-37.

Leblanc, Y. 1987. Intraclutch variation in egg size of Canada geese. Canadian Journal of Zoology 65:3044-3047.

Loos, E. R. and F. C. Rohwer. 2004. Laying-stage nest attendance and onset of incubation in prairie nesting ducks. Auk 121(2):587-599.

MacCluskie, M. C., P. L. Flint, J. S. Sedinger. 1997. Variation in incubation periods and egg metabolism in mallards: Intrinsic mechanisms to promote hatch synchrony. Condor 99:224-228.

Moe, B., E. Stølevik, and C. Bech. 2005. Ducklings exhibit substantial energy-saving mechanisms as a response to short-term food shortage. Physiological and Biochemical Zoology 78(1):90-104.

Nice, M. M. 1962. Development of behavior in precocial birds. Transactions of the Linnean Society of New York. 81:1-211.

Nicolai, C. A., J. S. Sedinger, and M. L. Wege. 2004. Regulation of development time and hatch synchronization in black brant (*Branta bernicla nigricans*). Functional Ecology 18:475-482.

Paganelli, C. V., A. Ar, and H. Rahn. 1973. Relative water vapor permeabilities of the shell and shell membranes of the hen's egg. Physiologist 16:415.

_____, C.V., R. A. Ackerman, and H. Rahn. 1978. The avian egg: In vivo conductance to oxygen, carbon dioxide, and water vapor in late incubation. Pages 212-218 in J. Piiper, ed. Respiratory functions in birds, adult and embryonic. Springer, Berlin.

Rahn, H. and A. Ar. 1974. The avian egg: Incubation and water loss. Condor 76:147-152.

_____, H., C. V. Paganelli, and A. Ar. 1974. The avian egg: Air-cell gas tension, metabolism and incubation time. Respiration Physiology 22:297-309.

_____, H., A. Ar, and C. V. Paganelli. 1979. How bird eggs breathe. Scientific American. 240:46-55.

_____, H., C. V. Paganelli, and A. Ar. 1987. Pores and gas exchange of avian eggs: a review. Journal of Experimental Zoology Supplement 1:165-172.

_____, H. and C. V. Paganelli. 1990. Gas fluxes in avian eggs: Driving forces and the pathway for exchange. Comparative Biochemistry and Physiology 95A(1):1-15.

Seymour, R. S. and A. H. J. Visschedijk. 1988. Effects of variation in total and regional shell conductance on air cell gas tensions and regional gas exchange in chicken eggs. Journal of Comparative Physiology B 158:229-236.

Turner, J. S. 2002. Avian Incubation: Behaviour, environment, and evolution. "Maintenance of egg temperature". edited by D. C. Deeming, Oxford University Press Inc., New York.

Tyler, C. 1964. A study of the egg shells of the anatidae. Proceedings of the Zoological Society of London 142:547-583.

Vleck, C. M., D. F. Hoyt, and D. Vleck. 1979. Metabolic of avian embryos: Patterns in altricial and precocial birds. Physiological Zoology 52(3):363-377.

_____, C. M. and D. Vleck. 1987. Metabolism and energetics of avian embryos. American Zoologist 20:405-416.

_____, D., C. M. Vleck, and D. F. Hoyt. 1980a. Metabolism of avian embryos: Ontogeny of oxygen consumption in the rhea and emu. Physiological Zoology 53(2):125-135.

_____, C. M., D. Vleck and D. F. Hoyt. 1980b. Patterns of metabolism and growth in avian embryos. American Zoologist 20:405-416.

_____, D. 1987. Measurement of O_2 consumption, CO_2 production, and water vapor production in a closed system. Journal of Applicational Physiology 62(5):2103-2106.

CHAPTER 3. EFFECTS OF ALTERNATIVE DEVELOPMENT IN SYNCHRONOUS HATCHING ON GOSLING SURVIVAL IN CANADA GEESE

ABSTRACT

Synchronous hatching when nest incubation is initiated prior to clutch completion indicates that, from the first to the last laid eggs, different developmental rates occur among the offspring. Costs associated with synchronized hatching of differentially developing embryos may have immediate consequences on offspring survival. Using a cross-fostering design, I created experimental clutches of giant Canada geese (*Branta canadensis maxima*) to accelerate, delay, or not change the nominal 28-day incubation stage to determine effects on gosling survival immediately following hatching. I used a mark-recapture approach and resighted goslings daily in the first two weeks following hatching to estimate survival rates. No difference in survival in the first two weeks post-hatching was detectable among clutches accelerated to hatch approximately two days early, clutches delayed to hatch approximately two days late, and clutches that hatched without changes in incubation stage. Hence, alternative embryonic development associated with synchronous hatching does not appear to affect offspring performance immediately after hatching. However, further studies are needed to address potential long-term effects associated with alternative embryonic development.

INTRODUCTION

Hatching can be synchronized at numerous levels, ranging from the population level (e.g., synchronous hatching of broods within a colony), to the individual level (e.g., synchronous hatching of chicks in a clutch). At the population level, extrinsic factors such as weather (Veiga 1992; Hébert 2002), food availability (Lamey and Lamey 1994), intraspecific behavioral signals (Lambrechts et al. 1996), photoperiod (Raveling 1978, Sockman and Schwabl 2001), and predation (Clark and Wilson 1981, Arnold et al. 1987, Briskie and Sealy 1989, Hébert 2002), regulate the timing of egg laying and hatching synchrony. Intrinsic regulation at the population level is relatively unknown; however, genetic factors (e.g. Findlay and Cooke 1982), and female condition and age (Findlay and Cooke 1982, Farner et al. 1985, Ellis et al. 2001, and references therein) may play an important role. Within individual clutches, synchronous hatching is regulated by a combination of intrinsic and extrinsic factors.

In waterfowl, hatching is considered synchronous because all viable offspring hatch typically within a 24 hour period. In these species, eggs are laid at a rate of one every one to two days (Bellrose 1980). Over the course of laying, females increase the amount of time spent on the nest performing nest maintenance and egg laying causing nest temperatures to extend above the physiological threshold temperature (25-27°C, referenced in Loos and Rohwer 2004), initiating embryonic development in eggs laid prior to clutch

completion. In fact, the developmental stage of initially laid northern shoveler (*Anas clypeata*), mallard (*Anas platyrhynchos*), and wood duck (*Aix sponsa*) eggs can be as much as two to three days of incubation prior to the final eggs being laid (Caldwell and Cornwell 1975, Afton 1979, Kennamer et al. 1990). This could lead to asynchronous hatching, which occasionally occurs in mallards (Prince et al. 1969), lesser snow geese (*Anser c. caerulescens,* Cargill and Cooke 1981), and Canada geese (*Branta canadensis;* Cooper and Hickin 1972, Cooper 1978), depending on other regulatory mechanisms to promote hatching synchrony.

Constraints on egg viability may result in selective pressure favoring onset of incubation prior to clutch completion in waterfowl. Traditionally, synchronous hatching was thought to only arise from incubation initiation after the clutch is completed (Kear 1970). Although this occurs in many species, there are costs to postponing incubation (e.g., Arnold 1987, 1993), notably egg viability. Asynchronous incubation may have evolved through females initiating incubation prior to clutch completion to minimize the loss of offspring due to diminishing viability because egg viability decreases the longer eggs sit without incubation (Arnold et al. 1987, references therein; Arnold 1993, Stoleson and Beissinger 1999). Reduced egg viability probably contributes to reduced hatching success observed in first laid eggs of larger clutches in Canada geese (Cooper 1978). Ambient temperatures may also affect egg viability, depending on the timing of egg laying (Batt and Cornwell 1972, Arnold 1993, Vinuela 2000). In dabbling ducks, loss in egg viability occurs

following five to ten days of no incubation (Arnold et al. 1987). Daily cooling of eggs may extend viability of unincubated eggs (Arnold 1993, Stoleson and Beissinger 1999); however, annual timing of nest initiation and environmental conditions ultimately determine the duration of viability of unincubated eggs.

