REPRODUCTIVE ENERGETICS OF FEMALE GREATER SCAUP (*AYTHYA MARILA*): NUTRITIONAL AND PHYSIOLOGICAL CORRELATES OF TIMING AND STATE OF REPRODUCTION

by

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B.S. Biology, Dickinson College 1996

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In the

Department of

Biological Sciences

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SIMON FRASER UNIVERSITY

Summer 2005

APPROVAL

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Degree: Master of Science

Title of Thesis:

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Date Approved   June 30, 2005

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Abstract

I studied variation in energetics and physiology of female Greater Scaup (*Aythya marila*) during arrival and egg production on the Yukon-Kuskokwim Delta, Alaska, specifically in relation to timing and state of reproduction. I quantified ovarian follicle dynamics to assign reproductive states, estimate the duration of rapid follicle growth (RFG) (5.2 ± 0.52 days), and derive predictive models to assign RFG initiation dates for reproductive females. I determined that female Greater Scaup, unlike many other duck species, did not use endogenous lipid, protein, or mineral reserves during egg production, nor did nutritional status at RFG initiation vary by date. Nutrient reserves were smaller in nonreproductive than reproductive females. These results suggest that nutritional status influences timing of reproduction via condition thresholds for RFG initiation, yet acquired reserves are not used during egg production. Circulating concentrations of plasma vitellogenin and very low-density lipoprotein were characterized as accurate physiological predictors of reproductive state.

Dedication

In memory of my mother, Sandra Gorman, who provided the most excellent example of positivity and courage in the face of adversity during her battle with cancer over the last 18 months. I am fortunate to have had such a role model, and will do my best to remember her stellar attitude whenever my life and future scientific endeavours prove difficult.

Acknowledgements

Studying female Greater Scaup during the breeding season on the Yukon-Kuskokwim Delta, in western Alaska was an experience of a lifetime, through which I grew tremendously as a scientist. This project enabled me to explore my interests in ecological energetics, become familiar with new physiological and biogeochemical techniques, in addition to learning more about information-theoretic methods for model selection and multi-modal inference. I sincerely hope that the results from this thesis and extended projects prove important for conserving Greater Scaup, in addition to advancing our understanding of avian reproductive energetics and physiology.

I am greatly indebted to my supervisory committee Drs. Dan Esler, Paul L. Flint, Lance F. W. Lesack, and Tony D. Williams for their scientific guidance throughout the various stages of this thesis. Furthermore, Drs. Esler, Flint, and Williams provided financial support for this work. I thank Dr. Sean Boyd for serving as Public Examiner during the thesis defense. Dr. Esler served as primary advisor, and always provided tremendous help with presentations, data analysis, and manuscript preparations. Dr. Flint deserves much credit for providing a high quality research camp through which this thesis work was conducted, as well as rigorous feedback on manuscripts. Dr. Williams provided an invaluable introduction to the fields of ecological and evolutionary physiology. I consider myself extremely fortunate to have had the opportunity to work with these scientists, and hope that I have met their expectations for the work they envisioned for this project.

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Chapter 1.

Avian reproductive energetics: implications for North American scaup declines

The metabolic cost of egg production,
fitness consequences, and the role of female condition

Primary reproductive effort in birds (i.e., egg synthesis) involves the production of yolk, albumen, and eggshell which are derived from lipid, protein, and mineral macronutrients (Carey 1996). Theoretical models of the metabolic cost of this physiological process estimate that passerine oogenesis results in basal metabolic rate (BMR) increases of 45-60% (Ricklefs 1974, Carey 1996, Monaghan and Nager 1997), while such estimates can exceed 200% of the female's BMR for precocial species due to the production of large clutches of energy-dense eggs (Alisauskas and Ankney 1992, Robbins 1993). Recent empirical studies (e.g., Nilsson and Raberg 2001, Vézina and Williams 2002) have found that the metabolic cost of passerine egg production is lower (22-27%) than that estimated by theoretical models, based on actual measurement of resting metabolic rate (RMR) during oogenesis. Similar studies have not been conducted on precocial species, thus, more accurate estimates of the metabolic cost of egg production for the waterfowl are not available. Nevertheless, primary reproductive effort is increasingly recognized as an important component of the energetic cost of reproduction in avian systems (Monaghan and Nager 1997), particularly for ducks and geese (Alisauskas and Ankney 1992).

The nutritional and energetic costs of egg production can have important consequences for individual fitness (Ricklefs 1996, Zera and Harshman 2001) often via effects on primary reproductive effort decisions such as nest initiation date and clutch size. These two traits have been shown to covary across an array of species such that a decline in clutch size is associated with later nest initiation dates (Lack 1968, Reynolds 1972, Birkhead and Nettleship 1982, Toft et al. 1984, Murphy 1986). In addition to the fitness consequences of reduced clutch sizes, young hatched from late laid nests

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typically exhibit reduced post-fledging survival and recruitment probabilities (Perrins 1966, Cooke et al. 1984). Delayed nest initiation also results in fewer opportunities for renesting (Rohwer 1992). Thus, understanding the proximate factors driving intraspecific variation in timing of reproduction has been a longstanding objective among evolutionary ecologists, and is particularly important for considering population demographics and conservation implications.

Darwin (1871) and Fisher (1958) both proposed an influence of female "health" on the date at which birds breed and their fecundity. Lack (1968), and in particular Perrins (1970), hypothesized that timing of reproduction is constrained by the energetic and nutritional costs of egg synthesis early in the season when food is less available, preventing some females from breeding at an optimal date for hatchling survival. In fact, a number of experimental studies involving supplemental feeding have addressed the role of food availability in timing of avian reproduction (reviewed in Daan et al. 1988, Boutin 1990), demonstrating a strong proximate role of food resources in breeding phenology; additional food advances nest initiation dates in a number of taxa including the Red-winged blackbird (*Aeglaius phoeniceus*), Dunnock (*Prunella modularis*), Blue Tit (*Parus caeruleus*), and the Eurasian Kestrel (*Falco tinnunculus*) (Ewald and Rohwer 1982, Davies and Lundberg 1985, Nilsson and Svensson 1993, Aparicio 1994).

In light of the relationship between food availability, breeding phenology, and subsequent fecundity, strategies for acquiring reproductive nutrients such as lipid, protein, and mineral are an important consideration. In birds, the nutrients required for synthesis of reproductive tissues are derived from endogenously stored nutrient reserves, exogenous food resources, or a combination of these sources. Drent and Daan (1980) introduced the concepts of *capital* and *income* breeding tactics as strategies used in primary reproductive effort decisions concerning nest initiation date

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and clutch size. A capital breeder would base decisions concerning egg production on an absolute level of body reserves in relation to a changing threshold, while an income breeder would base decisions on the rate of reserve accumulation in relation to a fixed threshold. More recent interpretations of these ideas include that capital and income breeding are extremes in a continuum of strategies (Thomas 1989), and that capital breeders "use stored energy for reproduction" while income breeders "use energy acquired during the reproductive period" (Stearns 1989, 1992 p. 221-222, Jönsson 1997). Altricial birds are generally limited in their ability to carry endogenous nutrient reserves due to small structural sizes, and thus are largely dependent on exogenous food sources for nutrient allocation to the clutch (Perrins 1970, Perrins 1996). Precocial birds, such as larger-bodied waterfowl, use endogenous nutrient reserves to varying degrees from almost complete reliance (Lesser Snow Geese (*Chen caerulescens caerulescens*): Ankeny and MacInnes 1978), to mixed approaches using both endogenous reserves and exogenous food sources (Greater Snow Geese (*Chen caerulescens atlanticus*): Choinière and Gauthier 1995, Lesser Scaup (*Aythya affinis*): Esler et al. 2001), to complete reliance on exogenous food (Northern Shoveler (*Anas clypeata*): MacCluskie and Sedinger 2000). The relative contribution of these various nutrient sources for reproduction can also vary intraspecifically as the breeding season progresses (Northern Pintail (*Anas acuta*): Esler and Grand 1994).

Conceptual models have been developed to address relationships between nutrient acquisition, timing of breeding, and the quantity of offspring. Reynolds (1972) hypothesized that a seasonally declining threshold of endogenous nutrient reserves is associated with delayed nest initiation and smaller clutch sizes. Drent and Daan (1980) proposed the *cost of delay* hypothesis which suggested that optimal timing of reproduction and clutch size were the resolution of a conflict between the advantage of

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greater offspring value associated with early breeding and the advantage of a delay in breeding (i.e., accumulation of condition for increased clutch size). The important prediction from this hypothesis is that poor condition females gain more from a delay in breeding than birds in better condition. More recently, Rowe et al. (1994) formalized the ideas of Drent and Daan (1980) in a condition-dependent dynamic model for adaptive seasonal decline in clutch size. This theory predicts optimal combinations of nest initiation date and clutch size based on initial female condition as well as the onset and rate of condition gain.

In summary, female birds meet the energetic and nutritional costs of primary reproductive effort through a variety of strategies, from capital to income breeding tactics. However, if nutritional resources (i.e., exogenous or endogenous sources) are limited, reproductive performance may be constrained via effects on decisions concerning nest initiation date and clutch size. A large body of empirical work on both altricial and precocial species provides evidence for these relationships, furthermore, state-dependent theoretical models (Rowe et al. 1994) predict an influence of female condition on nest initiation date and clutch size such that birds in relatively poor condition breed later and produce smaller clutches than those in relatively better condition. Thus, within the context of declining populations, consideration of these topics lends insight into proximate influences of productivity, and therefore population dynamics.

Conservation rationale: numerical declines in North American scaup

The combined North American estimate of Greater and Lesser Scaup (*A. marila* and *affinis*) numbers has declined from 6.0 million in 1978 to a record low of 3.47 million estimated in 1998, representing an average decline of approximately 150,000 individuals

per year (Austin et al. 2000, Afton and Anderson 2001). Recent population estimates remain below the goal of 6.3 million established by the North American Waterfowl Management Plan (United States Fish and Wildlife Service and Canadian Wildlife Service 1986, United States Fish and Wildlife Service 1998). Conservation concerns over declining North American scaup populations have prompted scientists to identify a number of important research directions to better understand population dynamics such as associations between wintering grounds, migration routes, and nesting areas, annual survival and productivity in the major breeding regions, as well as the role of contaminants and nutritional status in reproductive performance (Austin et al. 2000).

Population trajectories differ markedly between the 3 major scaup breeding regions with the most serious decline estimated in boreal forest nesting scaup of subarctic Canada and eastern Alaska. This population is thought to consist primarily of Lesser Scaup. The prairie-parkland nesting population in the north central U.S. and south central Canada, also thought to be mostly Lesser Scaup, has increased recently (i.e. 1993-1997) while the tundra nesting population of western Alaska (Figure 1.1), considered primarily Greater Scaup, has remained stable since 1955 when breeding waterfowl surveys began (Austin et al. 2000, Afton and Anderson 2001). Austin et al. (2000) and Afton and Anderson (2001) suggested that numerical declines, particularly for Lesser Scaup, may be attributed to effects of (1) reduced female survival and (2) low recruitment to breeding populations, as indicated by an increase in male : female sex ratios and a decrease in immature : adult age ratios in U.S. harvest survey data. These population-constraining mechanisms are potentially consequences of (1) increased predation of females, (2) reduced female breeding propensity, clutch size, or renesting attempts, and / or (3) lowered nesting success or duckling survival (Afton 1984). Population status of Greater Scaup is unclear because Lesser Scaup are more

numerous, and the two species are not separated during annual surveys because of difficulties in species identification from survey aircraft (Austin et al. 2000). Thus, an important research direction is to develop an understanding of life history strategies from declining and stable breeding regions to highlight differences. Therefore, investigations of factors related to (1) reduced female survival and (2) lowered recruitment to breeding populations in Greater Scaup are warranted (Austin et al. 2000).

The study system: female Greater Scaup
breeding on the Yukon-Kuskokwim Delta, Alaska

Given waterfowl young are highly precocial, the metabolic costs of egg production are considered high in comparison with other avian taxa. Thus, quantifying strategies of nutrient acquisition to meet the demands of egg production by waterfowl has been a longstanding topic of interest. Many studies have addressed the role of female condition in the regulation of clutch size, and several have shown that nutritional status is correlated with a larger clutch size or a greater absolute and proportional lipid content as well as energy density of eggs (King 1973, Ankney and MacInnes 1978, Krapu 1981, Arnold et al. 1991). Fewer studies have addressed the role of nutritional status in timing of reproduction (e.g., Esler and Grand 1994, Esler et al. 2001), despite the important associated fitness consequences.

Female Greater Scaup breeding on the Yukon-Kuskokwim Delta (YKD), Alaska (Figure 1.1), the study species for this thesis work, provide an excellent opportunity to investigate relationships between female condition, timing of reproduction, and individual fitness. The energetic and nutritional costs of clutch formation by this species are high even among waterfowl. Alisauskas and Ankney (1992) report a maximum daily energetic cost of egg production that is 234% of the female's basal metabolic rate for

this species, as Greater Scaup produce clutches comprised of many, large, energy-dense eggs (Flint and Grand 1999). Thus, female condition may play a role in timing of reproduction as evidence for these relationships exists for the closely related Lesser Scaup (Esler 2001). Current data suggest positive relationships between early nesting and clutch size for this species (Flint et al. 2005). Despite the selective pressure for early nesting, Greater Scaup are among the latest nest initiating duck species in western Alaska (Flint et al. 2005). Finally, variation in nutrient reserve use during egg production between female Greater and Lesser Scaup are of particular interest given the current differences in breeding population trajectories of these closely related species (see Esler et al. 2001 for results of Lesser Scaup nutrient reserve use during clutch formation).

The fieldwork for this thesis was conducted at the Hock Slough research camp, operated and funded by the Alaska Science Center – USGS, Anchorage, Alaska, between May and June, 2002 and 2003. Hock Slough is located along the lower Kashunuk River (60°20'N, 165°35'W) (Figure 1.1 and 1.2), in the center of the coastal fringe of the YKD. Land elevations are low, approximately 0-3 m above sea level, and the study site consists of sedge meadows and numerous small lakes and ponds (Grand et al. 1997, Flint and Grand 1999) used by breeding waterfowl for sources of food and brood-rearing habitat.

Thesis objectives

The project described in this thesis was designed to contribute to the field of avian reproductive energetics, and to offer specific insights into the proximate factors affecting Greater Scaup productivity on the YKD, Alaska. The thesis is organized into three data chapters, each of which addresses a particular aspect of the overall topic. Chapter 2 describes physiological changes in female Greater Scaup reproductive organs through

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the egg production cycle. The information presented in this chapter is required for analyses presented in Chapters 3 and 4, and thus represents an important aspect of the overall project. Chapter 3 is an investigation of the nutrient reserve dynamics of female Greater Scaup during egg production, with particular emphasis on intraspecific variation in nutrient reserve use in relation to timing of reproduction. The data presented in this chapter provide an important description of scaup life history strategies from a stable breeding population. Chapter 4 is a study characterizing the plasma yolk precursors vitellogenin and very low-density lipoprotein during egg production in female Greater Scaup. The data presented in this chapter provide useful, non-lethal physiological indices for identifying reproductive state in female waterfowl. Chapters 2-4 were prepared as manuscripts for journal submission. Finally, Chapter 5 is a general synthesis of the thesis work.

This project also involved several studies that are not included in the formal thesis. First, stable isotope analysis was used to directly trace the allocation of nutrients used in reproduction by female Greater Scaup. Second, in collaboration with scientists at Bird Studies Canada, a study was initiated to examine selenium burdens in breeding female Greater Scaup. Finally, heart tissue samples were collected for genetic analysis and archiving at the Alaska Science Center-USGS.