While the mechanisms regulating synchronous hatching in clutches with differential incubation are not completely known, there is considerable selective pressure for synchronized hatching in waterfowl broods. Females often leave the nest shortly (approximately 24 hours) after hatching (Cooper and Hickin 1971, Cooper 1978), reducing costs to the female associated with incubation and brooding behaviors. Predation pressure increases over the course of incubation (Clark and Wilson 1981, Briskie and Sealy 1989), encouraging females to leave the nest site quickly as hatching occurs. Therefore, it is advantageous for offspring to hatch as synchronously as possible (which minimizes risk of abandonment by departing females). The energy reserves of hatchlings are usually depleted within one to two days of hatching (Ankeny 1980, Badzinski et al. 2002), and hatchlings need to be guided to feeding areas by the adult. Presumably, females time nest departure to optimize the tradeoff between risks to themselves (e.g., predation) and risks to offspring (e.g., starvation).

Embryo-embryo and embryo-female communication may have evolved as mechanisms facilitating hatch synchrony (Vince 1966, 1968; McCoshen and Thompson 1968; Hess 1972; Orcutt 1974; Davies and Cooke 1983; Brua 2002). External vibrations and vocalization alter developmental rates in

precocial embryos (Vince 1966, 1968; Orcutt 1974; Davies and Cooke 1983; Cannon et al. 1986). During the final two to three days of incubation, vibrations between eggs result from increased embryo movements as hatching begins (Vleck et al. 1985). Approximately one day before hatching, breaking of the chorioallantoic membrane (internal pipping, Vleck et al. 1979) exposes the chick to the air cell and a direct source of oxygen prior to breaking through the shell. While located in the air cell, clicking and vocalizations are prominent (perhaps contributed to increasing airflow over the syrinx, Forsythe 1971). Playbacks of recordings of low intensity vibrations and clicking to bobwhite quail (*Colinus virginianus*) and Japanese quail (*Coturnix japonica)* eggs accelerated hatching by two days, while the absence of these stimuli delayed hatching for a similar period (Vince 1966, 1968). Embryo-female communication can have similar effects on synchronization of hatching (Hess 1972). In lesser snow geese, females altered their incubation behavior to accommodate late hatching offspring (Davies and Cooke 1983), presumably using sound or movement cues to identify later developing embryos.

Intrinsic mechanisms also facilitate within clutch hatching synchrony. In black brant (*Branta bernicla nigricans*), relatively smaller eggs laid later in the laying sequence have higher metabolic rates (Nicolai et al. 2004), presumably indicating faster developmental rates. Embryonic metabolic rates in precocial species increase exponentially, but plateau approximately 80 percent of the way through incubation (Vleck et al. 1979). Precocial offspring approach

hatchling mass approximately 80 percent of the way through incubation (Vleck et al. 1980), shortening or lengthening of the plateau duration may help to accommodate synchronous hatching. Delayed development during the final days of incubation and early hatching can both be detrimental to offspring condition at hatching (Vleck et al. 1985, Nilsson and Persson 2004), but the consequences of lengthening or shortening the developmental period associated with synchronous hatching on offspring performance after hatching are not known.

In this study, I assessed the repercussions of alternative development on initial offspring survival in Canada geese. I employed a cross-foster design to create foster clutches of eggs such that time to hatch was accelerated for some clutches, delayed for other clutches, and not changed in other clutches. Following hatching, I assessed gosling survival for two weeks using mark-recapture methods to determine effects of altered development on early offspring survival.

MATERIALS AND METHODS

Study Site

I manipulated Canada goose nests from a breeding population located in Moorhead, Minnesota (46° 54.139´ N, 96° 45.020´ W) nesting on constructed ponds owned by the American Crystal Sugar Company during the 2005 breeding season (mid March through the end of May). Minimal predator management is implemented on plant property, possibly aiding in the survivability of over 200 pairs of Canada geese that nest and raise broods within the area of the constructed wetlands.

Nest Monitoring and Cross-Foster Design

I located and monitored nests at the onset of laying to provide a pool of nests to be used in a cross-foster design. After locating nests at the onset of laying, I monitored nests daily to determine the order of laying for all eggs in the clutch (see Chapter one for a complete description of nest searching and monitoring methods). Eggs were marked to identify position and natal nest, and at least two weeks prior to hatching, eggs were assigned and moved to a foster nest.

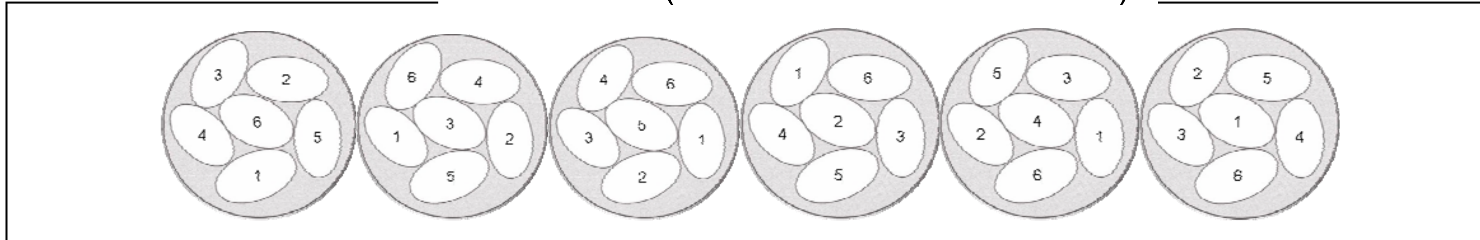
To manipulate the alternative development offspring may encounter during synchronous hatching, I used a cross-foster design to accelerate, delay, or not change embryonic growth during the latter half of incubation. To create foster nests, I first back calculated and categorized clutches from predicted hatch dates for all eggs. Hatch dates were determined 28 days (Cooper 1978) from third laid eggs, the estimated time of onset of female

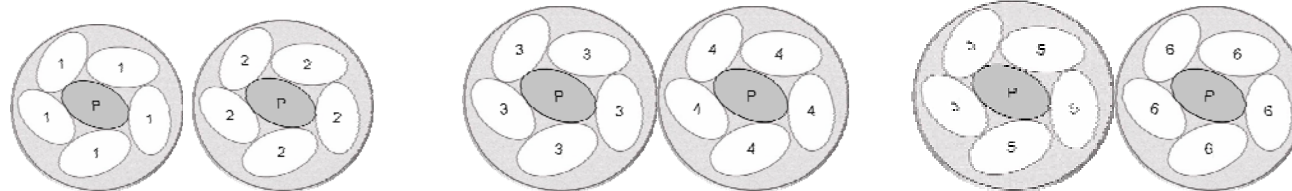
incubation. The predicted hatch dates were then used to rearrange clutches into foster clutches consisting of six eggs, five of which were randomly selected with the constraint that the predicted hatch dates were within one to two days of each other (Figure 3.1). The sixth egg of the foster clutch was a randomly selected primer egg to set a hatch date for the foster clutch. The primer egg was always placed in the middle of the nest and in contact with all eggs in the clutch to ensure vocalizations or other physical cues associated with the primer egg hatching were equally transmitted to all of the eggs in the clutch. Primer eggs were assigned to set hatch dates for the clutch relative to the expected hatch dates of the five non-primer eggs in three groups: accelerated development, delayed development, and no change (i.e., control) development. Primer eggs in accelerated foster nests had predicted hatch dates approximately five days earlier than expected hatch dates for surrounding non-primer eggs; in delayed foster nests, primer eggs had expected hatch dates approximately five days later than expected hatch dates for surrounding non-primer eggs; and control foster nests had primer eggs with expected hatch dates that were the same as expected hatch dates of other eggs in the nest.

Gosling Survival Rates

I assessed the effect of developmental rate on survival using a mark-recapture design. In the final week of incubation, I checked the foster nests daily to determine the hatching status of the goslings. All goslings present in

Natal Nests (Predicted Hatch Date = 125)





Delayed Foster | Control Foster | Accelerated Foster

(Predicted Hatch Date = 125 + 2) | (Predicted Hatch Date = 125 + 0) | (Predicted Hatch Date = 125 – 2)

Figure 3.1. Diagram representing the design of foster nests to manipulate hatch date. Clutches in the top row represent six natural clutches estimated to hatch on the same day (predicted clutch hatch dates based on third laid eggs; average Julian Date = 125). Intraclutch egg numbering refers to observed laying sequence with identical numbers between clutches indicating similar hatching dates. The bottom row represents six foster clutches created from the natal clutches in the top row such that A) eggs with earlier expected hatching dates have a primer egg (middle, in gray) due to later hatch (and are thus delayed), B) eggs with similar expected hatching dates have a primer egg due to hatch at the same date (and thus experience no change, i.e., control), and C) eggs with later expected hatching dates have a primer egg due to hatch at an earlier date (and are thus accelerated). In reality, expected hatch dates do vary in natal nests; therefore, any particular foster nest may be comprised of eggs from multiple positions dependent on predicted hatch dates.

the nest were captured and individually colored using a non-toxic, semi-permanent marker (Sharpie®).

Once goslings were present in a nest, I continued visiting the nest every two to three hours until all goslings had hatched and were marked, or until the female departed the nest with the brood. Coloring locations were restricted to the top of the head, left cheek, and right cheek. I used one of four contrasting colors (Black, Green, Red, and Blue) or no color to mark each location such that each individual was given a unique color pattern. After the initial color marking, I conducted daily observations for the initial two weeks following hatching to record presence or absence of each individual gosling marked from the brood. After one week of observations, a recapture attempt was conducted to touch-up any fading color marks.

Statistical Analysis

I analyzed the effect of the cross-foster design on mean hatch dates for the experimental groups using Analysis of Variance (ANOVA) with effect detection limits at the $\alpha = 0.05$ level. The Tukey-Kramer test was used to compare differences between pairs of groups.

Gosling survival rates were estimated using the Cormack-Jolly-Seber method (Lebreton et al. 1992) via Program MARK (White and Burnham 1999). This method estimates both a probability of gosling survival between encounters, as well as the probability of recapture per encounter. I developed a suite of candidate models of survival and ranked models using the Akaike Information

Criterion adjusted for small sample size (AIC_c , Burnham and Anderson 2002). Parameter estimates and AIC_c values for all models were obtained from Program MARK (White and Burnham 1999).

RESULTS

Observed hatch dates of eggs in foster nests differed from their expected hatch dates. I established 22 foster nests (six accelerated foster nests, seven control foster nests, and nine delayed foster nests) that survived to the hatching stage. Mean hatch dates for the foster nests were significantly different among foster groups ($F=12.238$, $p=0.0004$, $df = 21$). Results of the Tukey test indicate that eggs in delayed foster nests hatched detectably later (mean of 2.89 ± 1.04 days from the expected hatch date) than eggs from both the control (mean of 1.21 ± 1.18 days from the expected hatch date) and accelerated foster nests (mean of -1.00 ± 1.28 days from the expected hatch date), but eggs from the control and delayed foster nests did not hatch at significantly different dates (Figure 3.2). Estimated gosling survival over the initial two weeks following hatching did not differ among foster nest groups. The most parsimonious statistical model of gosling survival assumed a constant survival rate for all foster nest groups (Table 3.1). Within the candidate set of models, the six top ranked models (based on AIC_c) all assumed a single constant rate of survival for all foster nest groups (Table 3.1). Furthermore, these models collectively accounted for over 90 percent of the evidence (represented by AIC_c weight, Burnham and Anderson 2002), given the data (Table 3.1). The only competitive model with foster group effects on survival ({ϕ(A,C,D),p(A,C,D)}, Table 3.1) had less than 2 percent of the evidence and was almost 20 times less likely than the highest ranked model (Table 3.1). The estimate of the probability of survival was approximately 0.955 for the most parsimonious model (Table 3.2), which yields approximately 52 percent survival over the two-week period.

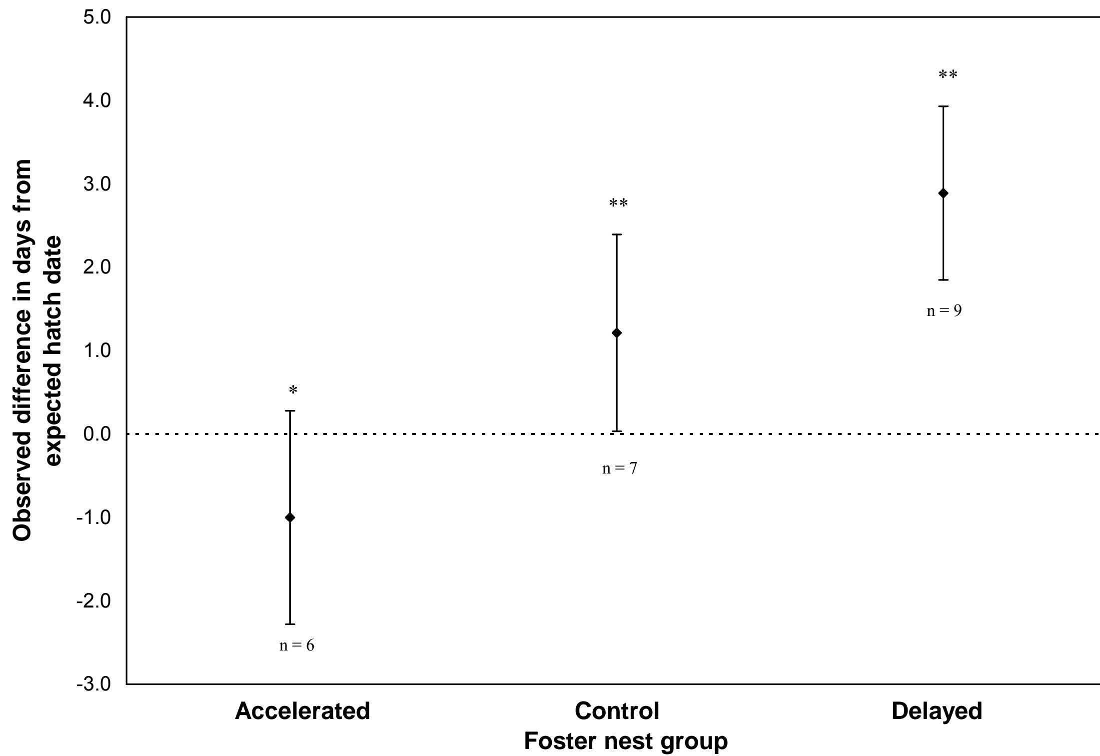


Figure 3.2. Mean difference in days from predicted hatch dates for eggs from the three foster nest groups with standard errors. ANOVA indicates a detectable difference among foster groups (F = 12.238, p-value = 0.0004, df = 21), with pairwise differences (via Tukey-Kramer test, $\alpha = 0.05$) between the accelerated group (indicated by *) and control and delayed groups, but no difference between control and delayed groups (indicated by **).

Table 3.1. Relative AIC_c values, AIC_c weights, and model likelihoods for the candidate models of gosling survival. Notation represents survival (ϕ), capture probability (p), and foster nest group (A, accelerated; C, control; and D, delayed). Goodness-of-fit was confirmed for a singular global model, {(ϕ (A,C,D),p(A,C,D)}, via a bootstrap comparison of total deviance in Program MARK.