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Figure 1.1. State of Alaska showing the location of the Yukon-Kuskokwim Delta
(YKD) and general location of the Hock Slough study area (•). Map
after http://geography.about.com/library/blank/blxusak.htm.
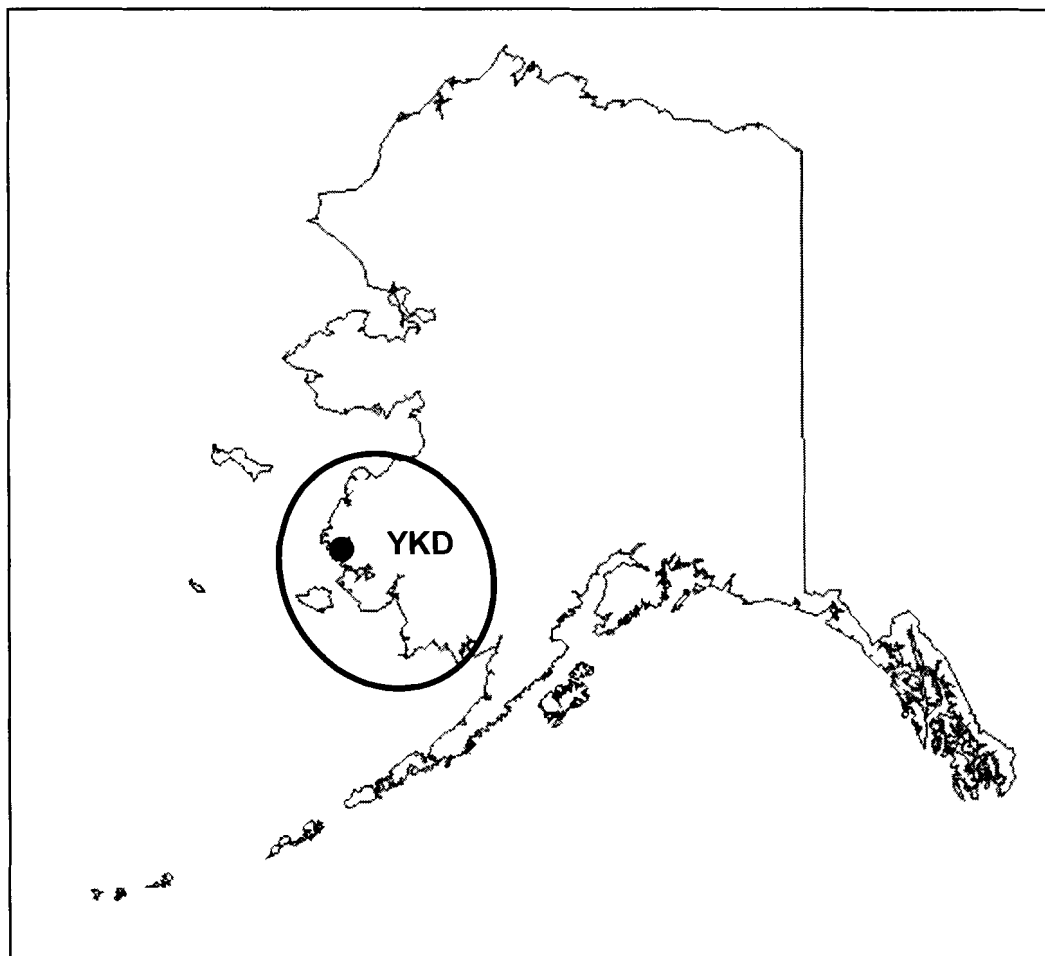
Figure 1.2. The lower Kashunuk River showing the detailed location of the Hock Slough study area (HS). Modified map produced using ESRI GIS and mapping software (http://www.esri.com/index.html).
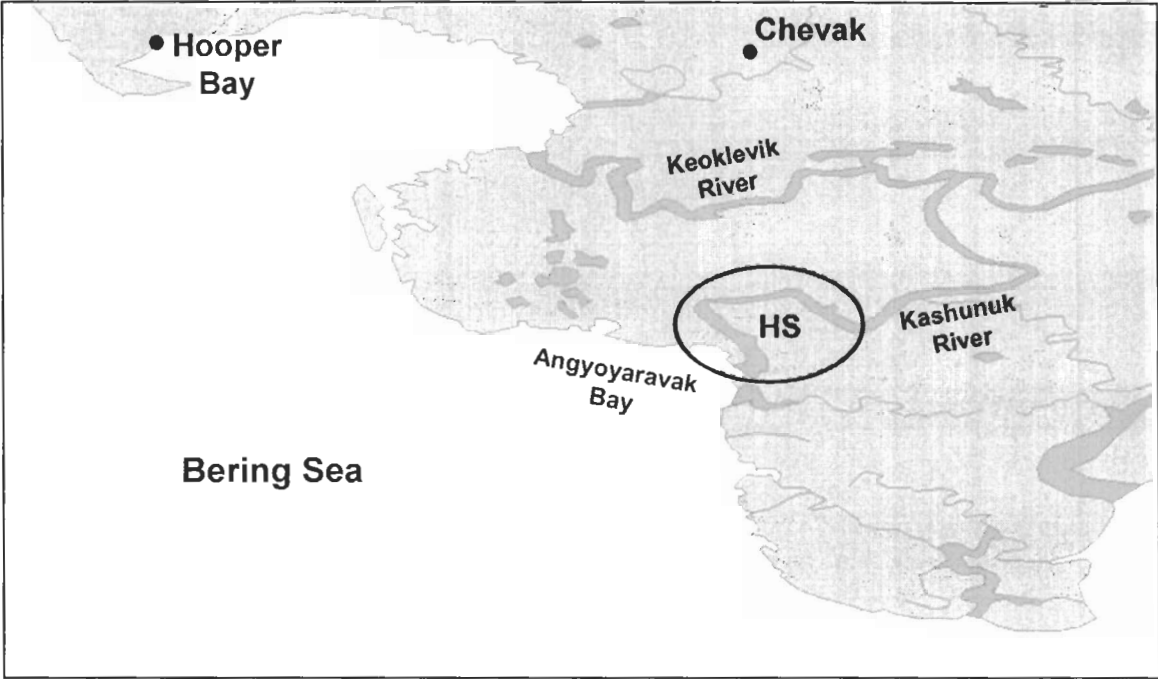
Chapter 2.

Reproductive organ dynamics of female Greater Scaup (*Aythya marila*) during egg production

Prepared as a manuscript for journal submission with the following authorship:

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Abstract

Concerns over declining North American scaup populations have called for research regarding the role of female condition in reproductive performance. Such studies of waterfowl nutrient reserve use during reproduction require a precise understanding of reproductive organ dynamics during egg production. Thus, we quantified ovarian follicle dynamics in female Greater Scaup (*Aythya marila*) breeding in western Alaska, using a method that accounts for within day variation in follicle size. We estimated that the duration (± 95% confidence interval) of rapid follicle growth (RFG) was 5.2 ± 0.52 days for each developing ovarian follicle. Average diameter and dry mass of preovulatory follicles at the onset of RFG were estimated to be 9.36 mm and 0.26 g, respectively, while average diameter and dry mass of preovulatory follicles at ovulation were estimated to be 41.47 mm and 15.57 g, respectively. Average diameter of postovulatory follicles immediately following ovulation was estimated to be 18.62 mm, and postovulatory follicles regressed quickly over several days. Furthermore, we derived predictive models to estimate the number of days before and after ovulation based on individual ovarian follicle characteristics, as well as to estimate dry mass of damaged follicles. Our results have important utility for discerning RFG and nest initiation dates, clutch size, and the daily energetic and nutritional demands of egg production, providing necessary information for additional work on Greater Scaup reproductive ecology and energetics.

Introduction

Studies of energetics have contributed significantly towards understanding avian life history strategies (Ricklefs 1996). For example, understanding energetic strategies for the meeting the energy and nutrient costs of reproduction has received much attention in

waterfowl as the demands of egg production are considered high relative to other avian taxa (King 1973, Alisauskas and Ankney 1992, Robbins 1993, Carey 1996). Such studies of female waterfowl nutrient reserve dynamics during reproduction (see Alisauskas and Ankney 1992, Rohwer 1992 for reviews) have provided insights regarding proximate constraints of clutch size (e.g., Ankney et al. 1991, Arnold and Rohwer 1991) and more recently, timing of reproduction (e.g., Esler and Grand 1994, Esler et al. 2001). Furthermore, these studies have important implications for the management and conservation of waterfowl populations.

A basic knowledge of the morphological changes in reproductive organs during the breeding cycle is essential for deriving proper inference from nutrient reserve studies regarding the influence of female condition on reproductive performance. For example, quantifying the growth of ovarian follicles during the rapid follicle growth (RFG) phase of oogenesis (see Johnson 2000 for review of avian egg production) allows for definition of the onset and duration of this process, which is a variable trait across taxa that has a strong influence on the daily energetic and nutritional demands of egg production (Alisauskas and Ankney 1992). The resulting information can be used to assign dates of RFG and nest initiation, determine clutch size, as well as allow for a precise understanding of the reproductive state of individuals.

Current research directions for better understanding North American scaup declines have emphasized a need for examining the role of female condition in reproductive performance among declining and stable segments of the breeding population (Austin et al. 2000). Thus, we quantified reproductive organ dynamics during egg production of female Greater Scaup (*Aythya marila*) breeding in subarctic, western Alaska to provide the necessary underpinning for addressing more complex questions regarding scaup reproductive energetics and physiology. In this study, we define the

onset and duration of RFG, as well as quantify the regression rate of postovulatory follicles. We generate models for predicting reproductive state, as well as the number of days before and after ovulation of females. In addition, we develop a predictive model for estimating dry mass of individual ovarian follicles, which can be used to estimate contents of follicles damaged during collection or dissection.

Methods

Field and laboratory methods

This study was conducted on female Greater Scaup (*Aythya marila*) breeding near the lower Kashunuk River (60°20'N, 165°35'W) on the Yukon-Kuskokwim Delta, Alaska during the breeding seasons of 2002 and 2003. This study was performed in accordance with permits from the U.S. Fish and Wildlife Service, the State of Alaska Department of Fish and Game, and the Yukon Delta National Wildlife Refuge. In addition, this study conformed to guidelines of the Canadian Committee on Animal Care (Simon Fraser University Animal Care Permit 637B-02). Approximately 2 female Greater Scaup were collected each day between 19 May - 21 June 2002 (n = 58) and 15 May - 19 June 2003 (n = 54), as these dates included the egg production phase of the breeding cycle for most individuals. Reproductive organs including ovaries, oviducts and oviductal eggs were dissected within 24 hours of collection. Follicles damaged during collection, or dissection, were noted as to their position in the hierarchy. Ovaries were weighed and subsequently preserved in 10% formalin.

In the laboratory, formalin preserved ovaries were dissected. Damaged follicles noted during field collections were removed and discarded. Diameters of the remaining 12 largest preovulatory and all postovulatory follicles were measured with digital calipers

in the plane of the stigma. The 12 preovulatory follicles were oven dried individually, as well as the remaining ovary.

Statistical methods: developing continuous models of follicle growth

All statistical analyses were performed in SAS (SAS Institute 1999) or SigmaPlot (SPSS Inc. 2000). Females that had ovulated at least one follicle (i.e., laying females) were used for the following analyses (n = 25), as only these ovary samples had RFG follicles in a hierarchy where each follicle could be assigned a particular number of days before ovulation, assuming a laying rate of 1 egg per 24 hours (Alisauskas and Ankney 1992). Ovary samples with damaged follicles were used if the position of the damaged follicle in the hierarchy was known. However, damaged follicles were excluded from analyses, in addition to entire ovary samples for which the largest follicle was damaged. For females late in their laying cycle (i.e., a gap in size between developing and nondeveloped follicles), only developing follicles in a consistent hierarchy were used in these analyses.

To quantify preovulatory follicle dynamics, the number of days before ovulation for the first 12 preovulatory follicles of each ovary sample was first estimated. Individual follicles were assigned a number of days before ovulation (i.e., assigning the largest follicle within an ovary sample as DAY 1 and the remaining follicles as DAY 2 – DAY 12 based on their position in the follicle hierarchy as determined by diameter measurements).

More precise estimates of the number of days before ovulation for each follicle were derived based on methods described by Esler (1994), which account for within day variation in follicle size. Correcting for this variation allows for the use of continuous models of follicle growth, as opposed to simply estimating mean follicle size for a

particular day. A correction value for the number of days before ovulation (*PreovCorr*) for the largest preovulatory follicle of each ovary sample was calculated following Equation 2.1.

PreovCorr = (estimated dry mass of an individual's follicle at ovulation – dry mass of an individual's DAY 1 follicle) / (estimated dry mass of an individual's follicle at ovulation – dry mass of the smallest DAY 1 follicle of the dataset).

(Equation 2.1)

Estimates for dry mass of an individual's ovulated follicle were based on the dry mass of that individual's oviductal egg yolk when possible or an average from a sample of oviductal and laid egg yolk dry masses (n = 31) when necessary. Thus, values of *PreovCorr* for DAY 1 follicles ranged from 0 for follicles just prior to ovulation, to 1 for follicles 24 hours from ovulation. *PreovCorr* estimates for the remaining follicles (i.e., DAY 2 – 12) within each ovary were calculated as that follicle's original DAY assignment plus the *PreovCorr* correction value from the DAY 1 follicle of that ovary. For example, if a DAY 1 follicle's *PreovCorr* was estimated to be 0.25 days, DAY 2 follicle's *PreovCorr* would be 1.25.

Similar to the methods used for quantifying preovulatory follicle dynamics, postovulatory follicles were first assigned a number of days after ovulation, based on their position in the postovulatory follicle hierarchy as determined by diameter measurements. Then, correction values for within day variation in postovulatory follicle diameters (*PostovCorr*) were also based on methods described by Esler (1994) where the precise number of days after ovulation for the largest postovulatory follicle was calculated following Equation 2.2.

PostovCorr = (1 – *PreovCorr* of the largest preovulatory follicle)

(Equation 2.2)

PostovCorr estimates for the remaining postovulatory follicles were calculated as the

PostovCorr correction value for the largest postovulatory follicle plus the appropriate

number of days after ovulation. For example, an ovary sample with a *PreovCorr* of 0.25

would be estimated as having a *PostovCorr* for its largest postovulatory follicle diameter

as 0.75, and 1.75 for the second largest postovulatory follicle diameter.

Statistical methods: defining RFG and predictive models

To quantify the onset and duration of RFG (i.e., *PreovCorr* at which follicles begin to

rapidly accumulate yolk lipid and protein macronutrients), we determined the most

parsimonious solution to the definition of 2 linear regressions through the relationship

between *PreovCorr* and follicle diameter. The relationship between *PreovCorr* and

follicle diameter could be precisely defined as 2 linear relationships, one prior to RFG

and one during RFG, based on previously described patterns (Esler 1994) and review of

our data. Therefore, models were iteratively generated using data assigned to either

preRFG or RFG groups, based on a number of *PreovCorr* values that putatively

corresponded to RFG initiation. The *PreovCorr* demarcation that best explained the

variation in these data (i.e., the model that maximized the r^2 and minimized the sum of

squares error values), determined the onset and duration of RFG for female Greater

Scaup. 95% confidence intervals (CI) were calculated for estimates of the duration of

RFG following the methods of Sokal and Rohlf (1981 p. 498).

Linear and polynomial models using data with *PreovCorr* less than the estimate

of the onset of RFG, as determined above, were derived to describe relationships

between *PreovCorr* and preovulatory follicle diameter and dry mass, respectively. In

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addition, an exponential decay model was derived to describe the relationship between *PostovCorr* and postovulatory follicle diameter. Finally, linear and polynomial models were derived to predict *PreovCorr* and *PostovCorr* from these same ovary characteristics.

Estimates of damaged follicle dry masses were derived by describing the relationship between dry mass of a follicle and dry mass of the next smallest follicle in the hierarchy of developing follicles from ovary samples with intact ovaries (n = 81 pairs of RFG follicles).

Results

The onset and duration ± 95% CI of RFG was estimated to be 5.2 ± 0.52 days for Greater Scaup (Figure 2.1), as this demarcation point maximized the r^2 and minimized the sum of squares error values for all possible preRFG and RFG data groupings. Using the linear relationship between *PreovCorr* and follicle diameter for follicles in RFG, average follicle diameters at the onset of RFG and at ovulation were estimated to be 9.36 and 41.47 mm, respectively (Figure 2.1).

The relationship between *PreovCorr* and follicle dry mass, for follicles in RFG, was fit with a second order polynomial model (Figure 2.2). Based on this model, follicle dry masses at initiation of RFG and ovulation were estimated to be 0.26 g and 15.57 g, respectively (Figure 2.2).

The relationship between *PostovCorr* and postovulatory follicle diameter was fit with an exponential decay function which estimated the average postovulatory follicle diameter at ovulation to be 18.62 mm (Figure 2.4).

Predictive models estimated *PreovCorr* with linear and third order polynomial models of diameter and dry mass of preovulatory follicles, respectively, and an

exponential decay model of postovulatory follicle diameter (Table 2.1). These equations can be used to precisely estimate the number of days prior to, or following, ovulation for any preovulatory follicle in RFG or any postovulatory follicle. Predictive models estimated preovulatory follicle dry mass with a third order polynomial model of dry mass of the next smallest follicle in the hierarchy (Table 2.1). This model allows for appropriate corrections in energetic studies for follicles that are burst or damaged during collection or dissection.

Discussion

This study expands previous work on reproductive organ dynamics in waterfowl (Esler 1994), by applying similar objective methods to quantify the onset and duration of RFG in female Greater Scaup. Studies of female waterfowl reproductive energetics have used an array of methods for defining the onset of RFG including total ovary mass or dry mass of the second smallest developing follicle from ovary samples with a full follicle hierarchy (e.g., Krapu 1974, Afton and Ankney 1991, Young 1993). We used a method that corrects for within day variation in follicle mass, allowing for the use of continuous models of follicle growth. Such models are advantageous as they can be used to precisely estimate basic breeding biology parameters at the individual level including RFG and nest initiation dates, clutch size, the identification of reproductive state, time of day of ovulation, renesting intervals, and daily nutrient and energy commitment to egg production of collected females.

Our analyses describe relationships between follicle characteristics and the number of days before and after ovulation at the population level, that is, the relationships we present describe an "average" female within the population. Flint and Grand (1999a) demonstrated that female Greater Scaup from the same breeding

population show less variation in egg size at the population level (CV: 3%) than that which has been reported for other duck species (e.g., Flint and Grand 1999b, Rohwer 1986). Although Flint and Grand (1999a) demonstrated that total egg lipid increased with egg size, lipid composition did not vary among female Greater Scaup. Thus, among female variation in both egg size and lipid composition is small in this population. Therefore, the models of preovulatory follicle growth we present presumably even more accurately describe these processes than similar models would for other species.

Our estimate for the duration of RFG by female Greater Scaup, 5.2 ± 0.52 days, is similar to that reported for female Lesser Scaup, 5.0 days (Esler 1994), but shorter than the 6.0 days reported for *Aythya* spp. (Alisauskas and Ankney 1992). Estimates of ovarian follicle size at the onset of RFG, 9.36 mm and 0.26 g, have not previously been reported for this species, nor have estimates of ovarian follicle size at ovulation, 41.47 mm and 15.57 g which are slightly larger than that reported for female Lesser Scaup, 37.13 mm and 11.02 g (Esler 1994). These interspecific differences in ovarian follicle size at ovulation are presumably related to the fact that female Greater Scaup are structurally larger than female Lesser Scaup, and have larger egg and yolk sizes. We present predictive models for estimation of the number of days before, and after, ovulation based on ovarian follicle characteristics. These models are useful for estimating RFG and nest initiation dates from ovaries of collected females. Our predictive model for dry mass estimation of follicles damaged during collection has important utility for studies of reproductive energetics where precise estimates of nutrients committed to clutch formation are necessary, yet can be difficult to obtain if follicles are damaged during collection.

Intraspecific variation in nutrient reserve use during reproduction by female waterfowl remains one of the more poorly understood aspects of waterfowl nutrient

reserve dynamics (Alisauskas and Ankney 1992). Our results are particularly applicable for advancing such studies. Dates of RFG initiation can be assigned to females collected for nutrient reserve studies based on the models we present. This parameter is increasingly incorporated in nutrient reserve studies to help explain variation in nutrient reserve use by breeding waterfowl across the nesting season (e.g., Alisauskas and Ankney 1992, Esler and Grand 1994, MacCluskie and Sedinger 2000, Esler et al. 2001). Finally, these data have utility in toxicological studies of Greater Scaup during reproduction. The role of contaminants both in breeding propensity and nesting success have been identified as important research directions for better understanding North American scaup declines (Austin et al. 2000). As the duration of RFG has implications for the daily energetic and nutritional demands of egg production, this parameter could be used in energetic models examining contaminant uptake during egg production.

Acknowledgements

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Figure 2.1 RFG of female Greater Scaup based on ovarian follicle diameter. Vertical dashed line represents the onset and defines the duration of RFG. Linear function is based on data with a *PreovCorr* value less than the estimate of the onset of RFG.

$$Y = 41.4731 - 6.1755\ X$$

$$r^2 = 0.97$$

Preovulatory Follicle Diameter (mm)

Days Before Ovulation
(*PreovCorr*)
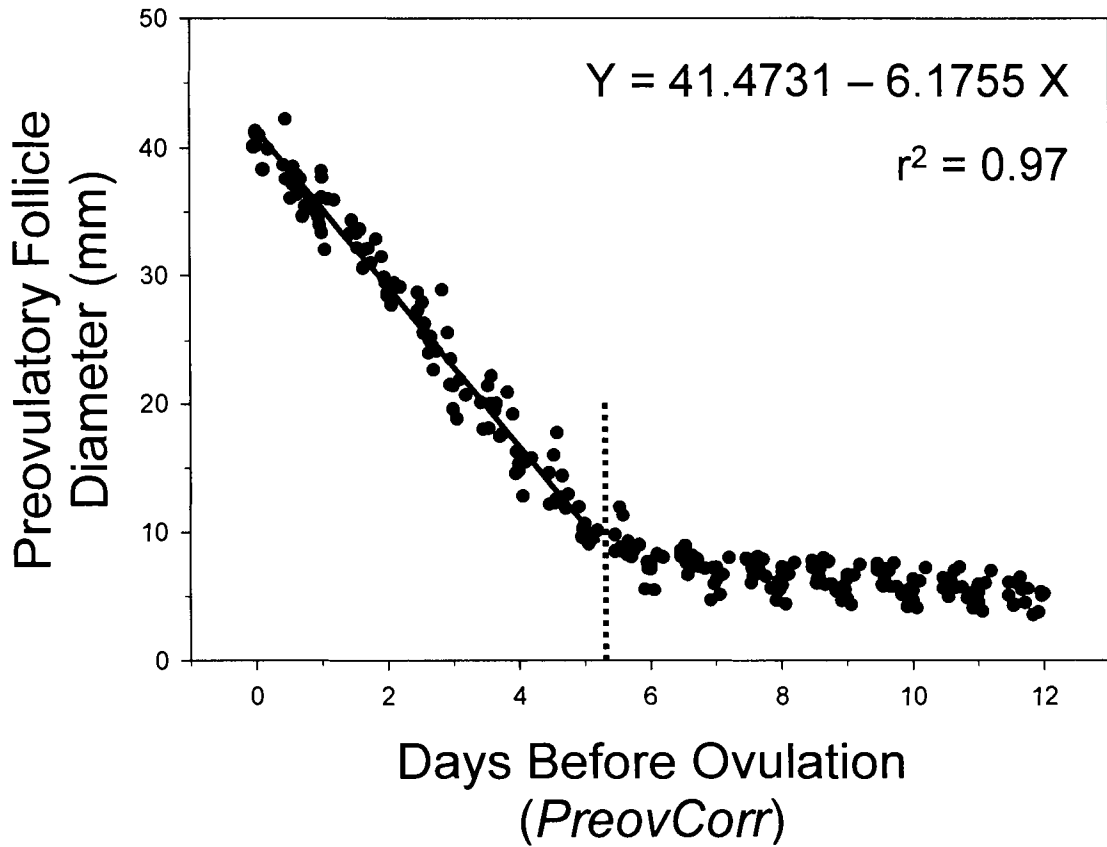
Figure 2.2 RFG of female Greater Scaup based on ovarian follicle dry mass.
Vertical dashed line represents the onset and defines the duration of
RFG as calculated from follicle diameter analyses. Polynomial function
is based on data with a *PreovCorr* value less than the estimate of the
onset of RFG.

$$Y = 15.5663 - 6.0120 \, X + 0.5900 \, X^2$$

$$r^2 = 0.97$$

Preovulatory Follicle
Dry Mass (g)

Days Before Ovulation
(*PreovCorr*)
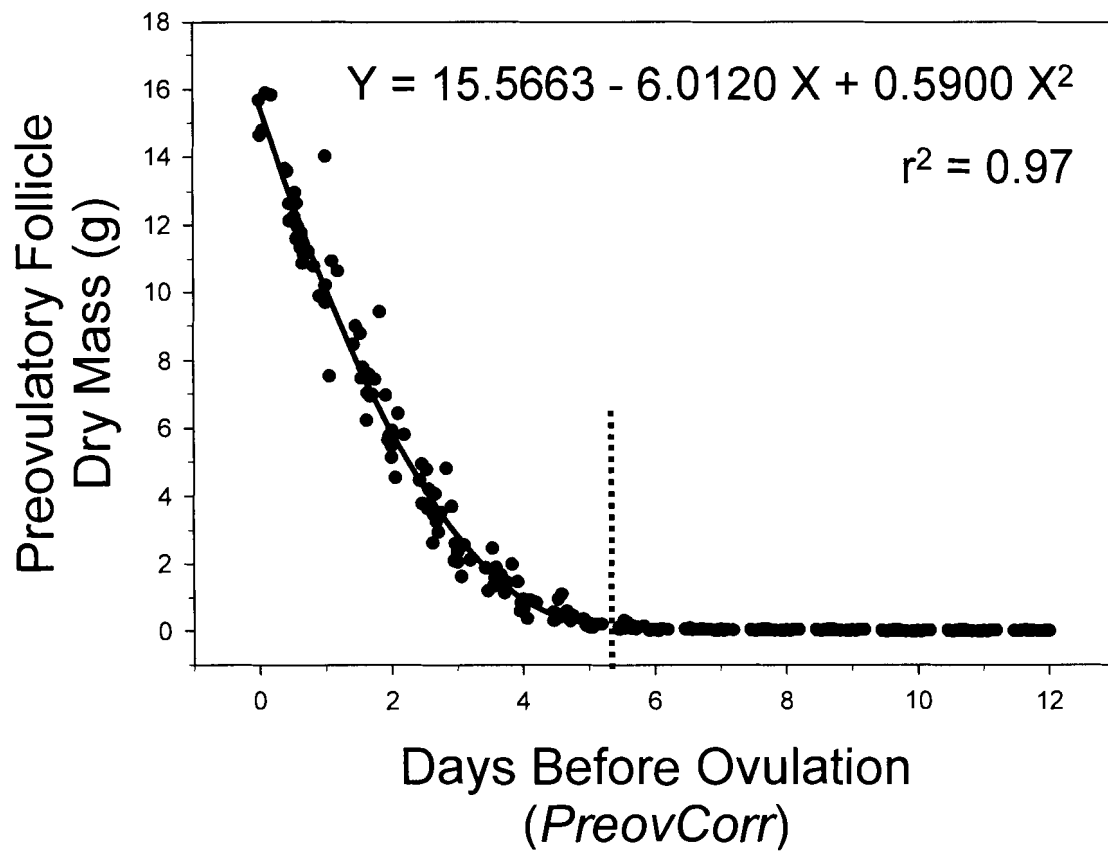
Figure 2.3 Postovulatory follicle regression of female Greater Scaup based on diameter measurements.

$$Y = 18.6215 \times \exp(-0.3142\ X)$$

$$r^2 = 0.89$$

Postovulatory Follicle Diameter (mm)

Days After Ovulation
(*PostovCorr*)
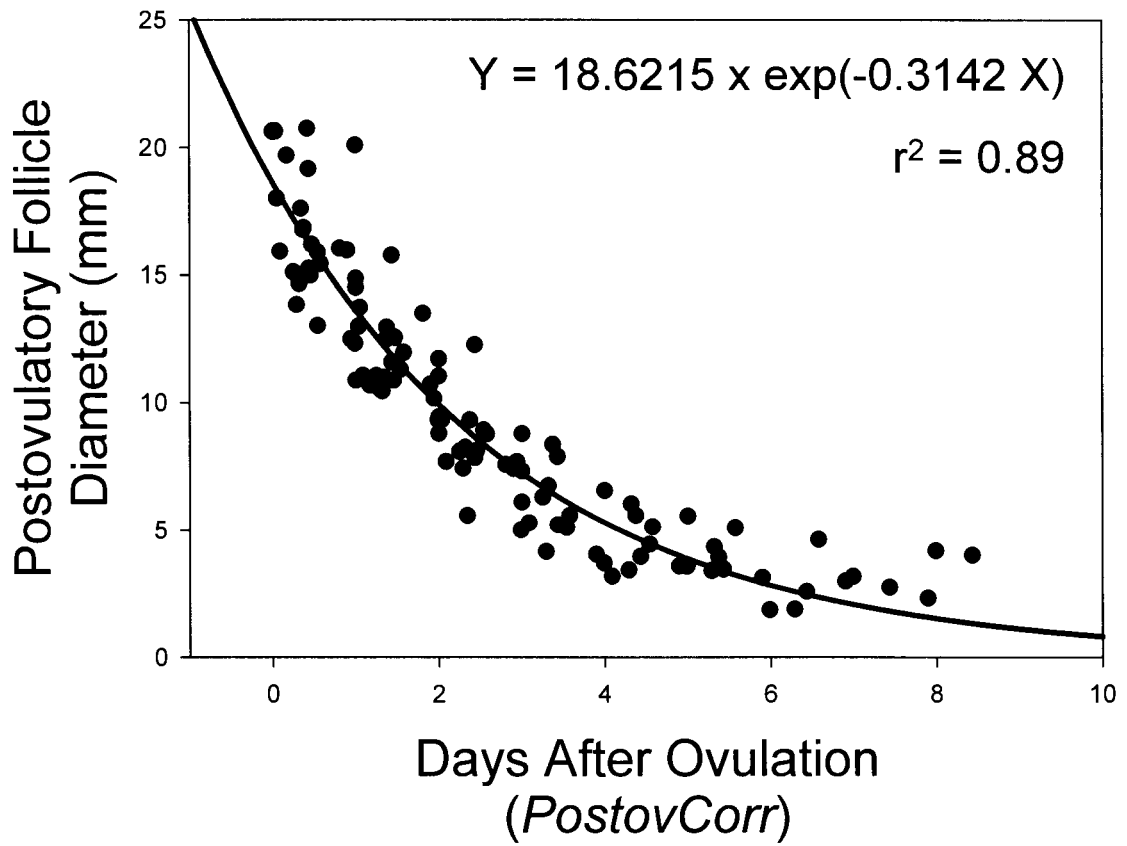
Table 2.1 Predictive models estimating days prior to, and following, ovulation (*Preov-* and *PostovCorr*, respectively) from follicle characteristics, as well as preovulatory follicle dry mass from dry mass of the next smallest follicle.

Follicle Characteristic	Equation
Preovulatory Follicle Diameter	$PreovCorr = 6.5986 - 0.1574\ Dia$ $r^2 = 0.97$
Preovulatory Follicle Dry Mass	$PreovCorr = 4.8635 - 0.8014\ DM + 0.0614\ DM^2 - 0.0019\ DM^3$ $r^2 = 0.97$
Postovulatory Follicle Diameter	$PostovCorr = 9.8564 \times exp(-0.1662\ PODia)$ $r^2 = 0.86$
Preovulatory Follicle Dry Mass	$Follicle\ Dry\ Mass = 0.2745 + 2.7616NSF - 0.2614NSF^2 + 0.0129NSF^3$ $r^2 = 0.99$

Abbreviations: Days before ovulation (*PreovCorr*), Days after ovulation (*PostovCorr*), Preovulatory follicle diameter (Dia), Preovulatory follicle dry mass (DM), Postovulatory follicle diameter (PODia), Next small follicle (NSF).

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Chapter 3.

Nutrient reserve dynamics during egg production by female Greater Scaup (*Aythya marila*): relationships with timing of reproduction

Prepared as a manuscript for journal submission with the following authorship:

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Abstract

We studied nutrient reserve dynamics of female Greater Scaup (*Aythya marila*) to quantify intraspecific strategies of nutrient acquisition for egg production, specifically in relation to the seasonal timing of reproduction (i.e., date of rapid follicle growth initiation). Female Greater Scaup incur relatively high energetic and nutritional costs of egg production, and initiate nesting late in comparison with other waterfowl breeding in subarctic western Alaska. Similar to most other ducks, Greater Scaup clutch sizes decline with delayed nest initiation. Thus, selective pressures for early nesting should operate, but energetic or nutritional constraints may be important proximate determinants of reproductive phenology. Therefore, we collected female Greater Scaup on the Yukon-Kuskokwim Delta, Alaska from arrival through clutch formation during the 2002 and 2003 breeding seasons and conducted standard proximate body composition analysis. Unlike most waterfowl studies, we found that endogenous lipid, protein, and mineral reserves did not decline during egg production, indicating that female Greater Scaup do not rely on endogenous nutrient reserves during egg production, and are therefore dependent on exogenous food sources to meet the energetic cost of clutch formation. Furthermore, endogenous nutrient reserves did not decline with date of clutch formation, indicating a seasonally static body condition threshold for initiation of egg production. We found evidence that females must attain nutrient reserve thresholds prior to initiation of egg production; lipid, protein, and mineral reserves were smaller in nonreproductive than reproductive females. Our results suggest that both early and late nesting female Greater Scaup employ income breeding strategies to meet the energy and nutrient costs of egg production, subsequently investing acquired reserves in reproductive phases other than egg production. Furthermore, we suggest that female condition drives intraspecific variation in timing of reproduction via initiation thresholds

for egg production. Finally, these data contrast with nutrient reserve dynamics described for Lesser Scaup (*A. affinis*), and should be considered in light of recent conservation concerns over declining North American scaup populations.

Introduction

The seasonal timing of reproduction is an important aspect of avian reproductive ecology, given the relationship with female reproductive performance. Numerous studies have demonstrated a decline in clutch size with delayed date of nest initiation (see Perrins 1970, Martin 1987, Daan et al. 1988 for reviews) (e.g., Dijkstra et al. 1982, Toft et al. 1986, Murphy 1986, Hamann and Cooke 1989). A decrease in offspring value, via reduced post-fledging survival and recruitment probability, is also generally associated with later nesting (Perrins 1966, Cooke et al. 1984, Hochachka 1990, Lepage et al 2000); furthermore, delayed reproduction results in fewer opportunities for renesting (Rohwer 1992). Given these fitness consequences, understanding the proximate mechanisms driving variation in timing of breeding has been of interest to both theoretical ecologists, as well as applied conservation scientists.

Recent studies have highlighted primary reproductive effort (i.e., egg production) as an important component of the energetic cost of reproduction (Monaghan and Nager 1997). Perrins (1970) proposed that timing of reproduction may be constrained by the female's ability to meet the energetic and nutritional demands of egg production when food resources are scarce. A large body of correlational and experimental evidence suggests strong proximate effects of food availability on reproductive timing (see Daan et al. 1988, Boutin 1990 for review). Given that sources of energy and nutrients used for egg production can originate from stored endogenous nutrient reserves and / or exogenous food resources, often referred to as *capital* or *income* breeding tactics (sensu

Stearns 1992, Jönsson 1997), body condition is considered an important proximate

factor in reproductive timing for some avian taxa (Drent and Daan 1980, see Rowe et al.

1994 for theoretical model).

The energetic and nutritional costs of primary reproductive effort are considered

high in the waterfowl relative to other birds based on theoretical estimates of

reproductive tissue energy content (King 1973, Alisauskas and Ankney 1992, Robbins

1993, Carey 1996), primarily due to production of large clutches of large, energy-dense

eggs (Sotherland and Rahn 1987). Thus, identifying strategies waterfowl use to meet

the demands of egg production has been a long-standing topic of interest, in particular

quantifying the use of endogenous nutrient reserves or exogenous food sources during

clutch formation (Alisauskas and Ankney 1992). Much of this work has been framed in

the context of clutch size limitation (e.g., Drobney 1991, Ankney et al. 1991, Arnold and

Rowher 1991); however, this area of research has also led to conceptual (Reynolds

1970, Rowe et al. 1994) and empirical (Esler and Grand 1994, Esler et al. 2001)

considerations of the proximate influence of female condition in driving variation in

reproductive timing.