Model	K	AIC_c	Δ AIC_c	w_i AIC_c	Model Likelihood	Deviance
{ϕ(),p(A&C, D)}	3	628.62	0.000	0.3900	1.0000	479.17603
{ϕ(),p(A, C, D)}	4	630.54	1.920	0.1494	0.3829	479.02922
{ϕ (),p(A&C*age, D)}	4	630.64	2.022	0.1419	0.3638	479.13186
{ϕ (),p(A&D, C)}	3	630.87	2.253	0.1264	0.3242	481.42909
{ϕ (),p(A&C*age, D*age)}	5	632.12	3.498	0.0679	0.1740	478.52365
{ϕ (),p()}	2	633.45	4.837	0.0347	0.0891	486.06229
{ϕ (A, C, D),p(A, C, D)}	6	634.58	5.959	0.0198	0.0508	478.88403
{ϕ (),p(A, C&D)}	3	634.97	6.350	0.0163	0.0418	485.52557
{ϕ (),p(age)}	3	635.08	6.461	0.0154	0.0395	485.63722
{ϕ (A, C+D),p()}	3	635.23	6.614	0.0143	0.0366	485.79008
{ϕ (age),p()}	3	635.49	6.877	0.0125	0.0321	486.05302
{ϕ (),p(A*age, C*age, D*age)}	7	636.20	7.584	0.0088	0.0225	478.3904
{ϕ (A, C, D),p(age)}	5	638.64	10.026	0.0026	0.0066	485.05213

Table 3.2. Parameter estimates based on most parsimonious model {ϕ ()p(A&C, D)}. Phi = survival probability, p = detection probability, A = accelerated, D = delayed, and C = controlled foster groups.

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:Phi	0.955191	0.012324	0.92380	0.974014
2:p (A&C)	0.466892	0.038199	0.39330	0.541944
3:p (D)	0.611168	0.039246	0.53209	0.684798

Capture success was higher for goslings from the delayed foster nests than for goslings from the accelerated and control foster nests. The top five ranked candidate models all assumed capture probability varied between foster nest groups (Table 3.2), and these models collectively accounted for over 87 percent of the evidence. The capture probability (which is a resighting probability) was lower for the goslings from accelerated and control foster nests compared to goslings from delayed foster nests (Table 3.2). Sample size was also smaller for the accelerated (n = 12) and control (n = 21) foster nest groups than from delayed foster nest group (n= 34) because there were fewer accelerated and control foster nests than delayed foster nests, and because more of the accelerated and control foster broods hatched and departed before I was able to capture and mark them.

DISCUSSION

Synchronous hatching of avian offspring within a clutch when incubation is initiated prior to clutch completion indicates that differential developmental rates occurs from the first to the last laid eggs in the clutch. Although not directly measured in this study, offspring development can vary substantially within a clutch depending on when incubation is initiated (Caldwell and Cornwell 1975, Afton 1979, Kennamer et al. 1990). Several extrinsic and intrinsic mechanisms regulate avian embryonic development and provide sufficient plasticity for synchronously hatching clutches in species such as waterfowl. However, this plasticity in development may result in costs to individual offspring following hatching (Vleck et al. 1985, Nilsson and Persson 2004). In this study, I measured the costs of plasticity in development to offspring survival by experimentally manipulating the incubation period in Canada geese such that offspring development was accelerated, delayed, or not changed from the standard 28-day incubation period and using mark-recapture methods to estimate survival in the first two weeks following hatching. I found that gosling survival immediately following hatching did not differ among goslings experiencing accelerated, delayed, or no change in embryonic development. While small sample size may have limited the power to detect differences in survival in my study, estimates of survival probability in a model assuming differences among foster nest groups (model {ϕ(A,C,D),p(A,C,D)}; Table 3.1) were similar for all groups (0.94, 0.96, and 0.96 for accelerated, control, and delayed foster nests, respectively). Based on

these results, differential survival costs to offspring within a clutch due to synchronous hatching do not appear to be significant in Canada geese.

Several costs of alternative development within clutches of synchronously hatching broods other than survival may be incurred that were not measured in this study. Nilsson and Persson (2004) reported an increased occurrence of leg deformities and dehydration in synchronously hatching broods of mallards and pheasants (*Phasianus colchicus*). While these conditions may reduce survival in the post-hatching environment, they may also reduce hatching efficiency. Canada geese will depart nests within 24 hours of eggs hatching (Cooper and Hickin 1972, Cooper 1978), and this may be a strong limiting factor on selective pressure acting on mechanisms regulating synchronous hatching in waterfowl. Moreover, there may be long-term costs associated with differential rates of development in the early life stages (Metcalfe and Monaghan 2001) that are not detectable in the first few weeks following hatching.

The lack of differences in early gosling survival came despite significant differences in the length of the developmental period among foster nest groups. Goslings hatched from eggs from the accelerated foster nests experienced an incubation period over three days shorter than goslings hatched from eggs from the delayed foster nests (Figure 3.1). Moreover, the changes in the duration of the incubation stage are similar to maximum limits (in each direction) reported for other species (mallards, ring-necked pheasants, Japanese quail, and bobwhite quail, Vince 1966, 1968; Persson and Andersson 1999; Nilsson and Persson 2004). Therefore, my finding that gosling survival after hatching was not related to

developmental rate was not due to an insufficient change in the duration of the incubation period.

In conclusion, alternative development plays a critical role in facilitating hatching synchrony in Canada goose broods, yet differential costs in survival among siblings immediately following hatching appear negligible. Other costs such as the risk of female departure prior to complete hatching of the brood appear to be greater than costs in early survival. Moreover, there may be long-term consequences (e.g., maturation rate, longevity) to alternative rates of embryonic development that have bearing on recruitment. Therefore, further understanding the mechanisms regulating development may be useful to a complete understanding of the ultimate factors affecting changes in waterfowl populations.

LITERATURE CITED

Afton, A. D. 1979. Incubation temperatures of the northern shoveler. Canadian Journal of Zoology 57:1052-1056.

Ankeny, C. D. 1980. Egg weight, survival, and growth of lesser snow goose goslings. Journal of Wildlife Management 44:174-182.

Arnold, T. W., F. C. Rohwer, and T. Armstrong. 1987. Egg viability, nest predation, and the adaptive significance of clutch size in prairie ducks. American Naturalist 130(5):643-653.

_____, T. W. 1993. Factors affecting egg viability and incubation time in prairie dabbling ducks. Canadian Journal of Zoology 71:1146-1152.

Badzinski, S. S., C. D. Ankeny, J. O. Leafloor, and K. F. Abraham. 2002. Growth and development of prefledging Canada geese and lesser snow geese: Ecological Adaptation or Physiological Constraint? Auk 119(1):221-227.

Batt, B. D. J. and G. W. Cornwell. 1972. The effects of cold on mallard embryos. Journal of Wildlife Management 36(3):745-751.

Bellrose, F. C. 1980. Ducks, Geese and Swans of North America. Stackpole Books. Harrisburg, Pa.

Briskie, J. V. and S. G. Sealy. 1989. Nest-failure and the evolution of hatching asynchrony in the least flycatcher. Journal of Animal Ecology 58:653-665.

Brua, R. B. 2002. Avian Incubation: Behaviour, Environment, and Evolution. “Parent-embro interactions.” edited by D. C. Deeming, Oxford University Press Inc., New York.

Burnham, K. P. and D. R. Anderson. 2002. Model selection and multi-model inference: a practical information-theoretic approach. Second edition. Springer Verlag, New York.

Caldwell, P. J. and G. W. Cornwell. 1975. Incubation behavior and temperatures of the mallard duck. Auk 92:706-731.

Cannon, M. E., R. E. Carpenter, and R. A. Ackerman. 1986. Synchronous hatching and oxygen consumption of darwin's rhea eggs (*Pterocnemia pennata*). Physiological Zoology 59(1):95-108.

Cargill, S. M. and F. Cooke. 1981. Correlation of laying and hatching sequences in clutches of the lesser snow goose (*Anser caerulescens caerulescens*). Canadian Journal of Zoology 59:1201-1204.

Clark, A. B. and D. S. Wilson. 1981. Avian breeding adaptations: Hatching asynchrony, brood reduction, and nest failure. Quarterly Review of Biology 56:253-277.