Greater Scaup (*Aythya marila*) females incur a particularly high energetic and

nutritional cost of egg production in comparison with other waterfowl species (Flint and

Grand 1999). Alisauskas and Ankney (1992) report a maximum daily energetic cost of

egg production that is 234% of the female's basal metabolic rate for this species,

primarily due to a large clutch size and an energy-dense egg composition. Furthermore,

for subarctic tundra breeding ducks in western Alaska, Greater Scaup are among the

latest to initiate nesting, and current data indicate seasonal declines in clutch size for this

species (Flint et al. 2005). Thus, we initiated a study to understand the proximate

influence of nutrition on reproductive phenology of female Greater Scaup breeding on the Yukon-Kuskokwim Delta, Alaska.

In this paper, we consider intraspecific variation in nutrient reserve use in relation to timing of reproduction (i.e., date of rapid follicle growth initiation) by female Greater Scaup. Along with providing insights into avian reproductive strategies, this study is an important component of current research designed to understand factors associated with North American scaup (*A. marila* and *A. affinis*) numerical declines. Recent conservation concerns have highlighted the need for studies of life history strategies affecting demography between declining and stable breeding populations (Austin et al. 2000). Since the late-1970's, the most serious scaup breeding population decline has occurred in the boreal forest of subarctic Canada and eastern Alaska, while coastal tundra populations of western Alaska have remained stable (Afton and Anderson 2001). Thus, we discuss our data on Greater Scaup nesting in western Alaska in comparison with results from closely related boreal forest and mid-continent nesting Lesser Scaup (*A. affinis*) (Esler et al. 2001).

Methods

Field methods

This study was conducted on female Greater Scaup breeding near the lower Kashunuk River (60°20'N, 165°35'W) on the Yukon-Kuskokwim Delta, Alaska (YKD) during the breeding seasons of 2002 and 2003. This study was performed in accordance with permits from the U.S. Fish and Wildlife Service, the State of Alaska Department of Fish and Game, and the Yukon Delta National Wildlife Refuge. In addition, this study conformed to guidelines of the Canadian Committee on Animal Care (Simon Fraser University Animal Care Permit 637B-02). Approximately two female Greater Scaup were

collected each day between 19 May - 21 June 2002 (n = 58) and 15 May - 19 June 2003 (n = 54), as these dates included the egg production phase of the breeding cycle for most individuals. At the time of collection, birds were immediately weighed and a blood sample was taken (≤ 5 ml).

Within 24 hours of collection, small (~1.0 g) tissue samples were dissected and weighed for stable isotope analysis: left pectoral muscle, abdominal lipid, and liver. A small sample of lipid-free heart tissue also was collected and weighed for genetic archiving. In addition, reproductive organs including ovaries, oviducts, and oviductal eggs were dissected. Ovaries were weighed to obtain total ovary wet mass. If more than one ovarian follicle had initiated rapid follicle growth (RFG) (i.e., phase of avian oogenesis when ovarian follicles uptake yolk lipid and protein, see Johnson 2000 for review) one RFG follicle was collected, weighed, and stored frozen for stable isotope analysis; when possible yolk for stable isotope analysis was collected from oviductal eggs as an alternative to follicle collection. However, stable isotope analysis results are not presented in this thesis. Remaining ovary tissue was subsequently preserved in 10% formalin. Follicles damaged during collection, or dissection, were noted as to their position in the hierarchy so that estimates of their nutrient contents could be calculated (see Chapter 2). Oviducts, oviductal eggs, and any laid eggs from known nests of collected individuals were weighed and frozen along with the remaining carcass.

Laboratory methods

In the laboratory, formalin preserved ovaries were dissected and individual follicles were measured, dried, and weighed. Based on findings from Chapter 2, reproductive states were defined as nondeveloped: largest ovarian follicle < 9.36 mm and < 0.26 g dry mass (n = 32); rapid follicle growth (RFG): largest ovarian follicle ≥ 9.36 mm, ≥ 0.26 g dry

mass and no postovulatory follicles present (n = 32); laying: ≥ 1 postovulatory follicle and a preovulatory follicle to be ovulated within 24 hours (> 35.6 mm) or the presence of an oviductal egg (n = 30); incubation: a hierarchy of regressed postovulatory follicles and the presence of a brood patch (n = 10); renesting: a series of completely regressed postovulatory follicles, the presence of ≥ 1 recent postovulatory follicle, and / or a preovulatory follicle to be ovulated within 24 hours (> 35.6 mm) or the presence of an oviductal egg (n = 4). Dates of RFG initiation were estimated based on models of ovarian follicle growth (Chapter 2, Esler 1994), number of postovulatory follicles, and a laying rate of one egg per day (Esler 1999, Flint et al. 2005).

Somatic nutrients (i.e., S-NUTRIENTS: S-LIPID, S-PROTEIN, S-MINERAL) were estimated using standard proximate body composition analysis. Somatic tissue composition analysis first involved thawing, plucking, and dissection of carcasses. We dissected organs and tissues including the proventriculus, liver, heart, pancreas, spleen, gizzard, small intestine, ceca, large intestine, kidneys, right breast, and right leg which were removed of connective tissue and external lipid, placed in preweighed plastic bags for lyophilization (i.e., freeze drying), and weighed. 3.0 g samples of liver tissue were collected from each liver for a study of selenium burdens in scaup prior to lyophilization of liver tissue. The remaining carcass also was placed in a preweighed plastic bag and weighed. Somatic tissues were lyophilized to a constant dry mass (± 0.01 g for organs, ± 1.0 g for carcasses).

Nutrient content of the liver, heart, right breast, right leg, and the remaining carcass were determined using standard procedures of lipid extraction using petroleum ether in a soxhlet apparatus for 8 hours to remove neutral storage lipids (Dobush et al. 1985) and combustion of lipid-free samples in a muffle furnace for 24 hours to determine proportions of protein and mineral (Ankney and Afton 1988). After reaching a constant

dry mass, the liver, heart, right breast, and right leg were homogenized individually using a mortar and pestle, and one small aliquot of each organ or tissue was taken for nutrient determination. Dried carcasses were homogenized using a Cuisinart food processor (Mini-Prep Plus), and three aliquots (~10.0 g) were taken for nutrient determination. Total somatic nutrient content for each bird was estimated by first calculating the nutrient content of the liver, heart, right breast, and right leg samples, followed by estimation of the total nutrient content for each organ or tissue based on the total dry and lean masses of that organ or tissue (Equations 3.1, 3.2, and 3.3).

Total Tissue Lipid = (extracted lipid in sample / sample tissue dry mass) x

total tissue dry mass

(Equation 3.1)

Total Tissue Protein = (ash free lean dry mass in sample / sample tissue lean mass) x

total tissue lean mass

(Equation 3.2)

Total Tissue Mineral = (ash mass in sample / sample tissue lean mass) x

total tissue lean mass

(Equation 3.3)

Nutrient content of homogenized carcasses was determined by first calculating the nutrient content of the three aliquot samples, followed by estimation of the total nutrient content for each carcass based on the nutrient content of each aliquot sample and the total dry and lean masses of that carcass. Finally, the three estimates of total nutrient content for each bird from the aliquot samples were averaged (AVE) (Equations 3.4, 3.5, and 3.6).

Total Carcass Lipid = AVE (extracted lipid in aliquot / aliquot tissue dry mass) x

total carcass dry mass

(Equation 3.4)

Total Carcass Protein = AVE (ash free lean dry mass in aliquot / aliquot tissue lean

mass) x total carcass lean mass

(Equation 3.5)

Total Carcass Mineral = AVE (ash mass in aliquot / aliquot tissue lean mass) x

total carcass lean mass

(Equation 3.6)

Small samples removed for separate analyses, including left pectoral muscle, liver, and

heart, were accounted for in body composition analyses by calculating the wet mass of

removed samples, then estimating the dry mass as well as nutrient content for each

removed sample based on the nutrient estimation for that tissue for the same individual.

These values were then added to the total nutrient content for that tissue. Abdominal

lipid samples removed for stable isotope analysis were assumed to be entirely lipid,

therefore, the mass of the sample was added to the final estimate of total lipid for each

bird. Finally, all tissue and carcass nutrient values were added to obtain total somatic

nutrient composition for each bird.

Reproductive nutrients (i.e., R-NUTRIENTS: R-LIPID, R-PROTEIN, R-MINERAL)

committed to the ovary, oviduct, oviductal eggs, and any laid eggs also were estimated

based on standard proximate body composition analysis, using petroleum ether in a

soxhlet apparatus for 8 hours (Dobush et al. 1985) and combustion of lipid-free samples

in a muffle furnace for 24 hours to determine proportions of protein and mineral (Ankney

and Afton 1988). Reproductive tissue composition analysis first required oven drying

formalin preserved ovarian follicles as described in Chapter 2. Oviductal tissue was

lyophilized to obtain dry mass estimates. Damaged follicle nutrient content was

estimated by describing the relationship between the dry weight of a follicle and the dry

weight of the next smallest follicle, from ovary samples with intact follicles in RFG

(Chapter 2, n = 81 pairs of RFG follicles, r^2 = 0.99, Equation 3.7).

Follicle Dry Mass = 0.2745 + (2.7616 x Small Follicle Dry Mass) – (0.2614 x Small

Follicle Dry Mass^2) + (0.0129 x Small Follicle Dry Mass^3)

(Equation 3.7)

Total ovary dry mass was calculated by adding the dry masses of dissected individual

ovarian follicles, the remaining ovary, and any estimated dry masses from damaged or

sampled follicles. All ovarian tissue was homogenized using a mortar and pestle, and

used in nutrient determination for each ovary. All oviductal tissue was homogenized

using a mortar and pestle, and used in nutrient determination for small oviducts. Large

oviducts were aliquoted and nutrient determination proceeded as described for somatic

tissues. Final estimates of ovarian and oviductal nutrient contents were calculated

based on total dry and lean masses of these tissues following Equations 3.1, 3.2, and

3.3.

To estimate nutrients committed to laid eggs, an individual's egg nutrients (i.e.,

nutrients committed to oviductal or laid eggs) were used when possible. Average egg

nutrient composition was used when an individual's egg nutrients could not be obtained.

Nutrient contents of 27 collected eggs were estimated using the same techniques

described for both somatic and reproductive tissues. Average (± 1 SE) egg lipid content

was 9.45 ± 0.22 g, average protein content was 5.49 ± 0.17 g, and average mineral

content was 5.98 ± 0.11 g. These average egg nutrients were multiplied by the number

of postovulatory follicles to obtain estimates of total nutrients committed to eggs laid.

For individuals with an oviductal egg, average egg nutrients were multiplied by the

number of postovulatory follicles minus 1 to obtain estimates of total nutrients committed

to eggs laid. For these birds, oviductal egg nutrients were added to R-NUTRIENTS.

Measurements (± 0.01 mm) of the right wing chord, right tarsus, culmen, bill width, bill nail width, bill nail length, and keel length were obtained for principal components analysis to quantify body size variation for subsequent analyses (Sedinger et al. 1997). The first principal component explained 25% of the total variance. Scores from the first principal component (PC1) were used as an index of body size.

Statistical methods – nutrient reserve dynamics during egg production

All statistical analyses were performed in SAS (SAS Institute 1999). Least-squares general linear models were used to examine variation in somatic nutrient reserve levels (S-LIPID, S-PROTEIN, S-MINERAL) of females that had initiated egg production (n = 63). Explanatory variables considered as main effects included investment into the corresponding reproductive nutrient (R-LIPID, R-PROTEIN, R-MINERAL), date of RFG initiation (RFGINIT), principal components score (PC1), and YEAR. The YEAR term was treated as a categorical variable with 2003 as the reference value. RFG initiation dates for each individual were calculated relative to the earliest RFG initiation date for both years, (19 May 2002) which was set at 0. Reproductive nutrient parameter estimates with negative values indicated a maximum percentage of that nutrient committed to reproduction that could be derived from endogenous nutrient reserves, averaged across individuals. RFG initiation parameter estimates were interpreted as the change in endogenous nutrient reserve levels each day females delay egg production. Interaction terms for R-NUTRIENT x RFGINIT, R-NUTRIENT x YEAR, and RFGINIT x YEAR also were included as explanatory variables. Parameter estimates for R-NUTRIENT x RFGINIT interactions were interpreted as the change in the amount of endogenous nutrient reserves used during clutch formation with increasing initiation date. Parameter estimates for R-NUTRIENT x YEAR interactions and RFGINIT x YEAR

interactions were interpreted as the difference in the proportional amount of endogenous nutrient reserves used during clutch formation between years and interannual variation in the amount of endogenous nutrient reserves at RFG initiation between years, respectively.

Information-theoretic methods were used to direct model selection and parameter estimation (Burnham and Anderson 2002). This approach to model selection is increasingly recommended, particularly for observational studies (Anderson et al. 2000). The set of candidate models for describing variation in each somatic nutrient reserve level included all possible combinations of explanatory variables as well as an equal means model, resulting in 34 candidate models each for S-LIPID, S-PROTEIN, and S-MINERAL (Table 3.1); we used this approach because all models described realistic biological possibilities, based on the array of strategies used by female waterfowl in the literature. Inference was based largely on the relative support for parameters across models and weighted parameter estimates (i.e., multimodal inference). For each model, Akaike's Information Criterion including a correction for small sample size (AICc) was calculated. Candidate models were compared using ΔAICc values, which is the difference between the AICc value for a particular model and the lowest AICc value within the set (i.e., the AICc value of the best fitting model). We also calculated AICc weights (AICcW), which represented the likelihood that a particular model was the best model, given the data and set of candidate models.

Parameter estimation included calculation of model-averaged parameter estimates based on AICcW for all candidate models (Burnham and Anderson 2002). Standard errors for parameter estimates were based on unconditional variances calculated across the same models. Model parameters were evaluated by summing AICcW for all models that included each parameter; these parameter likelihood values

can range from 0 to 1, indicating very weak to very strong support, respectively, for the explanatory value of the parameter.

Statistical methods – nutrient reserve thresholds for egg production

We also examined differences in nutrient reserve levels between nonreproductive females (i.e., those with nondeveloped ovaries) and reproductive females (i.e., RFG and laying individuals) to evaluate body condition thresholds for egg production (Alisauskas and Ankney 1992). To evaluate the presence of body condition thresholds, we quantified variation in somatic lipid, protein, and mineral nutrient reserve levels in relation to the following explanatory variables: reproductive state (R-STATE) (i.e. nonreproductive vs. reproductive), principle components score (PC1), YEAR, and date of collection (C-DATE) (i.e., Julian date). Our candidate model set included combinations of the main effects of these variables and an equal means model (Table 3.2). The RFGINIT term was not included in these analyses. Information-theoretic methods were again used for model selection, and summed AICcW were calculated to assess parameter likelihood values. A difference in reserve levels between nonreproductive and reproductive females, after accounting for other effects, was interpreted as evidence for the existence of a nutrient threshold that must be attained prior to initiation of reproduction.

Results

We collected 112 female Greater Scaup during the arrival and egg production phases of the 2002 and 2003 breeding seasons on the YKD. Of these, 8 females were excluded from analyses; 4 were identified as renesting individuals, 3 females had ovaries damaged upon collection which precluded accurate reproductive state assignments, and

1 female was thought to be a Lesser Scaup based on structural measurements. The remaining 104 individuals were assigned to the various reproductive states as noted in the previous section on ovary dissections and data from these individuals were used in analyses.

Nutrient reserve dynamics - lipid reserves

Two models describing variation in lipid reserve levels had ΔAICc values $\leq$ 2.0 (Table 3.3). AICcW for both of these models were relatively low, indicating a high degree of model uncertainty. Both of these models included PC1 terms, while the best supported model also included the YEAR term. However, over 87% of the variation in the data was not explained by these models (Table 3.3). Parameter likelihood values indicated moderate support for PC1 and YEAR terms by the data, given the set of candidate models (Table 3.4). The positive parameter estimates for PC1 and YEAR suggested that larger bodied females had more endogenous lipid, and that 2002 females had higher levels of endogenous lipid than 2003 females, although 95% confidence intervals (CI: parameter estimate $\pm$ 1.96*SE) overlapped 0 (Table 3.4). No other explanatory variables were strongly supported by the data, including R-LIPID and RFGINIT main effects as well as all interaction terms. In summary, there was no evidence that female Greater Scaup relied on endogenous lipid reserves to meet the energy and nutrient demands of egg production, or that lipid reserve levels differed among females initiating RFG across the nesting season.

Nutrient reserve dynamics - protein reserves

Four models describing variation in protein reserve levels received ∆AICc values ≤ 2.0, although again model uncertainty was high given the low AICcW values for these models (Table 3.3). The best fitting model included PC1 and R-PROTEIN terms. PC1 was included in all models receiving ∆AICc values ≤ 2.0 and was substantially supported by the data, while R-PROTEIN was included in 2 of the top 4 models and only received moderate support (Table 3.4). The positive parameter estimate for PC1 indicated that larger bodied females had more endogenous protein. The parameter estimate for R-PROTEIN indicated that no endogenous protein reserves were used during clutch formation as 95% CI overlapped 0 (Table 3.4). RFGINIT and R-PROTEIN x RFGINIT interaction terms also were included in models with ∆AICc values ≤ 2.0 (Table 3.3), although they were not well supported by the data (Table 3.4). There was no evidence that YEAR as a main effect or the interaction terms including YEAR were important for describing variation in protein reserve levels. Thus, female Greater Scaup did not rely on endogenous protein reserves to meet the energy and nutrient demands of egg production, and there was no evidence that protein reserve levels differed among females initiating egg production across the nesting season.