Cooper, J. A. and J. R. Hickin. 1972. Chronology of hatching by laying sequence in Canada geese. Wilson Bulletin 84(1):90-92.

_____, J. A. 1978. The history and breeding biology of the Canada geese of Marshy Point, Manitoba. Wildlife Monographs 61:1-87.

Davies, J. C. and F. Cooke. 1983. Intraclutch hatch synchronization in the lesser snow goose. Canadian Journal of Zoology 61:1398-1401.

Ellis, L. A., J. D. Styrsky, R. C. Dobbs, and C. F. Thompson. 2001. Female condition: A predictor of hatching synchrony in the house wren? Condor 103:587-591.

Farner, D. S., J. R. King, and K. C. Parkes. 1985. Avian Biology Volume VIII, Academic Press, Inc., New York.

Findlay, C. S. and F. Cooke. 1982. Breeding synchrony in the lesser snow goose (*Anser caerulescens caerulescens*). I. Genetic and environmental components of hatch date variability and their effects on hatch synchrony. Evolution 36(2):342-351.

Forsythe, D. M. 1971. Clicking in the egg-young of the long-billed curlew. Wilson Bulletin 83:441-442.

Hébert, P. N. 2002. Avian Incubation: Behaviour, Environment, and Evolution. “Ecological factors affecting initiation of incubation behaviour.” edited by D. C. Deeming, Oxford University Press Inc., New York.

Hess, E. H. 1972. Imprinting in a natural laboratory. Scientific American. 227:24-31.

Kear, J. 1970. The adaptive radiation of parental care in waterfowl. “Social behavior of birds and mammals.” Edited by J. H. Crook. Academic Press. New York, NY.

Kennamer, R. A., W. F. Harvey IV, and G. R. Hepp. 1990. Embryonic development and nest attentiveness of wood ducks during egg laying. Condor 92(3):587-592.

Lambrechts, M. M., P. Perret, and J. Blondel. 1996. Adaptive differences in the timing of egg laying between different populations of birds results from variation in photoresponsiveness. Proceedings of the Royal Society of London B 263:19-22.

Lamey, T. C. and C. S. Lamey. 1994. Hatch synchrony and bad food years. American Naturalist 143:734-738.

Lebreton, J-D., K. P. Burnham, J. Clobert, and D. R. Anderson. 1992. Model survival and testing biological hypothesis using marked animals: A unified approach with case studies. Ecological Monograph 62:67-118.

Loos, E. R. and F. C. Rohwer. 2004. Laying-stage nest attendance and onset of incubation in prairie nesting ducks. Auk 121(2):587-599.

McCoshen, J. A. and R. P. Thompson. 1968. A study of clicking and its source in some avian species. Canadian Journal of Zoology 46:169.

Metcalfe, N. B. and P. Monaghan. 2001. Compensation for a bad start: Grow now, pay later? Trends in Ecology and Evolution 16:254-260.

Nicolai, C. A., J. S. Sedinger, and M. L. Wege. 2004. Regulation of development time and hatch synchronization in black brant (*Branta bernicla nigricans*). Functional Ecology 18:475-482.

Nilsson, J-A. and I. Persson 2004. Postnatal effects of incubation length in mallard and pheasant chicks. OIKOS 105:588-594.

Orcutt, A. B. 1974. Sound produced by hatching Japanese quail as potential aids to synchronous hatching. Behavior 50:173-184.

Persson, I. and G. Andersson. 1999. Intraclutch hatch synchronization in pheasants and mallard ducks. Ethology 105:1087-1096.

Prince, H. H., P. B. Siegel, and G. W. Cornwell. 1969. Hatchability, clutch position, and hatching sequence in mallards. Auk 86:762-763.

Raveling, D. G. 1978. The timing of egg laying by northern geese. Auk 95:294-303.

Sockman, K. W. and H. Schwabl. 2001. Covariation of clutch size, laying date, and incubation tendency in the American kestrel. Condor 103 (3):570–578.

Stoleson, S. H. and S. R. Beissinger. 1999. Egg viability as a constraint on hatching synchrony at high ambient temperatures. Journal of Animal Ecology 68:951-962.

Veiga, J. P. 1992. Hatching asynchrony in the house sparrow: A test of the egg viability hypothesis. American Naturalist 139:669-675.

Vince, M. A. 1966. Artificial acceleration of hatching in quail embryos. Animal Behaviour 14:389-394.

_____, M. A. 1968. Retardation as a factor in the synchronization of hatching. Animal Behaviour 16:332-335.

Vinuela, J. 2000. Opposing selective pressures on hatching asynchrony: Egg viability, brood reduction, and nestling growth. Behavioral Ecology and Sociobiology 48:333-343.

Vleck, C. M., D. F. Hoyt, and D. Vleck. 1979. Metabolic of avian embryos: Patterns in altricial and precocial birds. Physiological Zoology 52(3):363-377.

_____, C. M., D. Vleck and D. F. Hoyt. 1980. Patterns of metabolism and growth in avian embryos. American Zoologist 20:405-416.

_____, D., C. M. Vleck, and D. F. Hoyt. 1985. Physiological correlates of synchronous hatching in Rhea eggs (*Rhea americana*). Proc. XVIII Int. Ornithol. Cong. 18:864-872.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study. Vol. 46, supplement 120-139.

OVERALL SUMMARY

Avian eggs develop externally; therefore, allocation of egg constituents, egg construction, and incubation behavior can have significant consequences for offspring development and performance that follow hatching. A female's ability to allocate resources to offspring during egg formation is directly related to her fitness, and the amount of resources available to offspring is directly related to their fitness. Hence, patterns in resource allocation presumably reflect an optimal allocation for female fitness but provide offspring with differential likelihoods for success.

In Canada geese (*Branta canadensis maxima),* both size and hormone allocations decrease for offspring from eggs laid later in a clutch. I found that egg size is largest for eggs laid second and third in a clutch, and declines with subsequently laid eggs. I found that maternally deposited yolk testosterone follows a similar pattern, but maternally deposited yolk estradiol did not vary (and levels were low) across the laying sequence. Egg size and maternally derived yolk testosterone are known to affect development and performance in birds (Dawson and Clark 1996, Lipar et al. 1999b).

Offspring of Canada geese, like other waterfowl species, hatch within a 24-hour period despite coming from eggs that were laid as much as ten days apart (Cooper 1978, Cooper and Hickin 1972). Incubation begins shortly after laying (typically when the third egg is laid), and therefore, the embryos exhibit unequal developmental rates, indicating there are internal or external mechanisms to synchronize hatching (Vince 1966, 1968; MacCluskie et al. 1997; Nicolai et al.

2004). In this study, I tried to identify potential mechanisms that promote synchronous hatching of Canada geese, focusing on maternal yolk hormone deposition during egg formation. Variation in embryonic metabolic rates in Canada geese was best explained by position of the egg in the laying sequence (an intrinsic factor) rather than egg size (another intrinsic factor) or incubation site (an extrinsic factor). Because patterns in maternally derived yolk testosterone are similar to the patterns of egg size within a clutch, it appears that yolk testosterone does not regulate embryonic metabolism in geese. More research is needed on within-clutch variation in egg shell characteristics and their relationship with embryonic oxygen consumption rates.

Furthermore, the long-term implications of differential developmental rates are not known. However, I found that gosling metabolism within 24-hours following hatching was not related to embryonic metabolic rate or position in the laying sequence. Moreover, initial survival in the two weeks following hatching was not affected by acceleration or delay in the duration of the incubation period. A thorough understanding of the long-term implications of differential resource allocation among offspring and proximate mechanisms regulating alternative developmental rates among offspring is critical to understanding the ultimate factors driving recruitment in waterfowl populations.

LITERATURE CITED

Afton, A. D. 1979. Incubation temperatures of the northern shoveler. Canadian Journal of Zoology 57:1052-1056.

Ankeny, C. D. 1980. Egg weight, survival, and growth of lesser snow goose goslings. Journal of Wildlife Management 44:174-182.