Nutrient reserve dynamics - mineral reserves

Five models describing variation in mineral reserve levels received ∆AICc values ≤ 2.0 but model uncertainty was high, based on the similar AICcW values across these models (Table 3.3). The YEAR term was strongly supported by the data, while R-MINERAL, PC1, and RFGINIT terms received only moderate support (Table 3.4). The negative parameter estimate for YEAR indicated that 2002 female Greater Scaup had smaller endogenous mineral reserves than 2003 females. The parameter estimate for

R-MINERAL indicated that endogenous mineral reserves were not used during clutch formation as 95% CI overlapped 0. The parameter estimate for PC1 indicated that structurally larger females had more endogenous mineral reserves. The RFGINIT term was not included in the best supported model, but was included in all other models with ΔAICc values $\leq$ 2.0 and was moderately supported by the data (Table 3.4). No other explanatory variables were strongly supported by the data. Therefore, similar to lipid and protein reserve dynamics, females did not rely on endogenous mineral reserves during clutch formation, nor did their mineral reserves vary seasonally. However, there was evidence for an effect of structural size and year on mineral reserve levels.

Nutrient reserve thresholds for egg production

Lipid and mineral reserve levels were each best described by models including reproductive state, PC1, and YEAR terms; in both cases, the AICcW indicated this model structure received strong support and each of the parameter likelihood values indicated support by the data. No other models received ΔAIC values $\leq$ 2, and the AICcW for the best fit models indicated approximately 5 times the support of the next best model (Table 3.5). Protein reserve levels were best described by a model including only reproductive state and PC1 terms, and these parameters were highly supported by the data (Table 3.5). Again, no other models received ΔAIC values $\leq$ 2, and the AICcW indicated the best fit model received more than 4 times the support of the next best model (Table 3.5). Lipid, protein, and mineral nutrient reserve levels were higher in reproductive than nonreproductive females in both years, suggesting that initiation thresholds exist for these nutrients, particularly for lipid (Figure 3.1).

Discussion

Intraspecific variation in condition and timing of reproduction

In this study, we assessed intraspecific variation in nutrient acquisition strategies for meeting the nutritional and energetic costs of egg production, specifically in relation to the seasonal timing of RFG initiation by female Greater Scaup breeding in subarctic, western Alaska. We found that endogenous lipid, protein, and mineral nutrient reserve levels did not decline during egg production. Thus, females apparently employed an income breeding strategy (i.e., they relied on exogenous food resources) to meet the energy and nutrient costs of egg production, despite the relatively high cost of clutch formation for this species. Furthermore, we found that female condition at RFG initiation did not decline with later dates of clutch formation; RFG initiation dates varied 25 days in our dataset suggesting a lack of a proximate effect of nutritional status on timing of reproduction. However, we argue that nutrition does influence the seasonal timing of breeding via seasonally static body condition thresholds for RFG initiation. We also suggest that these thresholds ensure availability of nutrients and energy during reproductive stages following egg production.

Most waterfowl species studied to date rely to some degree on endogenous reserves as sources of energy and nutrients during clutch formation. Large-bodied arctic nesting geese are thought to employ highly capital breeding strategies (e.g., Ankney and MacInnes 1978, Raveling 1979, Drent and Daan 1980, but see Choinière and Gauthier 1995, Ganter and Cooke 1996, Gauthier et al. 2003). Breeding ducks also appear to rely on endogenous nutrients to varying degrees during egg production. For example, Alisauskas and Ankney (1992) reviewed data on prairie nesting species and found net declines in lipid reserves over the laying cycle in Mallards (*Anas platyrhynchos*), Canvasbacks (*Aythya valisineria*), Ring-necked Ducks (*Aythya collaris*), Blue-winged

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Teal (*Anas discors*), and Lesser Scaup. Studies of nutrient reserve dynamics of ducks breeding at high latitudes are more limited (e.g., Mann and Sedinger 1993, Esler and Grand 1994, MacCluskie and Sedinger 2000, Esler et al. 2001), but indicate that female Northern Pintails and Lesser Scaup rely heavily on lipid reserves during egg production, although work on female Northern Shovelers (*Anas clypeata*) demonstrated no use of lipid reserves during egg production (MacCluskie and Sedinger 2000).

Other studies have addressed reproductive timing issues with respect to nutritional status (Krapu 1981, Alisauskas and Ankney 1994). Esler and Grand (1994) demonstrated that lipid reserve levels at RFG initiation and rate of reserve use by female Northern Pintails both declined with date of RFG initiation, while protein reserves contributed minimally to the clutch but also declined with timing of clutch formation. Female Northern Shovelers initiating nests early had larger lipid reserves than late nesting females, despite their lack of reserve use during clutch formation (MacCluskie and Sedinger 2000). Furthermore, Esler et al. (2001) showed that lipid and protein reserve levels of female Lesser Scaup declined with date of RFG initiation, yet the proportion of endogenous nutrients in the clutch did not change over the nesting season. Taken together, these results have been interpreted as providing evidence for seasonally declining nutrient reserve thresholds for initiation of egg production (sensu Ryder 1970, Reynolds 1972).

Inconsistent with the conceptual models of Ryder (1970) and Reynolds (1972), and contrary to the body of empirical work described above, our data indicate that nutritional status of female Greater Scaup at RFG initiation did not vary seasonally, which could be interpreted to indicate that nutritional status is not a proximate determinant of reproductive timing. However, our data suggest that females need to attain some minimum level of nutrient reserves to initiate egg production. Therefore, we

interpret our data to indicate that female condition does have an effect on timing of reproduction, and that variation in RFG initiation is related to interindividual differences in timing of achieving condition thresholds, seasonally static in this case, which in turn is a function of timing of arrival on breeding areas, condition status upon arrival, and rates of nutrient reserve accumulation after arrival.

These relationships can be understood following theory described by Rowe et al. (1994) in a state-dependent dynamic model for reproductive timing and clutch size in birds. The model presented by Rowe and coworkers effectively formalized the ideas of Drent and Daan (1980), and predicts optimal decisions of lay date and clutch size by birds based on the initial body condition of females at arrival on the breeding grounds, time of arrival relative to other conspecifics, and the rate of condition gain. For example, female birds arriving on the breeding grounds at the same time, but in relatively different condition (i.e., one female arrives with higher reserve levels than another) will have different lay dates and clutch sizes, such that a female in relatively better condition will breed earlier and lay a larger clutch than a female in worse condition (Figure 3.2a). These decisions are considered to be resolutions to a conflict between the advantages of greater offspring value via seasonally earlier breeding and the advantages of accumulation of condition for potentially larger clutch sizes via seasonally later breeding (i.e., the *cost of delay* hypothesis proposed by Drent and Daan (1980)).

In Greater Scaup, females arriving on the breeding grounds in relatively poor condition likely take longer to reach the minimum condition threshold required for initiation of clutch formation, assuming constant rates of condition gain among individuals. Thus, nutritional status at arrival may be a more important determinant of reproductive timing than nutritional status at RFG initiation. Interestingly, we found some evidence that larger females, based on PC1 score for structural size, had earlier RFG

initiation dates (RFGINIT = PC1: ΔAICc = 0.00, AICcW = 0.96, r^2 = 0.13; equal means model: ΔAICc = 6.25, AICcW = 0.04). We suggest that structurally larger females may be able to carry more endogenous reserves to the breeding areas, and thus reach condition thresholds faster than smaller females, resulting in earlier dates of egg production. This idea is supported by the positive parameter estimates for PC1 score in analyses of condition thresholds for egg production for lipid and protein nutrient reserves (lipid: PC1 = 5.58 ± 2.29, protein: PC1 = 3.93 ± 0.72, values are best fit model parameter estimates ± 1SE); structurally larger birds had higher endogenous lipid and protein reserve levels. Given the fitness benefits of early reproduction, there should be selective pressure leading to larger body sizes. Although Greater Scaup are relatively large ducks, in relation to their congener Lesser Scaup, there presumably are counteracting pressures limiting body size increases.

Although we found strongly supported differences in reserve levels between reproductive states for all nutrients in condition threshold analyses, this difference was most marked with respect to lipid reserves suggesting that lipid is an important nutrient dictating initiation of egg production (Figure 3.1). This result is consistent with other studies suggesting lipid control of productivity by high latitude breeding ducks (e.g., Esler and Grand 1994, Esler at al. 2001). A recent study on nutrient reserve dynamics of Lesser Scaup during migration in the Mississippi flyway supported a "spring condition" hypothesis, which suggests that reproductive performance had declined across years due to decreases in female body condition during migration and subsequent arrival on the breeding grounds (Anteau and Afton 2004). Migrating females at late spring staging areas had smaller lipid reserves than historic data suggest from the 1980's. Nest initiation dates of Lesser Scaup breeding near these northern staging areas were

markedly later during this study than historical records indicate (Koons and Rotella 2003).

We found interannual variation in lipid reserve levels between the two years of our study as indicated by both nutrient reserve dynamics during egg production and condition threshold results where year terms were included in best fitting models (Table 3.3 and 3.4). In 2002, lipid reserve levels were higher in both nonreproductive and reproductive females than in 2003 (Figure 3.1). Furthermore, the proportion of females collected with nondeveloped ovaries was much lower in 2002 (19%) than 2003 (41%), and mean RFG initiation date was approximately 3.5 days earlier in 2002. Observationally, water levels at the beginning of the 2003 breeding season when birds arrived on the breeding grounds were much lower than the 2002 conditions, due to a lack of over-winter precipitation in western Alaska prior to the 2003 nesting season. Previous work on variability of wetlands and potential food resources for waterfowl from our study area indicates that the abundance of aquatic invertebrates (e.g., chironomidae larvae) and plant seeds are strongly affected by water regimes, particularly among years (W.T. O'Connell Unpubl. Data). Given these results, we further examined relationships between YEAR, date of collection (C-DATE) of females, and the interaction of these variables (YEAR x C-DATE) to describe variation in somatic lipid reserve levels of both nonreproductive and reproductive females to better understand rates of lipid reserve accumulation across the nesting season and between years. Two models received support, the first model included YEAR and the interaction term (ΔAICc = 0.00, AICcW = 0.36, r^2 = 0.13), while the second model included C-DATE and the interaction term (ΔAICc = 0.20, AICcW = 0.32, r^2 = 0.13). Results from the highest weighted model indicated the slope of the relation between date of collection and somatic lipid level was higher in 2002 (1.3242 ± 0.59) than 2003 (0.1889 ± 0.50), suggesting a higher rate of

lipid accumulation by females in 2002. Thus, we suggest that acquisition of nutrient reserves, particularly lipid, which apparently influences timing of egg production by female Greater Scaup may be influenced by local food resource availability which is driven by variation in water regimes. Such relationships have particularly important implications for the effects of climate change on productivity of Greater Scaup breeding at high latitudes.

We have shown that female Greater Scaup build nutrient reserves after arrival on the breeding grounds and suggest that there are initiation thresholds which must be achieved prior to initiation of reproduction. This appears inconsistent with our finding that Greater Scaup females do not use acquired reserves during egg production. However, we suggest that these nutrient reserves are, instead, used during incubation and are a necessary component of Greater Scaup reproductive performance. Based on previous work conducted on this population, nutrient reserves built following arrival are likely used to some degree during incubation. Flint (2003) demonstrated that female Greater Scaup body mass declines 6.4 g / day during incubation and reported an incubation constancy of 83%, suggesting the use of both endogenous and exogenous sources of nutrients to meet the energetic demands of incubation. Interestingly, the interannual differences we documented with respect to lipid thresholds for egg production likely may also influence incubation constancy, where lower rates of reserve accumulation and initiation thresholds in 2003 would correspond to lower incubation constancies (Figure 3.2a, b). Thus, within the capital vs. income breeding spectrum, female Greater Scaup may be considered income layers, but use mixed capital and income approaches during incubation (sensu Thomas 1988, Meijer and Drent 1999).

Female condition, timing of reproduction, and effects on clutch size

There has been considerable debate in the waterfowl literature concerning the role of nutrient reserves in proximately limiting clutch size (e.g., Drobney 1991, Ankney et al. 1991, Arnold and Rohwer 1991); our data are inconsistent with the idea that seasonal declines in nutrient reserve levels at RFG initiation correspond to declines in clutch size as has been suggested by other researchers (e.g., Esler and Grand 1994, Esler et al. 2001). We found evidence that female Greater Scaup nutrient reserve levels at the onset of egg production are static across the nesting season, despite reported declines in clutch size at a rate of 0.100 ± 0.007 eggs / day based on long-term data from this population (Flint et al. 2005). This raises the important question, is there a proximate constraint of nutrition on clutch size of female Greater Scaup? Given that timing or reproduction and clutch size are tightly associated, and we have shown an influence of female condition on initiation of egg production, clutch size is at least indirectly affected by female condition. However, our data do not suggest that females terminate egg formation as a direct response to nutritional constraints.

Interspecific variation in timing of reproduction

Long-term data from our study site in western Alaska indicate that Greater Scaup are, on average, the latest nest initiating species (Flint et al. 2005). Other species breeding seasonally earlier than Greater Scaup include Northern Pintails, Common Eiders (*Somateria mollisma*), Spectacled Eiders (*Somateria fischeri*), and Long tailed Ducks (*Clangula hyemalis*). Sympatric nesting pintails were found to rely on endogenous lipid reserves during egg production early in the breeding season more than any other duck species studied to date (Esler and Grand 1994). Although data are lacking on nutrient reserve use for sympatric nesting eiders and Long-tailed Ducks during egg production at

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our study site, large seaducks such as eiders are speculated to have considerable

reliance on stored nutrient reserves during egg production due to their structural size

and relatively early initiation of clutch formation. Thus, female Greater Scaup are likely

late nest initiators due to their extreme reliance on exogenous food resources during egg

production which are presumably not available when sympatric nesting species begin

clutch formation. In addition, interspecific variation in timing of reproduction may be

influenced by variation in migration strategies as well as wing-loading. Eiders and Long-

tailed Ducks, species similar to Greater Scaup which have high wing-loading, may be

able to store reserves during winter and spring staging for use during breeding because

they have a much shorter migration than Greater Scaup which migrate along both

Atlantic and Pacific coasts.

Conservation implications

Recent conservation concerns over declining North American scaup populations (Austin

et al. 2000, Afton and Anderson 2001) have highlighted the need for studies addressing

the role of food resources and body condition in reproductive performance (e.g., Anteau

and Afton 2004. Our work compliments that by Esler et al. (2001) which evaluated the

importance of nutrient reserves in reproductive timing by female Lesser Scaup breeding

in interior Alaska and Manitoba, Canada. Population estimates of scaup breeding in

coastal tundra habitat of western Alaska, thought to be primarily Greater Scaup, appear

to have been stable since 1955 when breeding waterfowl surveys began. In

comparison, numerical declines have been described for boreal forest breeding

populations since the early 1980's, which are thought to be predominately Lesser Scaup

(Afton and Anderson 2001). The marked differences which have been documented in

nutrient reserve use during egg production between female Greater and Lesser Scaup

are clearly important in considering the different population trends of the 2 species. Female Lesser Scaup may face very different selective pressures than female Greater Scaup given their different nutrient acquisition strategies to meet the demands of egg production. Such, differences should be considered when developing strategies for managing Greater and Lesser scaup populations separately.

Acknowledgements

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Table 3.1 Candidate models for describing variation in somatic nutrient reserve levels of reproductive female Greater Scaup. S-NUTRIENT includes somatic lipid, protein, or mineral. R-NUTRIENT includes the corresponding reproductive nutrient according to the S-NUTRIENT in question.

Response Variable	Model	Explanatory Variable
S-NUTRIENT	1	Equal Means Model
	2	PC1
	3	YEAR
	4	R-NUTRIENT
	5	RFGINIT
	6	R-NUTRIENT x RFGINIT
	7	PC1, R-NUTRIENT
	8	PC1, RFGINIT
	9	PC1, R-NUTRIENT x RFGINIT
	10	R-NUTRIENT, RFGINIT
	11	R-NUTRIENT, R-NUTRIENT x RFGINIT
	12	RFGINIT, R-NUTRIENT x RFGINIT
	13	PC1, R-NUTRIENT, RFGINIT
	14	PC1, R-NUTRIENT, R-NUTRIENT x RFGINIT
	15	PC1, RFGINIT, R-NUTRIENT x RFGINIT
	16	R-NUTRIENT, RFGINIT, R-NUTRIENT x RFGINIT
	17	PC1, R-NUTRIENT, RFGINIT, R-NUTRIENT x RFGINIT
	18	PC1, YEAR
	19	R-NUTRIENT, YEAR
	20	RFGINIT, YEAR
	21	R-NUTRIENT x RFGINIT, YEAR
	22	PC1, R-NUTRIENT, YEAR
	23	PC1, RFGINIT, YEAR
	24	PC1, R-NUTRIENT x RFGINIT, YEAR
	25	R-NUTRIENT, RFGINIT, YEAR
	26	R-NUTRIENT, R-NUTRIENT x RFGINIT, YEAR
	27	RFGINIT, R-NUTRIENT x RFGINIT, YEAR
	28	PC1, R-NUTRIENT, RFGINIT, YEAR
	29	PC1, R-NUTRIENT, R-NUTRIENT x RFGINIT, YEAR
	30	PC1, RFGINIT, R-NUTRIENT x RFGINIT, YEAR
	31	R-NUTRIENT, RFGINIT, R-NUTRIENT x RFGINIT, YEAR
	32	PC1, R-NUTRIENT, RFGINIT, R-NUTRIENT x RFGINIT, YEAR
	33	R-NUTRIENT, R-NUTRIENT x YEAR, YEAR
	34	RFGINIT, RFGINIT x YEAR, YEAR

Abbreviations: Principal components score (PC1), RFG initiation date (RFGINIT).