Ar, A. and H. Rahn. 1985. Pores in avian eggshells: Gas conductance and embryonic growth rate. Respiration Physiology 61:1-20.

Arnold, T. W., F. C. Rohwer, and T. Armstrong. 1987. Egg viability, nest predation, and the adaptive significance of clutch size in prairie ducks. American Naturalist 130(5):643-653.

_____, T. W. 1993. Factors affecting egg viability and incubation time in prairie dabbling ducks. Canadian Journal of Zoology 71:1146-1152.

Badzinski, S. S., C. D. Ankeny, J. O. Leafloor, and K. F. Abraham. 2001. Composition of eggs and neonates of Canada geese and lesser snow geese. Auk 118(3):687-697.

_____, S. S., C. D. Ankeny, J. O. Leafloor, and K. F. Abraham. 2002. Growth and development of prefledging Canada geese and lesser snow geese: Ecological Adaptation or Physiological Constraint? Auk 119(1):221-227.

Bahr, J. M., S-C. Wang, M. Y. Huang, and F. O. Calvo. 1983. Steroid concentrations in isolated theca and granulose layers of preovulatory follicles during the ovulatory cycle of the domestic hen. Biology of Reproduction 29:26-334.

Batt, B. D. J. and G. W. Cornwell. 1972. The effects of cold on mallard embryos. Journal of Wildlife Management 36(3):745-751.

Bellrose, F. C. 1980. Ducks, Geese and Swans of North America. Stackpole Books. Harrisburg, Pa.

Bentley, P. J. 1998. Comparative Vertebrate Endocrinology. Cambridge University Press, United Kingdom.

Booth, D. T. and P. R. Sotherland. 1991. Oxygen consumption, air-cell gas tensions, and incubation parameters of mute swan eggs. Physiological Zoology 64(2):473-484.

Briskie, J. V. and S. G. Sealy. 1989. Nest-failure and the evolution of hatching asynchrony in the least flycatcher. Journal of Animal Ecology 58:653-665.

Brua, R. B. 2002. Avian Incubation: Behaviour, Environment, and Evolution. “Parent-embryo interactions.” edited by D. C. Deeming, Oxford University Press Inc., New York.

Burnham, K. P. and D. R. Anderson. 2002. Model selection and multi-model inference: A practical information-theoretic approach. Second edition. Springer Verlag, New York.

Caldwell, P. J. and G. W. Cornwell. 1975. Incubation behavior and temperatures of the mallard duck. Auk 92:706-731.

Cannon, M. E., R. E. Carpenter, and R. A. Ackerman. 1986. Synchronous hatching and oxygen consumption of darwin's rhea eggs (*Pterocnemia pennata*). Physiological Zoology 59(1):95-108.

Cargill, S. M. and F. Cooke. 1981. Correlation of laying and hatching sequences in clutches of the lesser snow goose (*Anser caerulescens caerulescens*). Canadian Journal of Zoology 59:1201-1204.

Clark, A. B. and D. S. Wilson. 1981. Avian breeding adaptations: hatching asynchrony, brood reduction, and nest failure. Quarterly Review of Biology 56:253-277.

Cooper, J. A. and J. R. Hickin. 1972. Chronology of hatching by laying sequence in Canada geese. Wilson Bulletin 84(1):90-92.

_____, J. A. 1978. The history and breeding biology of the Canada geese of Marshy Point, Manitoba. Wildlife Monographs 61:1-87.

Davies, J. C. and F. Cooke. 1983. Intraclutch hatch synchronization in the lesser snow goose. Canadian Journal of Zoology 61:1398-1401.

Dawson, R. D. and R. G. Clark. 1996. Effects of variation in egg size and hatching date on survival of lesser scaup (*Aythya affinis*) ducklings. Ibis 138(4):693-699.

Dietz, M. W., M. Kampen,M. J. M. van Griensven, and S. van Mourik. 1998. Daily energy budgets of avian embryos: The paradox of the plateau phase in egg metabolic rate. Physiological Zoology 71(2):147-156.

Eising, C., C. Eikenaar, H. Schwabl, and T. G. G. Groothuis. 2001. Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: Consequences for chick development. Proceedings of the Royal Society of London B 268:839-846.

_____, C. M., W. Müller, C. Dijkstra, and T. G. G. Groothuis. 2003. Maternal androgens in egg yolks: relation with sex, incubation time and embryonic growth. General and Comparative Endocrinology 132:241-247.

_____, C. M., G. H. Visser, W. Müller, and T. G. G. Groothuis. 2003. Steroids for free? No metabolic costs of elevated maternal androgen levels in the black-headed gull. Journal of Experimental Biology 206:3211-3218.

Ellis, L. A., J. D. Styrsky, R. C. Dobbs, and C. F. Thompson. 2001. Female condition: A predictor of hatching synchrony in the house wren? Condor 103:587-591.

Findlay, C. S. and F. Cooke. 1982. Breeding synchrony in the lesser snow goose (*Anser caerulescens caerulescens*). I. Genetic and environmental components of hatch date variability and their effects on hatch synchrony. Evolution 36(2):342-351.

Flint, P. L. and J. S. Sedinger. 1992. Reproductive implications of egg-size variation in the black brant. Auk 109 (4):896-903.

Forsythe, D. M. 1971. Clicking in the egg-young of the long-billed curlew. Wilson Bulletin 83:441-442.

Gil, D., J. A. Graves, N. Hazon, and A. Wells. 1999. Male attractiveness and differential testosterone investment in zebra finch eggs. Science 286:126-128.

_____, D., C. Heim, E. Bulmer, M. Rocha, M. Puerta, and M. Naguib. 2004. Negative effects of early development stress on yolk testosterone levels in a passerine bird. Journal of Experimental Biology 207:2215-2220.

Gille, U., F. V. Salomon, and J. Ronnert. 1999. Growth of the digestive organs in ducks with considerations on their growth in birds in general. British Poultry Science 40(2):194-202.

Grau, C. R. 1976. Ring structure of avian egg yolk. Poultry Science 55:1418-1422.

Groothuis, T. G. G. and H. Schwabl. 2002. Determinants of within- and among-clutch variation in levels of maternal hormones in black-headed gull eggs. Functional Ecology 16:281-289.

_____, T. G. G., W. Müller, N. von Engelhardt, C. Carere, and C. Eising. 2005. Maternal Hormones as a Tool to Adjust Offspring Phenotype in Avian Species. Neuroscience and Biobehavioral Reviews 29:329-352.

Hamilton, K. L. 1985. Metabolism of barn owl eggs. American Midland Naturalist 114(2):209-215.

Hébert, P. N. 2002. Avian Incubation: Behaviour, Environment, and Evolution. “Ecological factors affecting initiation of incubation behaviour.” edited by D. C. Deeming, Oxford University Press Inc., New York.

Hess, E. H. 1972. Imprinting in a natural laboratory. Scientific American. 227:24-31.

Hirschenhauser, K., E. Möstl, and K. Kotrschal. 1999. Seasonal patterns of sex steroids determined from feces of different social categories of greylag geese *Anser anser*. General Comparative Endocrinology 114:67-79.

Hoyt, D. F., D. Vleck, and C. M. Vleck. 1978. Metabolism of avian embryos: Ontogeny and temperature effects in the ostrich. Condor 80:265-271.

_____, D. F., R. G. Board, H. Rahn, and C. V. Paganelli. 1979. The eggs of the anatidae: Conductance, pore structure, and metabolism. Physiological Zoology 52:438-450.

Janoschek, A. 1957. Das reaktionskinetische Grundgesetz und seine Beziehungen zum Wachstums- und Ertragsgesetz. Stat. Vjschr. 10:25-37.

Johnson, A. L. and A. van Tienhoven. 1980. Plasma concentrations of six steroids and LH during the ovulatory cycle of the hen, *Gallus domesticus*. Biology of Reproduction 23:386-393.

Kear, J. 1970. The adaptive radiation of parental care in waterfowl. Social behavior of birds and mammals. Edited by J. H. Crook. Academic Press. New York, NY.

Kennamer, R. A., W. F. Harvey IV, and G. R. Hepp. 1990. Embryonic development and nest attentiveness of wood ducks during egg laying. Condor 92(3):587-592.

Lambrechts, M. M., P. Perret, and J. Blondel. 1996. Adaptive differences in the timing of egg laying between different populations of birds results from variation in photoresponsiveness. Proceedings of the Royal Society of London B 263:19-22.

Lamey, T. C. and C. S. Lamey. 1994. Hatch synchrony and bad food years. American Naturalist 143:734-738.

Leblanc, Y. 1987. Intraclutch variation in egg size of Canada geese. Canadian Journal of Zoology 65:3044-3047.

Lebreton, J-D., K. P. Burnham, J. Clobert, and D. R. Anderson. 1992. Model survival and testing biological hypothesis using marked animals: A unified approach with case studies. Ecological Monograph 62:67-118.

Loos, E. R. and F. C. Rohwer. 2004. Laying-stage nest attendance and onset of incubation in prairie nesting ducks. Auk 121(2):587-599.

Lipar, J. L., E. D. Ketterson, and V. Nolan Jr. 1999a. Intraclutch variation in testosterone content of red-winged blackbird eggs. Auk 116(1):231-235.

_____, J. L., E. D. Ketterson, V. Nolan Jr., and J. M. Casto. 1999b. Egg yolk layers vary in the concentration of steroid hormones in two avian species. General and Comparative Endocrinology 115:220-227.

_____, J. L. and E. D. Ketterson. 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-wing blackbird *Agelaius phoeniceus*. Proceedings of the Royal Society of London B 267:2005-2010.

_____, J. L. 2001. Yolk steroids and the development of the hatching muscle in nestling European starling. Journal of Avian Biology 32:231-238.

MacCluskie, M. C., P. L. Flint, and J. S. Sedinger. 1997. Variation in incubation periods and egg metabolism in mallards: Intrinsic mechanisms to promote hatch synchrony. Condor 99:224-228.

Mazuc, J., C. Bonneaud, O. Chastel, and G. Sorci. 2003. Social environment affects female and egg testosterone levels in the house sparrow (*Passer domesticus*). Ecology Letters 6:1084-1090.

Metcalfe, N. B., and P. Monaghan. 2001. Compensation for a bad start: Grow now, pay later? Trends in Ecology and Evolution 16:254-260.

Michl, G., J. Török, P. Péczely, L. Z. Garamszegi, and H. Schwabl. 2004. Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. Behavioral Ecology 16(2):383-388.

Moe, B., E. Stølevik, and C. Bech. 2005. Ducklings exhibit substantial energy-saving mechanisms as a response to short-term food shortage. Physiological and Biochemical Zoology 78(1):90-104.

McCoshen, J. A. and R. P. Thompson. 1968. A study of clicking and its source in some avian species. Canadian Journal of Zoology 46:169.

Nelson, R. J. 2000. Parental Behavior: An introduction to behavioral endocrinology. Sinauer Associates, Inc., Sunderland, Massachusetts.

Nice, M. M. 1962. Development of behavior in precocial birds. Transactions of the Linnean Society of New York. 81:1-211.

Nicolai, C. A., J. S. Sedinger, and M. L. Wege. 2004. Regulation of development time and hatch synchronization in black brant (*Branta bernicla nigricans*). Functional Ecology 18:475-482.

Nilsson, J-A. and I. Persson 2004. Postnatal effects of incubation length in mallard and pheasant chicks. OIKOS 105:588-594.

Orcutt, A. B. 1974. Sound produced by hatching Japanese quail as potential aids to synchronous hatching. Behavior 50:173-184.

Paganelli, C. V., A. Ar, and H. Rahn. 1973. Relative water vapor permeabilities of the shell and shell membranes of the hen's egg. Physiologist 16:415.

_____, C.V., R. A. Ackerman, and H. Rahn. 1978. The avian egg: in vivo conductance to oxygen, carbon dioxide, and water vapor in late incubation. Pages 212-218 in J. Piiper, ed. Respiratory functions in birds, adult and embryonic. Springer, Berlin.

Persson, I. and G. Andersson. 1999. Intraclutch hatch synchronization in pheasants and mallard ducks. Ethology 105:1087-1096.

Pilz, K. M., H. G. Smith, M. I. Sandell, and H. Schwabl. 2003. Interfemale variation in egg yolk androgen allocation in the European starling: Do high-quality females invest more? Animal Behaviour 65:841-850.

_____, K. M., M. Quiroga, H. Schwabl, E. Adkins-Regan. 2004. European starling chicks benefit from high yolk testosterone levels during a drought year. Hormones and Behavior 46:179-192.

Prince, H. H., P. B. Siegel, and G. W. Cornwell. 1969. Hatchability, clutch position, and hatching sequence in mallards. Auk 86:762-763.

Rahn, H. and A. Ar. 1974. The avian egg: Incubation and water loss. Condor 76:147-152.

_____, H., A. Ar, and C. V. Paganelli. 1979. How bird eggs breathe. Scientific American. 240:46-55.

_____, H. and C. V. Paganelli. 1990. Gas fluxes in avian eggs: Driving forces and the pathway for exchange. Comparative Biochemistry and Physiology 95A(1):1-15.

_____, H., C. V. Paganelli, and A. Ar. 1974. The avian egg: Air-cell gas tension, metabolism and incubation time. Respiration Physiology 22:297-309.

_____, H., C. V. Paganelli, and A. Ar. 1987. Pores and gas exchange of avian eggs: a review. Journal of Experimental Zoology Supplement 1:165-172.

Raveling, D. G. 1978. The timing of egg laying by northern geese. Auk 95:294-303.

Reed, W. L. and C. Vleck. 2001. Functional significance of variation in egg-yolk androgens in the American coot. Oecologia 128:164-171.

Romanoff, A. L. and A. J. Romanoff. 1949. The avian egg. Academic Press. New York.

Royle, N. J., P. F. Surai, and I. R. Hartley. 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects. Behavioral Ecology 12(4):381-385.

Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Science 90:11446-11450.

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

_____, H. 1996b. Maternal testosterone in the avian egg enhances postnatal growth. Comparative Biochemistry and Physiology 114A(3):271-276.

_____, H., D. W. Mock, and J. A. Gieg. 1997. A hormonal mechanism for parental favouritism. Nature 386:231.

Seymour, R. S. and A. H. J. Visschedijk. 1988. Effects of variation in total and regional shell conductance on air cell gas tensions and regional gas exchange in chicken eggs. Journal of Comparative Physiology B 158:229-236.

Shahabi, N.A., H. W. Norton, and A. V. Nalbandov. 1975. Steroid levels in follicles and the plasma of hens during the ovulatory cycle. Endocrinology 96:962-968.

Sidor, C. A. and D.G. Blackburn. 1998. Effects of testosterone administration and castration on the forelimb musculature of male leopard frog (*Rana pipiens*). Journal of Experimental Zoology 280:28-37.

Slagsvold, T. 1986. Asynchronous versus synchronous hatching in birds: Experiments with the pied flycatcher. Journal of Animal Ecology 55:1115-1134.

Sockman, K. W. and H. Schwabl. 1999. Daily estradiol and progesterone levels relative to laying and onset of Incubation in canaries. General and Comparative Endocrinology 114:257-268.

_____, K. W. and H. Schwabl. 2000. Yolk androgens reduce offspring survival. Proceedings of the Royal Society of London B 267:1451-1456.

_____, K. W. and H. Schwabl. 2001. Covariation of clutch size, laying date, and incubation tendency in the American kestrel. Condor 103(3):570–578.

Stoleson, S. H. and S. R. Beissinger. 1999. Egg viability as a constraint on hatching synchrony at high ambient temperatures. Journal of Animal Ecology 68:951-962.