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Table 3.2 Candidate models for describing variation in somatic nutrient reserve levels between nonreproductive and reproductive female Greater Scaup. S-NUTRIENT includes somatic lipid, protein, or mineral.

Response Variables	Model	Explanatory Variables
S-NUTRIENT	1	Equal Means Model
	2	R-STATE
	3	PC1
	4	YEAR
	5	C-DATE
	6	R-STATE, PC1
	7	R-STATE, YEAR
	8	R-STATE, C-DATE
	9	PC1, YEAR
	10	PC1, C-DATE
	11	YEAR, C-DATE
	12	R-STATE, PC1, YEAR
	13	R-STATE, PC1, C-DATE
	14	R-STATE, YEAR, C-DATE
	15	PC1, YEAR, C-DATE
	16	R-STATE, PC1, YEAR, C-DATE

Abbreviations: Reproductive state (R-STATE), Principal components score (PC1), Collection date (C-DATE).

Table 3.3 Candidate models describing variation in nutrient reserve levels of female Greater Scaup during egg production. Models presented are those best fit by the data as well as all models receiving ΔAICc values ≤ 2.

Response Variables	Model #	Explanatory Variables	# of Parameters	ΔAICc	AICcW	r^2
S-LIPID	1	PC1, YEAR	4	0.00	0.19	0.13
	2	PC1	3	1.15	0.10	0.08
S-PROTEIN	1	PC1, R-PROT	4	0.00	0.20	0.36
	2	PC1, RFGINIT, R-PROT x RFGINIT	5	0.52	0.15	0.38
	3	PC1, R-PROT, RFGINIT	5	0.83	0.13	0.38
	4	PC1, R-PROT x RFGINIT	4	1.91	0.08	0.34
S-MINERAL	1	PC1, R-MIN, YEAR	5	0.00	0.15	0.22
	2	PC1, R-MIN, RFGINIT, YEAR	6	0.02	0.15	0.25
	3	RFGINIT, R-MIN x RFGINIT, YEAR	5	0.10	0.14	0.22
	4	PC1, RFGINIT, R-MIN x RFGINIT, YEAR	6	0.34	0.12	0.25
	5	R-MIN, RFGINIT, YEAR	5	0.79	0.10	0.21

Abbreviations: Principal components score (PC1), R-PROTEIN (R-PROT), RFG initiation date (RFGINIT), R-MINERAL (R-MIN).

Table 3.4 Parameter estimates from candidate models describing variation in nutrient reserve levels of female Greater Scaup during egg production. Parameter estimates are weighted averages and standard errors are based on unconditional variances. Parameter likelihoods are summed AICc weights across all models including that particular variable.

Response Variables	Explanatory Variables	Parameter Likelihood	Parameter Est ± 1SE
S-LIPID	Intercept		117.22 ± 6.60
	PC1	0.7168	4.27 ± 2.36
	R-LIPID	0.2999	0.04 ± 0.06
	RFGINIT	0.2746	-0.08 ± 0.25
	R-LIPID x RFGINIT	0.2619	-0.0021 ± 0.0041
	YEAR	0.6485	8.37 ± 6.56
	R-LIPID x YEAR	0.0194	0.0061 ± 0.0073
	RFGINIT x YEAR	0.0242	0.0483 ± 0.0577
S-PROTEIN	Intercept		161.44 ± 2.82
	PC1	0.9998	3.96 ± 0.16
	R-PROT	0.5836	0.04 ± 0.03
	RFGINIT	0.4747	-0.16 ± 0.14
	R-PROT x RFGINIT	0.4521	0.0017 ± 0.0023
	YEAR	0.2366	-0.06 ± 0.53
	R-PROT x YEAR	0.0000	0.00 ± 0.00
	RFGINT x YEAR	0.0000	0.00 ± 0.0041
S-MINERAL	Intercept		33.75 ± 1.54
	PC1	0.6172	0.41 ± 0.08
	R-MIN	0.6074	0.04 ± 0.03
	RFGINIT	0.6466	-0.05 ± 0.12
	R-MIN x RFGINIT	0.4920	0.0023 ± 0.0026
	YEAR	0.9457	-2.61 ± 0.95
	R-MIN x YEAR	0.0090	-0.0001 ± 0.0006
	RFGINIT x YEAR	0.0063	0.0014 ± 0.0018

Abbreviations: Parameter Estimate (Parameter Est), Principal components score (PC1), RFG initiation date (RFGinit), R-PROTEIN (R-PROT), R-MINERAL (R-MIN).

Table 3.5 Candidate models models describing variation in somatic nutrient reserve levels between nonreproductive and reproductive female Greater Scaup. Parameter likelihoods are presented as well as all models receiving ΔAICc values $\leq$ 2.

Response Variables	Model #	Explanatory Variables	Parameter Likelihood	# of Parameters	ΔAICc	AICcW	r^2
S-LIPID	1	R-STATE, PC1, YEAR		5	0.0000	0.4749	0.34
		Intercept	1.0000				
		R-STATE	0.7127				
		PC1	0.6254				
		YEAR	0.5462				
		C-DATE	0.2873				
S-PROTEIN	1	R-STATE, PC1		4	0.0000	0.4738	0.28
		Intercept	1.0000				
		R-STATE	0.6296				
		PC1	0.7105				
		YEAR	0.1783				
		C-DATE	0.2895				
S-MINERAL	1	R-STATE, PC1, YEAR		5	0.0000	0.5425	0.18
		Intercept	1.0000				
		R-STATE	0.6054				
		PC1	0.6532				
		YEAR	0.7085				
		C-DATE	0.2764				

Abbreviations: Reproductive state (R-STATE), Principal components score (PC1), Collection date (C-DATE).

Figure 3.1 Somatic nutrient reserve variation in relation to reproductive state and year. Values are mean reserve levels ± 1SE for nonreproductive 2002 (•), reproductive 2002 (o), nonreproductive 2003 (▼), and reproductive 2003 (▽) female Greater Scaup controlling for structural size.
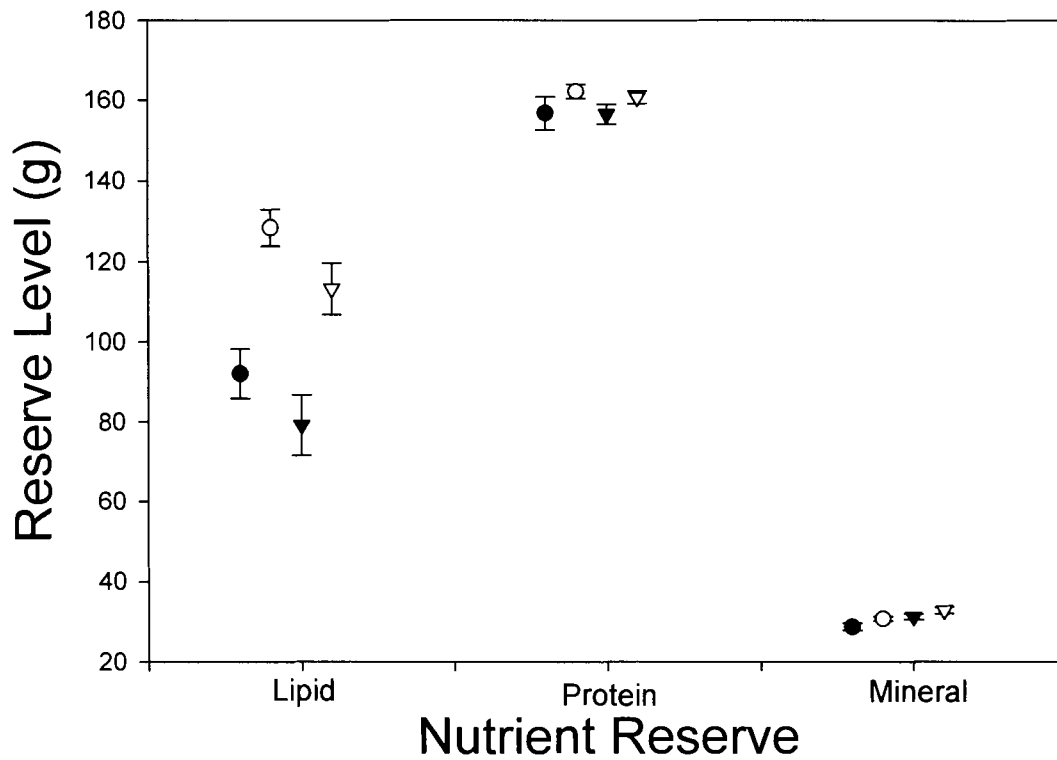
Figure 3.2 Conceptual models of the relationships between relative female condition at arrival, rate of nutrient reserve accumulation, and timing of reproduction, as well as variable nutrient reserve thresholds for initiation of egg production and subsequent effects on incubation constancy by female Greater Scaup. Females in relatively better condition at arrival (A) reach the minimum threshold for egg production faster than females in poorer condition (B) resulting in seasonally earlier reproduction. A higher rate of reserve accumulation and a higher minimum threshold for egg production in 2002 (Model a) would correspond with a higher incubation constancy, in comparison with Model b where a lower rate of reserve accumulation and minimum threshold in 2003 would correspond with a lower incubation constancy. Models after Bêty et al. (2003).
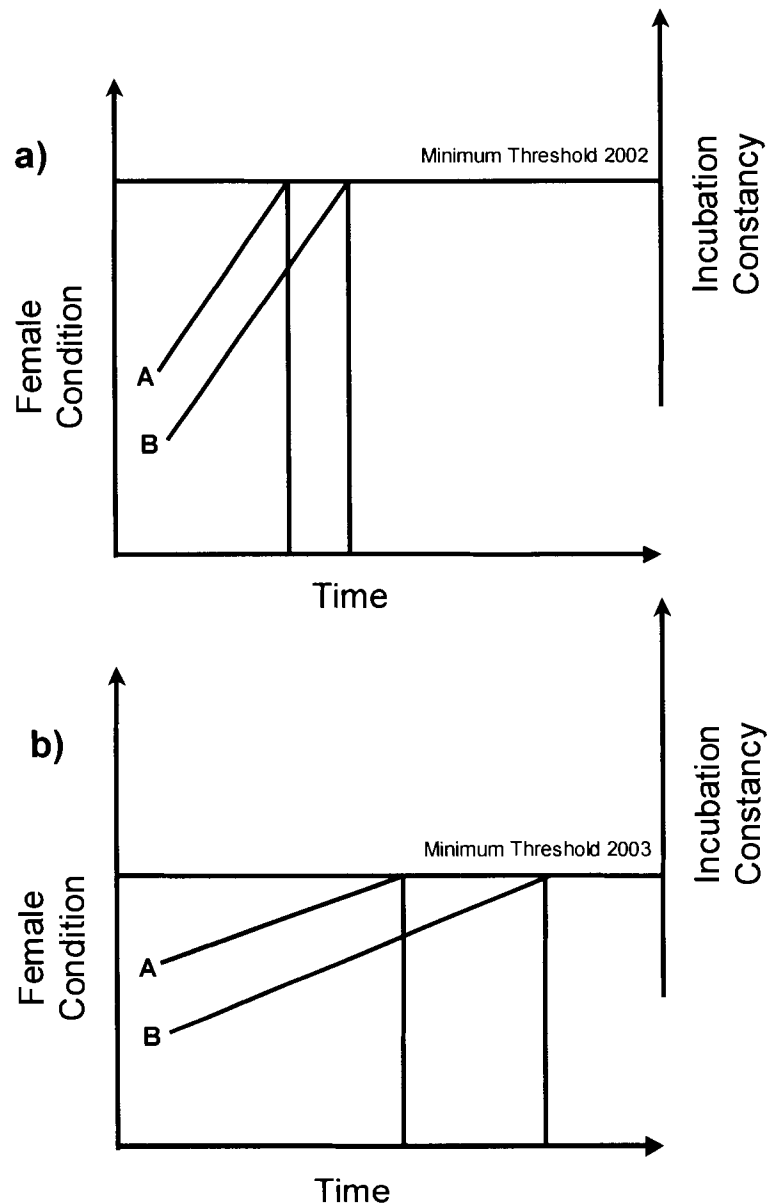
Chapter 4.

Plasma yolk precursor dynamics during egg production by female Greater Scaup (*Aythya marila*): characterization and indices of reproductive state

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Abstract

Physiological indices, based on well characterized hormonal or metabolite profiles, are a novel method which can be used noninvasively via blood sampling for discerning detailed information on individual state or condition. We characterized dynamics of the plasma yolk precursors vitellogenin (VTG) and very-low density lipoprotein (total VLDL) in female Greater Scaup (*Aythya marila*) during the egg production phase of the breeding cycle and evaluated yolk precursors as indices of reproductive state. Plasma concentrations of VTG and total VLDL increased rapidly as ovarian follicles initiated rapid follicle growth (RFG) prior to laying, from mean concentrations of 0.58 ± 0.05 *ug* / ml VTG and 3.75 ± 0.29 mmol / l total VLDL for females with nondeveloped ovaries to mean concentrations of 3.38 ± 0.40 *ug* / ml VTG and 7.31 ± 2.56 mmol / l total VLDL for females with a full follicle hierarchy. Concentrations of both yolk precursors remained elevated through the laying cycle and decreased markedly by three days into incubation. To describe concentrations of VTG and total VLDL as physiological indices of reproductive state, variation in VTG concentration was best explained by a model where WINTER and NONDEVELOPED states were grouped together, while RFG, LAYING, and INCUBATION reproductive states were grouped independently. Variation in total VLDL concentration was explained by models that grouped nonreproductive states (i.e., WINTER, NONDEVELOPED, INCUBATION) and reproductive states (i.e. RFG, LAYING). We tested the predictive capacity of both yolk precursors to identify course scale reproductive states (nonreproductive and reproductive). Demarcation values of VTG and total VLDL were established based on 2002 data and then tested on 2003 data where a VTG demarcation value of 1.4 *ug* / ml correctly classified 88% of 2003 females and a total VLDL demarcation value of 5.4 mmol / l correctly classified 72% of 2003 females. When demarcation values of VTG and total VLDL where established, based on

pooled data from 2002 and 2003, a demarcation value of 1.4 ug / ml VTG correctly classified the greatest proportion of females (90%). In comparison, a demarcation value of 5.3 mmol / l total VLDL correctly classified 74% of females. Our work suggests that plasma yolk precursor analysis can provide a noninvasive, reliable physiological index of egg production in female scaup. Thus, the method should be of great utility to a variety of research in evolutionary, ecological, and applied conservation studies of reproduction in waterfowl.

Introduction

Reproduction is a critical biological function, receiving attention in an array of disciplines including life history evolution (Stearns 1992, Roff 1992) and behavioural ecology (Krebs and Davies 1997), ecological and evolutionary energetics (Ricklefs 1996), as well as studies in population biology and environmental toxicology. Advances in these disciplines are often hindered by a lack of methodology allowing noninvasive, accurate assessment of reproductive parameters. The use of physiological indices to assess reproductive parameters, when based on measurement of well characterized hormonal or metabolite profiles (Goodman 1999, Williams 1999), is a novel approach that can provide detailed information on individual physiological state obtained simply through blood sampling.

Primary reproductive effort (i.e., the egg production phase of the breeding cycle) is a particularly important aspect of waterfowl reproductive biology given their developmental mode. Waterfowl young are precocial, therefore, adult females have a high investment of energy and nutrients directly into eggs and less in offspring after hatch, as young are nidifugous and self-feeding (Alisauskas and Ankney 1992). Thus, reproductive performance in these systems is often highly influenced during egg

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production as opposed to subsequent phases of the breeding cycle (i.e., brood-rearing). Consequently, many studies of waterfowl reproductive biology have focused on measuring parameters associated with primary reproductive effort (e.g., breeding propensity, nest initiation dates, clutch size, and renesting).

The use of noninvasive physiological methods for assessment of condition or state (sensu Houston and McNamara 1999) has been examined in many passerine and shorebird species (see Brown 1996 for review, plasma metabolites: Jenni-Eiermann and Jenni 1994, Williams et al. 1999, hematocrit: Piersma et al. 1996, and immune function: Ots et al. 1998). Despite the potential utility of these techniques to waterfowl research, proper characterization of many physiological parameters is lacking for this group. In this paper, we develop a physiological approach to assess reproductive state in female waterfowl, based on the relationship between circulating concentrations of the plasma yolk precursors vitellogenin (VTG) and very low-density lipoprotein (VLDL) with ovarian development during egg production given the focus by waterfowl researchers on this phase of the breeding cycle.

The two main plasma yolk precursors VTG and yolk targeted VLDLy are produced by the avian liver in response to increasing plasma estrogen concentrations during reproduction (see Johnson 2000 for review, Deely et al. 1975, Chan et al. 1976). VTG is the primary source of egg yolk protein, while VLDLy is the primary source of egg yolk lipid produced via physical and biochemical changes in hepatically produced generic VLDL (Walzem 1996). Both VTG and total VLDL (i.e., generic and VLDLy) are greatly elevated in circulation during egg production for transport to developing ovarian follicles (Deely et al. 1975, Chan et al. 1976). Thus VTG and total VLDL present noninvasively measurable physiological parameters potentially useful for identifying reproductive state in free-living birds.