Turner, J.S. 2002. Avian Incubation: Behaviour, environment, and evolution. “Maintenance of egg temperature”. edited by D. C. Deeming, Oxford University Press Inc., New York.

Tyler, C. 1964. A study of the egg shells of the anatidae. Proceedings of the Zoological Society of London 142:547-583.

Veiga, J. P. 1992. Hatching asynchrony in the house sparrow: A test of the egg viability hypothesis. American Naturalist 139:669-675.

Verboven, N., P. Monaghan, D. M. Evans, H. Schwabl, N. Evans, C. Whitelaw, and R. G. Nager. 2003. Maternal condition, yolk androgens and offspring performance: A supplemental feeding experiment in the lesser black-backed gull (*Larus fuscus*). Proceedings of the Royal Society of London B 270:2223-2232.

Vince, M. A. 1964. Potential stimulation produced by avian embryos. Animal Behaviour 14:34-40.

_____, M. A. 1966. Artificial acceleration of hatching in quail embryos. Animal Behaviour 14:389-394.

_____, M. A. 1968. Retardation as a factor in the synchronization of hatching. Animal Behaviour 16:332-335.

Vinuela, J. 2000. Opposing selective pressures on hatching asynchrony: Egg viability, brood reduction, and nestling growth. Behavioral Ecology and Sociobiology 48:333-343.

Vleck, C. M., D. F. Hoyt, and D. Vleck. 1979. Metabolic of avian embryos: Patterns in altricial and precocial birds. Physiological Zoology 52(3):363-377.

_____, D., C. M. Vleck, and D. F. Hoyt. 1980a. Metabolism of avian embryos: Ontogeny of oxygen consumption in the rhea and emu. Physiological Zoology 53(2):125-135.

_____, C. M., D. Vleck and D. F. Hoyt. 1980b. Patterns of metabolism and growth in avian embryos. American Zoologist 20:405-416.

_____, D., C. M. Vleck, and D. F. Hoyt. 1985. Physiological correlates of synchronous hatching in rhea eggs (*Rhea americana*). Proc. XVIII Int. Ornithol. Cong. 18:864-872.

_____, C. M. and D. Vleck. 1987. Metabolism and energetics of avian embryos. American Zoologist 20:405-416.

_____, D. 1987. Measurement of O_2 consumption, CO_2 production, and water vapor production in a closed system. Journal of Applicational Physiology 62(5):2103-2106.

Whittingham, L. A. and H. Schwabl. 2002. Maternal testosterone in tree swallow eggs varies with female aggression. Animal Behaviour 63:63-67.

Williams, T. D., A. S. Kitaysky, and F. Vézina. 2004. Individual variation in plasma estradiol-17β and androgen levels during egg formation in the European starling *Sturnus vulgaris*: implications for regulation of yolk steroids. General and Comparative Endocrinology 136:346-352.

Wingfield, J. C., and D. S. Farner. 1975. The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. Steroids 26:311-327.

_____, J. C., K. S. Matt, and D. S. Farner. 1984. Physiologic properties of steroid hormone-binding proteins in avian blood. General and Comparative Endocrinology 53:281-292.

Winkler, D. W. 1993. Testosterone in egg yolks: An ornithologist's perspective. Proceedings of the National Academy of Science USA 90:11439-11441.

White, G. C. and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study. Vol. 46, supplement 120-139.

APPENDIX A

Competitive Binding Radioimmunoassay for T and E_2

Individual standard curves and samples were conducted for each assay of T and E_2. Standard curves comprised of B1-3, {B1-total counts per min (CPM), B2- non-specific bound, and B3- maximum bound}, and S1-9 {serial dilution of cold "unlabeled" steroid-(500-1.953 pg)}. I used stripped buffer solution in place of PBSg buffer in the standard curve, simulating complete yolk samples and eliminating chromatography columns. The competitive binding portion of T and E_2 assays involved the addition of 100 µl of labeled steroids (500 pg) and antiserum to standard curve and samples (exception, B1 and B2s received no antiserum). Samples were stored overnight at 4°C to enhance equal proportions of labeled and unknown steroids to bind to antiserum. The following day, unbound hormones were removed from samples with the addition of 500 µl of dextran-charcoal in PBSg (B1s received only PBSg buffer). Samples were centrifuged 12 minutes at 2,500 rpm following the addition of charcoal to the last sample and decanted into scintillation vials with 4.5 ml of scintillation fluid.

Standard curve and sample counts were measured in five minute intervals at room temperature using a scintillation counter (Beckman Coulter, LS 6500 multipurpose). I averaged the counts per minute values for B1-3s and determined percentage bound in standard curve dilutions S1-9, along with yolk samples by the following:

$$\% Bound = \left(\frac{sample - B2}{B3 - B2} \right) \cdot 100$$

A logit transformation of percentage bound was applied to standard curve values as well as samples as follows:

$$Logit(\%Bound) = \ln\left(\frac{\%Bound}{100 - \%Bound}\right)$$

The logit transformation linearizes "S"-shaped curves (which is typical of the standard curve for steroid serial dilutions) such that a linear regression can be fit to the standard curve S1-9 duplicate count per minute values and the corresponding log-transformed level of hormone (pg hormone) determined in the serial dilution. The slope (A_1) and intercept (A_0) parameters from the regression were then used to calculate hormone levels (H, pg hormone) in the yolk samples via:

$$H = 10^{\left(\frac{Logit(Sample\%Bound) - A_0}{A_1}\right)}$$

To adjust calculations for hormone loss during extraction, percentage recovery rates (% Recovered) for samples was determined by:

$$\% \text{ Rec} = \left\{\frac{R_{CPM} \cdot \left(T_v / R_v\right)}{I_{CPM}}\right\} \cdot 100$$

where R_{CPM} represents the counts per minute from recovery vials, T_v is the total volume of PBSg in which buffer samples were reconstituted (recovery volume + 400 μl), R_v is remaining volume aliquoted to recovery scintillation vials, and I_{CPM} represents the initial counts per minute of labeled steroid from the initial spiking of samples.

An estimate of steroid concentration (Steroid, pg of hormone per mg of yolk) was then calculated based on recoveries and the initial amount of yolk as:

$$Steroid = \frac{H \cdot \left(T_v / 200\mu l \right)}{\left(100 / \%\,\mathrm{Re}\,c \right) \cdot I_y}$$

where H (pg) is the mass of hormone determined prior to calculating percent recovery, Tv is the total volume of PBSg in which buffer samples were reconstituted (recovery volume + 400µl), % Rec is the percentage recovery calculated for each sample, and I_y is initial amount of yolk (mg wet).

Statistical Analysis

I standardized relative hormone concentrations from intra-clutch concentrations using the z-score:

$$z_{Si} = \frac{Steroid_i - \overline{Steroid}_{clutch}}{sd\ Steroid_{clutch}}$$

where $Steroid_i$ is the steroid concentration of egg i within a clutch, $\overline{Steroid}_{clutch}$ is the mean concentration of steroid for the all eggs in the clutch, and $sd\ Steroid_{clutch}$ is the standard deviation of steroid concentrations for the clutch.

To explain patterns in hormone levels across the laying sequence, I used simple linear and nonlinear regressions to assess trends in standardized hormone concentrations. I used the Akaike information criterion adjusted for small sample size (AIC_c, Burham and Anderson 2002) to rank the regression models. AIC_c ranks models based on parsimony by simultaneously accounting for explained variance as well as uncertainty (i.e., variance) in model parameter estimates. Because eggs within a clutch may not be independent, AIC_c values were computed using a sample size based on the number of clutches rather than the number of eggs sampled. Models with the lowest AIC_c value represent the most parsimonious

model in the suite of models compared. Model goodness-of-fit was assessed using the coefficient of determination (R^2).

An additional comparison of hormone levels across the laying sequence was performed using a simple t-test. Relative hormone concentrations for eggs laid early in the sequence (positions one through three) were compared to concentrations for eggs laid late in the sequence (positions four through eight).