Although many aspects of yolk precursor production have been well studied in captive species such as the domestic hen (e.g., Deely et al. 1975, Chan et al. 1976, Wang and Williams 1982, Walzem 1996, Walzem et al. 1999) and Zebra Finch (Williams 2000, 2001, Salvante and Williams 2002, 2003), relatively little is known about yolk precursor dynamics in free-living species (e.g., Vanderkist et al. 2000, Challenger et al. 2001). Here we present the first characterization of plasma yolk precursor dynamics during the laying cycle of a free-living waterfowl species, female Greater Scaup (*Aythya marila*). We predicted that circulating concentrations of VTG and total VLDL were tightly coupled with the egg production phase of the breeding cycle, and therefore, provide accurate physiological indices of reproductive state. Thus, we describe circulating VTG and total VLDL concentrations among discrete reproductive states, and finally examine the predictive capacity of VTG and total VLDL indices to identify nonreproductive and reproductive females.

Methods

Field methods

This study was conducted on female Greater Scaup (*Aythya marila*) breeding along the lower Kashunuk River (60°20'N, 165°35'W) on the Yukon-Kuskokwim Delta, Alaska during the breeding seasons of 2002 and 2003. This study was performed in accordance with permits from the U.S. Fish and Wildlife Service, the State of Alaska Department of Fish and Game, and the Yukon Delta National Wildlife Refuge. In addition, this study conformed to guidelines of the Canadian Committee on Animal Care (Simon Fraser University Animal Care Permit 637B-02). Approximately two female Greater Scaup were collected each day between 19 May - 21 June 2002 (n = 58) and 15 May - 19 June 2003 (n = 54), as these dates included the egg production phase of the breeding cycle for most individuals. In addition, three female Greater Scaup wintering

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along the British Columbia coast were collected (8 March, 2003) to obtain yolk precursor data for wintering birds in accordance with a permit from Environment Canada. At the time of collection, a blood sample was immediately taken via a heparinized syringe to obtain plasma for yolk precursor analyses.

Reproductive organs including ovaries, oviducts and oviductal eggs were dissected within 24 hours of collection. Ovaries were weighed and subsequently preserved in 10% formalin. Plasma for VTG and total VLDL analyses was separated from heparinized treated blood via centrifugation also within the same day of collection and stored frozen.

Laboratory methods

Plasma samples were assayed for yolk precursors VTG and total VLDL using diagnostic kits for vitellogenic zinc (Zinc kit, Wako Chemicals) and total triglycerides (Triglyceride E kit, Wako Chemicals for 2002 samples and glycerol kit (reagents A and B), Sigma for 2003 samples), respectively at Simon Fraser University following the methods of Mitchell and Carlisle (1991). All assays were measured using a Biotel 340i microplate reader. Given that two different kits were used for estimation of plasma total VLDL, quantification of total VLDL by the Wako and Sigma kits was validated and there was no significant difference in the concentrations measured by the two kits (Wako: 11.91 ± 1.11 mg/ml, Sigma: 12.79 ± 1.19 mg/ml, values are means ± 1 SE, n = 29, T. D. Williams, unpubl. data). Intra-assay coefficients of variation for VTG using a laying hen plasma pool (n = 10) and total VLDL using a 19-week hen plasma pool (n = 40) were 7.5% and 7.9%, respectively. Inter-assay coefficients of variation were 9.7% and 6.3% for VTG (n = 15) and total VLDL (n = 7), respectively.

Formalin preserved ovaries were dissected and measured as described in

Chapter 2 to obtain data on reproductive state for individual females. Based on these

results reproductive states were defined as winter: females collected in coastal British

Columbia (n = 3); nondeveloped: largest ovarian follicle < 9.36 mm and < 0.26 g dry

mass (n = 32); rapid follicle growth (RFG) (i.e., phase of oogenesis where individual

ovarian follicles uptake yolk lipid and protein, see Johnson 2000 for review): largest

ovarian follicle ≥ 9.36 mm, ≥ 0.26 g dry mass and no postovulatory follicles present (n =

32); laying: ≥ 1 postovulatory follicle and a pre-ovulatory follicle of sufficient size to be

ovulated within 24 hours (> 35.6 mm) or the presence of an oviductal egg (n = 30); and

incubation: a hierarchy of regressing postovulatory follicles and the presence of a brood

patch (n = 10).

Measurements (± 0.01 mm) of the right wing chord, right tarsus, culmen, bill

width, bill nail width, bill nail length, and keel length of collected birds were obtained for

principal components analysis (PC1 score) to be used as an index of body size. The

first principal component explained 25% of the total variance.

Statistical methods

All statistical analyses were performed in SAS (SAS Institute 1999). To describe yolk

precursor concentrations as physiological indices of reproductive state, general linear

models were used to examine variation in yolk precursor concentrations (VTG, total

VLDL) in relation to discrete reproductive state explanatory variables (WINTER,

NONDEVELOPED, RFG, LAYING, INCUBATION) (n = 100 for these analyses). YEAR

also was included as an explanatory variable to assess interannual variation in yolk

precursor dynamics. Preliminary analyses indicated there was no relationship between

yolk precursor concentration (VTG, total VLDL) and PC1 score for structural size (r^2 <

0.005 in both cases), therefore PC1 score was not included as an explanatory variable. Information-theoretic methods were used to direct model selection (Burnham and Anderson 2002). The set of candidate models (Table 4.1) for each yolk precursor included biologically plausible combinations of explanatory variable groupings, as well as an equal means model, resulting in 34 candidate models to describe variation in VTG and total VLDL concentration, separately. For each model, Akaike's Information Criterion including a correction for small sample size (AICc) was calculated. Candidate models were compared using ΔAICc values; which is the difference between the AICc value for a particular model and the lowest AICc value within the set. Models with ΔAICc values ≤ 2 were considered to have strong support. In addition, AICc weight (AICcW) values were used to consider the likelihood that a particular supported model was the best model (Burnham and Anderson 2002). This modelling approach is analogous to performing a traditional ANOVA to determine differences in yolk precursor concentrations among discrete groups (i.e., reproductive states), where AIC model selection criteria are used instead of an alpha-level to determine which model best describes variation in these data.

We also tested the predictive capacity of yolk precursors (VTG and total VLDL, separately) to identify more course scale reproductive states, NONREPRODUCTIVE (i.e., females with nondeveloped ovaries and incubation) and REPRODUCTIVE (i.e., RFG and laying females) as these groups are likely more biologically relevant to researchers interested in using this technique. Our analysis first used yolk precursor data from 2002 to discern a VTG or total VLDL demarcation concentration (i.e., individuals with lower concentrations than the demarcation value were classified as nonegg producing while individuals with higher concentrations were classified as egg producing) that maximized the correct classification of reproductive state. This involved

iteratively assigning yolk precursor data to either nonreproductive or reproductive groups and comparing the predicted classification with the known classification for a particular individual. Then, this demarcation concentration was applied to 2003 data to test how well it predicted reproductive state. Finally, demarcation concentrations for both VTG and total VLDL that maximized the correct classification of reproductive state using pooled data from 2002 and 2003 were determined.

Results

Characterization of VTG and total VLDL dynamics

Circulating concentrations of VTG and total VLDL were related within individuals (r^2 = 0.33). Both yolk precursors increased rapidly as total ovary mass increased during RFG prior to laying. We described the relationship between total ovary mass and both yolk precursor concentrations prior to laying as a hyperbolic function (i.e., yolk precursor concentration = [a x total ovary mass] / [b + total ovary mass]) (VTG: r^2 = 0.71; VLDL: r^2 = 0.27) (Figure 4.1). Mean (± 1 SE) concentrations were low in females with nondeveloped ovaries and increased to 1.62 ± 0.29 *ug* / ml VTG and 5.91 ± 1.20 mmol / l total VLDL in females with 1 follicle in RFG (n = 11). Yolk precursor concentrations continued to increase through RFG, in females with ovarian development at a full follicle hierarchy (i.e., ≥ 5 developing follicles > 9.36 mm, n = 4) mean concentrations were 3.38 ± 0.40 *ug* / ml VTG and 7.31 ± 2.56 mmol / l total VLDL (Figure 4.1). Yolk precursor concentrations remained high through the laying cycle, and decreased markedly by 3 days into incubation (Figure 4.2).

Describing yolk precursors as physiological indices of reproductive state

Mean (± 1 SE) plasma concentrations for discrete reproductive states including

WINTER, NONDEVELOPED, RFG, LAYING, and INCUBATION were 0.13 ± 0.13, 0.58

± 0.05, 2.45 ± 0.24, 3.33 ± 0.21, and 1.73 ± 0.66 ug / ml respectively for VTG, and 1.10 ±

0.23, 3.75 ± 0.29, 6.76 ± 0.68, 7.64 ± 0.67, 4.85 ± 2.09 mmol / l respectively for total

VLDL (Figure 4.3).

Two models of reproductive state groupings received support for describing

variation in VTG concentration (ΔAICc ≤ 2) (Table 4.2). The best fitting model grouped

WINTER and NONDEVELOPED states together and indicated that this group was

different in mean VTG concentration from all other reproductive states. Mean VTG

concentration of RFG, LAYING, and INCUBATION reproductive states also were

different from all other reproductive states. The best supported model received a

substantially higher AICcW than the second supported model (i.e., 0.46 vs. 0.19). There

was no indication that YEAR was an important parameter in describing variation in VTG

concentration; the weighted parameter estimate for year was 0.0073 with an

unconditional SE of 0.0585.

Eight models of reproductive state groupings received support for describing

variation in total VLDL concentration (ΔAICc ≤ 2) (Table 4.2). There was a lack of a

clearly higher weighted model (i.e., AICcW values) among the eight reproductive state

models receiving support, indicating a high degree of model uncertainty. The best fitting

model grouped nonegg producing states together (i.e., WINTER, NONDEVELOPED,

and INCUBATION), as well as egg producing states (i.e., RFG and LAYING) which was

different from the nonegg producing state. In fact, all of the models receiving ΔAICc

values ≤ 2 indicated that egg producing females had a different mean total VLDL

concentration than the other groups (Table 4.2). Although the year parameter occurred

in two of the eight models with ΔAICc values ≤ 2, the weighted parameter estimate was small (0.1888) with a relatively large associated unconditional SE (0.2639) suggesting that year was not an important explanatory variable.

Testing yolk precursors as physiological predictors of reproductive state

VTG performed better than total VLDL at predicting NONREPRODUCTIVE and REPRODUCTIVE states of females. A VTG concentration of 1.4 *ug* / ml best identified reproductive state using 2002 data, with 92% of individuals correctly identified. Applying this demarcation value to 2003 data resulted in 88% of females being correctly classified. In comparison, a demarcation value of 5.4 mmol / l total VLDL, based on 2002 data, best identified 72% of females. When applied to 2003 data, this demarcation value correctly identified 74% of individuals. Using pooled data from both 2002 and 2003, demarcation values best identifying reproductive state were 1.4 *ug* / ml VTG (90% correct classification) and 5.3 mmol / l total VLDL (74% correct classification).

Discussion

Yolk precursor dynamics have not been characterized in many free-living avian taxa (e.g., Vanderkist et al. 2000, Challenger et al. 2001). Consequently, this work represents the first description of VTG and total VLDL dynamics in a precocial species, Greater Scaup. We found evidence for a strong coupling between circulating yolk precursor concentrations and ovarian follicular development during egg production. Plasma concentrations of both VTG and total VLDL were low in females with nondeveloped ovaries and rose markedly to maximum levels within an increase of only a few grams of ovary mass during RFG. VTG and total VLDL concentrations remained

high at maximum levels through the laying cycle and decreased to low concentrations by 3 days into incubation.

These dynamics are similar to those reported for other nondomestic birds in terms of the general onset, maintenance, and termination of yolk precursor production during the laying cycle (Cassin's Auklet: Vanderkist et al. 2000, European Starling: Challenger et al. 2001, Zebra Finch: Salvante and Williams 2002). Basal and maximum VTG concentrations were similar among species, where as maximum total VLDL concentrations were approximately 3-fold lower in comparison with starling females. However, Vanderkist et al. (2000) described a similar maximum total VLDL concentration for breeding female Cassin's Auklet as we found with scaup. Such interspecific differences in yolk precursor dynamics highlight the importance of properly characterizing these physiological parameters in order to be useful as a technique for discerning reproductive state. The extent to which our data are applicable to other waterfowl species remains unknown. However, given the limited inter-specific data available on yolk precursor dynamics in free-living birds it appears that VTG dynamics are highly conserved across taxa, while variation in total VLDL dynamics may be driven by developmental mode (i.e., altricial vs. precocial). Thus, it seems that our data, particularly for VTG, is likely applicable to other duck species, although it would be informative to obtain similar data from other waterfowl in order to better understand how general yolk precursor dynamics are across species.

It was clear from these analyses that VTG varied more discretely among reproductive states than total VLDL. The best supported model for VTG indicated that circulating concentrations were associated with a pre-reproductive state (i.e., winter and nondeveloped) as well as RFG, laying, and incubation states. In contrast, the best supported model describing variation in total VLDL concentrations grouped nonegg

producing (i.e., winter, nondeveloped, incubation) and egg producing (i.e., RFG, laying) reproductive states. Our results from examining the predictive capacities of VTG and total VLDL corroborate the conclusion that VTG is a more reliable index of reproductive state. We found that using a VTG demarcation value correctly classified up to 90% of females while total VLDL classified only 74% of females as either non-egg or egg producing.

These results are similar to those of Vanderkist et al. (2000), who demonstrated that VTG was a more reliable index of egg production. Explanations for these observed differences between VTG and total VLDL are likely due to the different physiological roles of each precursor. VTG and the yolk-targeted VLDLy component of total VLDL are effectively absent in male, immature, and non-breeding female birds, yet following estrogen treatment or the onset of egg production VTG and VLDLy are readily synthesized by the liver (Walzem 1996, Speake et al. 1998). Conversely, the generic VLDL component of total VLDL is present in circulation of non-laying and male birds, serving as a transport mechanism for energy to somatic tissues (Walzem 1996). Given that we measured total VLDL, including generic VLDL and yolk-targeted VLDLy, our measurement includes the portion of total VLDL which is used by the hen for maintenance metabolism during breeding, and is therefore not as specific a precursor to egg production as VTG. Some among-individual variation in total VLDL concentrations could therefore be due to maintenance metabolism.

Although our results indicate VTG is a stronger predictor of reproductive state, we advise researchers interested in using this technique to measure both yolk precursors when other a priori information on reproductive state is unavailable. The diagnostic kits used in both assays are relatively inexpensive and require very small amounts of plasma for analysis (VTG ~ 125 ul, total VLDL ~ 5 ul plasma per sample).

We showed that VTG and total VLDL are correlated within individuals. This relationship is particularly useful given that the VTG assay requires different plasma aliquots for non-egg and egg producing individuals, where as the total VLDL assay does not. Therefore, first analyzing samples for total VLDL provides an estimate of reproductive status on which to base VTG assay protocols. We do suggest basing final reproductive state assignments based on VTG data given we have shown it is a more accurate index of reproductive state with higher predictive capabilities.

Vanderkist et al. (2000), Lougheed et al. (2002), McFarlane-Tranquilla et al. (2003), and Perry et al. (2004) are excellent examples of the utility of yolk precursors as a tool for discerning reproductive parameters at the population level such as breeding phenology and proportion of fecund females in a species of concern, the Marbled Murrelet. Similarly, yolk precursor analysis can now be used by waterfowl researchers. Recent conservation concerns over declining North American scaup populations (i.e., *A. marila* and *affinis*) have prompted research in a number of areas including reproductive ecology (Austin et al. 2000). In conjunction with radio-telemetry studies, yolk precursor analysis can be used to more accurately discern where in the breeding cycle failed reproduction occurs (i.e., breeding propensity, failed nesting, or clutch loss due to predation or environmental conditions) (e.g., Perry et al. 2004). In conjunction with other physiological parameters such as plasma calcium (Newman et al. 1997) and / or prolactin such methods might aid in identifying all phases of the laying cycle from the onset of egg production during RFG, to laying, and incubation. Furthermore, yolk precursor analysis likely has important utility in studies of toxicological effects on reproduction in waterfowl. In summary, plasma yolk precursor analysis is a useful technique that should be applicable to a variety of research in evolutionary, ecological, and applied conservation studies of reproduction in waterfowl.

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Table 4.1   Candidate models for describing variation in plasma yolk precursor concentrations of female Greater Scaup.

Response Variable	Model	Explanatory Variable
Yolk Precursor	1	Equal Means Model
(VTG or total VLDL)	2	W ≠ (ND = RFG = L = I)
	3	W ≠ ND ≠ (RFG = L = I)
	4	W ≠ ND ≠ RFG ≠ (L = I)
	5	W ≠ ND ≠ (RFG = L) ≠ I
	6	W ≠ (ND = RFG) ≠ L ≠ I
	7	W ≠ (ND = RFG = L) ≠ I
	8	(W = ND) ≠ RFG ≠ L ≠ I
	9	(W = ND) ≠ (RFG = L = I)
	10	(W = ND = I) ≠ (RFG = L)
	11	(W = ND = I) ≠ RFG ≠ L
	12	(W = I) ≠ (ND = RFG = L)
	13	(W = I) ≠ ND ≠ RFG ≠ L
	14	W ≠ (ND = I) ≠ (RFG = L)
	15	W ≠ (ND = I) ≠ RFG ≠ L
	16	(W = ND) ≠ (RFG = L) ≠ I
	17	W ≠ ND ≠ RFG ≠ L ≠ I
	18	(W = ND = RFG = L = I), YEAR
	19	W ≠ (ND = RFG = L = I), YEAR
	20	W ≠ ND ≠ (RFG = L = I), YEAR
	21	W ≠ ND ≠ RFG ≠ (L = I), YEAR
	22	W ≠ ND ≠ (RFG = L) ≠ I, YEAR
	23	W ≠ (ND = RFG) ≠ L ≠ I, YEAR
	24	W ≠ (ND = RFG = L) ≠ I, YEAR
	25	(W = ND) ≠ RFG ≠ L ≠ I, YEAR
	26	(W = ND) ≠ (RFG = L = I), YEAR
	27	(W = ND = I) ≠ (RFG = L), YEAR
	28	(W = ND = I) ≠ RFG ≠ L, YEAR
	29	(W = I) ≠ (ND = RFG = L), YEAR
	30	(W = I) ≠ ND ≠ RFG ≠ L, YEAR
	31	W ≠ (ND = I) ≠ (RFG = L), YEAR
	32	W ≠ (ND = I) ≠ RFG ≠ L, YEAR
	33	(W = ND) ≠ (RFG = L) ≠ I, YEAR
	34	W ≠ ND ≠ RFG ≠ L ≠ I, YEAR

Abbreviations: Winter (W), Nondeveloped (ND), Rapid Follicle Growth (RFG), Laying (L), and Incubation (I).

Figure 4.1 Relationship between total ovary mass and (a) plasma VTG, (b) plasma
total VLDL concentration in female Greater Scaup before the onset of
laying. Δ denotes mean ovary mass and yolk precursor concentration
± 1 SE in females with a full follicle hierarchy.
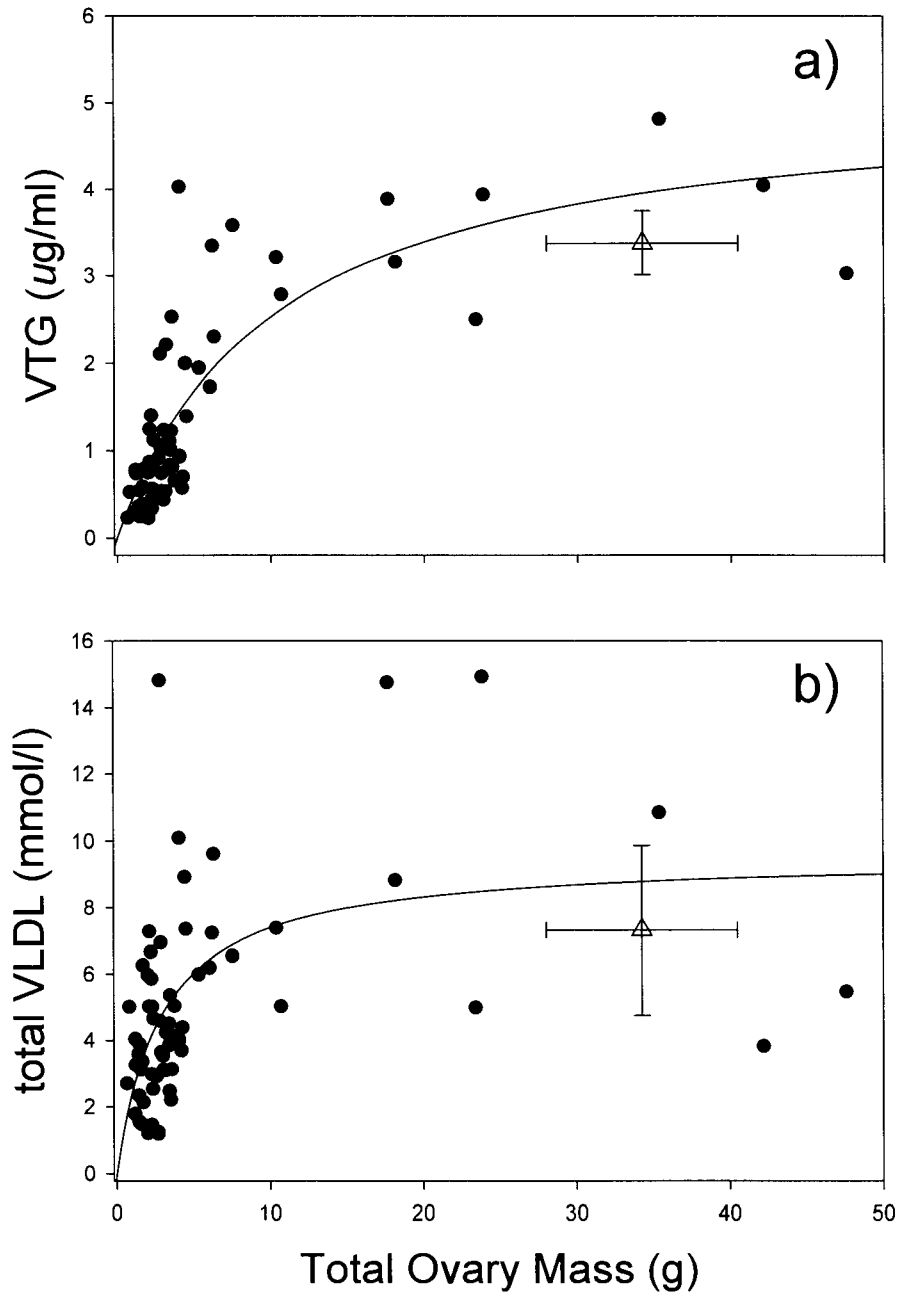
Figure 4.2 Variation in plasma concentration of VTG (•) and total VLDL (o) through
laying and early incubation of female Greater Scaup. Values are means
± 1 SE, sample size per group noted at the top.
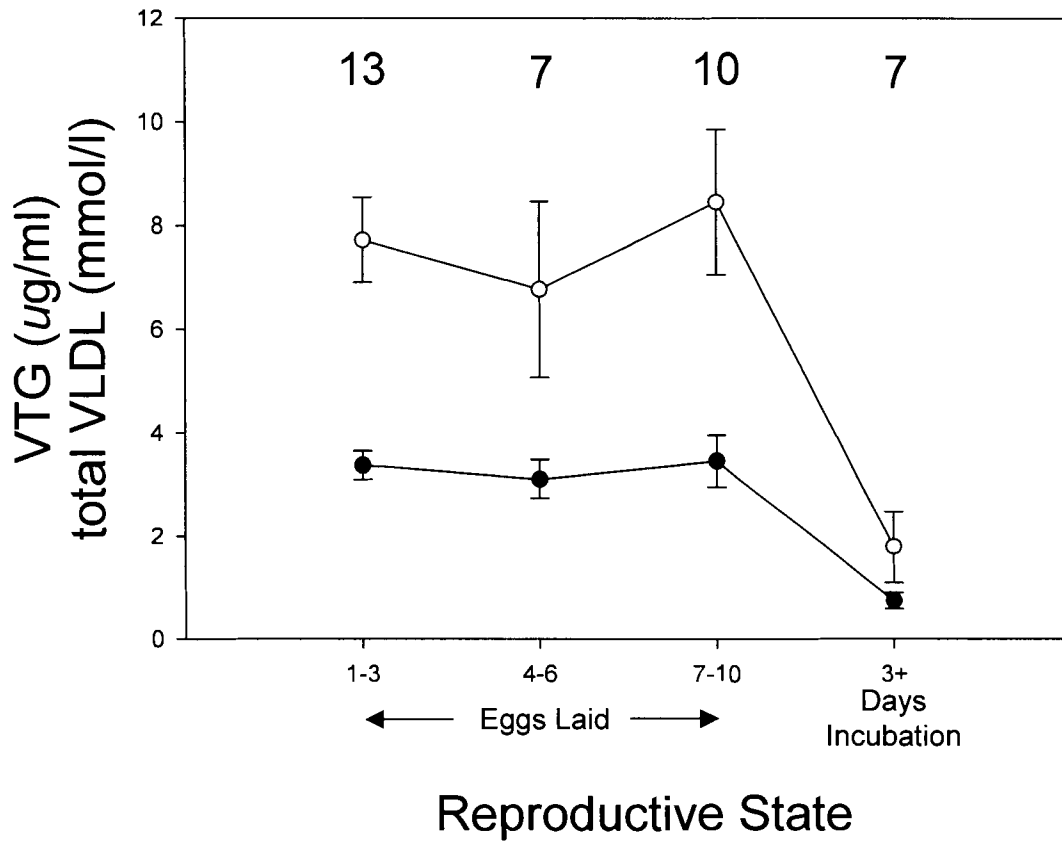
Figure 4.3 Mean yolk precursor concentration, VTG (•) and VLDL (o), for discrete reproductive states of female Greater Scaup. Values are means ± 1 SE, sample size per group noted at the top.
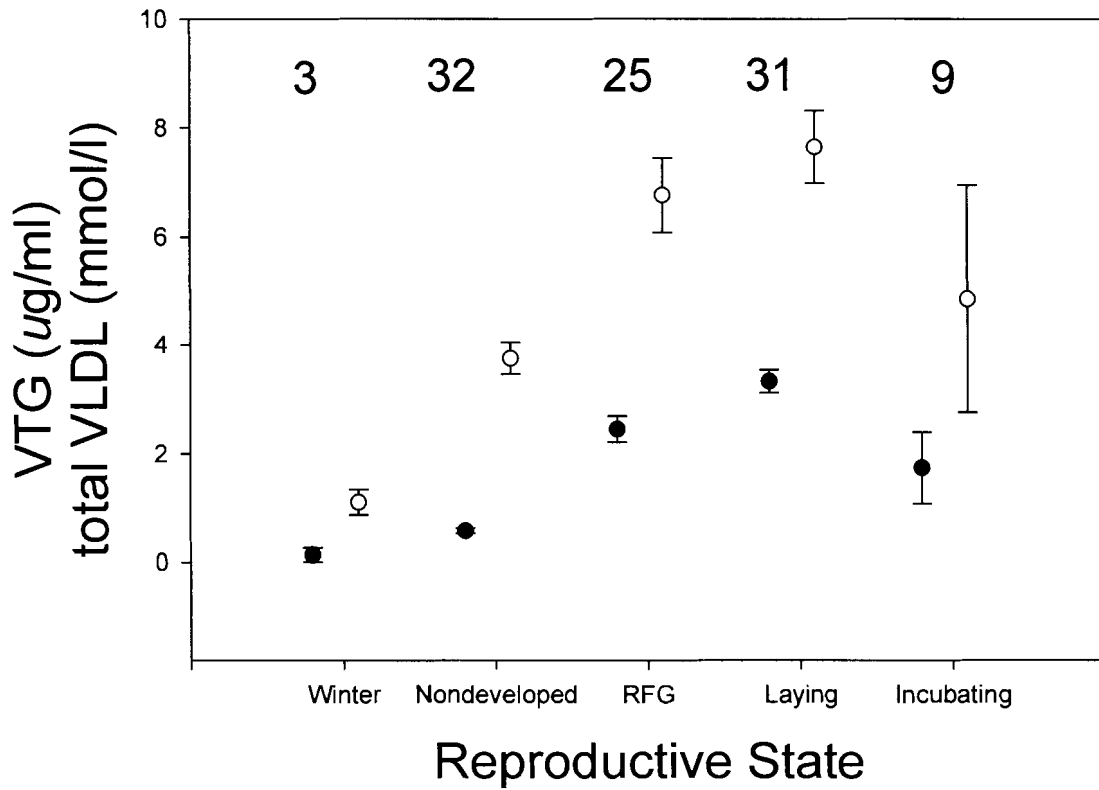
Table 4.2 Candidate models describing variation in VTG and total VLDL concentrations of female Greater Scaup. Models presented are those best fit by the data as well as all models receiving ΔAICc values ≤ 2.

Response Variables	Model #	Explanatory Variables	# of Parameters	ΔAICc	AICcW	r^2
VTG	1	(W = ND) ≠ RFG ≠ L ≠ I	5	0.0000	0.4633	0.83
	2	W ≠ ND ≠ RFG ≠ L ≠ I	6	1.7390	0.1942	0.83
total VLDL	1	(W = ND = I) ≠ (RFG = L)	3	0.0000	0.1279	0.76
	2	W ≠ (ND = I) ≠ (RFG = L)	4	0.0973	0.1218	0.76
	3	(W = ND = I) ≠ (RFG = L), YEAR	4	1.0339	0.0763	0.76
	4	(W = ND) ≠ (RFG = L) ≠ I	4	1.0630	0.0752	0.76
	5	(W = ND = I) ≠ RFG ≠ L	4	1.2329	0.0690	0.76
	6	W ≠ (ND = I) ≠ RFG ≠ L	5	1.3567	0.0649	0.77
	7	W ≠ ND ≠ (RFG = L) ≠ I	5	1.5542	0.0588	0.77
	8	W ≠ (ND = I) ≠ (RFG = L), YEAR	5	1.5740	0.0582	0.77

Abbreviations: Winter (W), Nondeveloped (ND), Rapid Follicle Growth (RFG), Laying (L), and Incubation (I).

Chapter 5.

General synthesis

Filling in the gaps to better understand
numerical declines in North American scaup

The current conservation concerns over declining North American scaup populations were a particularly important motivation for this thesis work. Continental numerical estimates have declined since the late-1970's, and much of this decline is not well understood in terms of the relative contribution of Greater vs. Lesser Scaup to the overall trend (Austin et al. 2000, Afton and Anderson 2001). Lesser Scaup are more numerous and therefore, thought to contribute more significantly to the population decline (Austin et al. 2000). There has been relatively more work conducted on Lesser Scaup biology (e.g., Afton and Ankney 1991, Dawson and Clark 2000, Esler et al. 2001, Rotella et al. 2003, Fast et al. 2004, Anteau and Afton 2004, Richman and Lovvorn 2004). Thus, the studies presented in this thesis and extended projects on Greater Scaup help fill some of the gap in our knowledge of this species. Most importantly, the data collected for this project from females breeding on the Yukon-Kuskokwim Delta, Alaska are representative of a stable breeding population (Afton and Anderson 2001), thus comparisons can be made with declining segments of the population with respect to life history strategies, particularly those affecting demography.

Austin et al. (2000) summarized research directions for better understanding North American scaup declines and outlined management needs. In particular, the following initiatives were highlighted, (1) examine the importance of food resources and body condition in reproductive performance, (2) investigate the effects of contaminants on body condition and reproductive performance, and (3) improve information needed to manage Greater and Lesser Scaup separately. This thesis work and extended projects broadly addressed these particular research needs. I found that female Greater Scaup breeding in western Alaska were highly dependent on exogenous food sources to meet

the energy and nutrient costs of clutch formation. Furthermore, there was evidence of a seasonally static body condition threshold for initiation of egg production. I suggested that this condition threshold is a proximate mechanism driving intraspecific variation in timing of reproduction and is therefore, associated with productivity via relationships with the seasonal decline in clutch size. These results differ markedly from the body of empirical work on female waterfowl nutrient reserve dynamics, and in particular the work by Esler et al. (2001) on the role of nutritional status in timing of reproduction by female Lesser Scaup breeding in the Alaska interior as well as midcontinent areas. Esler et al. (2001) found little evidence that nutrient reserve use during egg production differed between high latitude and midcontinent females. Endogenous lipid reserves contributed a significant proportion to reproductive lipid, although this proportion declined with later dates of nest initiation corresponding to smaller clutch sizes. Thus, female Greater and Lesser Scaup appear to use very different strategies for meeting the energy and nutrient costs of clutch formation, spanning capital to income breeding tactics. These results should be of great importance to those interested in links between female scaup condition and productivity, and in particular for managing the two species separately.

I also developed a physiological approach to detect reproductive state in female precocial birds based on the characterization of plasma yolk precursors vitellogenin (VTG) and very low-density lipoprotein (total VLDL) during the laying cycle of female Greater Scaup. This is a novel, noninvasive technique for detecting reproductive state via physiological parameters for waterfowl. This approach has only been used in a small number of free-living bird species (e.g., Vanderkist et al. 2000, Challenger et al. 2001, McFarlane-Tranquilla et al. 2003, Perry et al. 2004) and offers considerable promise for enhancing studies of reproduction not only of scaup, but also of other waterfowl species. Thus, this approach is an important advancement in monitoring techniques for waterfowl,

which during the course of this thesis work has already been adopted by other researchers interested in Common Eider, Harlequin Duck, and scoter reproductive biology.

In collaboration with scientists at Bird Studies Canada, Long Point Bird Observatory, liver tissue samples from the females collected for this project are being analyzed for selenium burdens. This work is part of a larger effort to understand the role of contaminants not only in aspects of scaup reproduction but other phases of the annual cycle. Some information is already available for Lesser Scaup regarding contaminant burdens (Custer et al. 2003), yet the presence and effects of such burdens on Greater Scaup remain largely unknown. Preliminary data from our samples of female Greater Scaup indicate selenium burdens are similar to that which has caused reproductive impairment in Mallards (S. Petrie, pers. comm.). In addition, samples of lipid-free heart tissue were archived for genetic analyses at the Alaska Science Center – USGS.

Finally, although not presented in this thesis, stable isotope analysis was used to directly trace the origin of nutrients used in reproduction by female Greater Scaup. These combined approaches from proximate body composition and stable carbon and nitrogen isotope analyses makes this dataset on female Greater Scaup highly unique. Furthermore, comparing the inference derived from these two approaches concerning the use of endogenous vs. exogenous sources of nutrients for clutch formation allows for the possibility of using stable isotope analysis as a reliable, nonlethal technique for directly tracing strategies of nutrient acquisition for egg production by female waterfowl, in addition to quantifying the relative contributions of these nutrient sources to reproductive nutrients (e.g., Gauthier et al. 2003).

In conclusion, thesis work and extended projects on female reproductive energetics of Greater Scaup breeding on the Yukon-Kuskokwim, Delta, Alaska are important steps in filling the knowledge gap of Greater Scaup reproductive biology, particularly given the identified research needs for better conserving North American scaup populations. In particular, the work on yolk precursor analysis and stable isotope analysis should prove highly useful in developing nonlethal methods for discerning parameters concerning waterfowl reproductive energetics and physiology.

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