J. Field Ornithol. 78(1):64–73, 2007 DOI: 10.1111/j.1557-9263.2006.00086.x

Ovarian follicle dynamics of female Greater Scaup during egg production

Kristen B. Gorman,[1,4] Paul L. Flint,[2] Daniel Esler,[3] and Tony D. Williams[1]

[1]*Centre for Wildlife Ecology, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia, V5A 1S6, Canada*
[2]*Alaska Science Center, U. S. Geological Survey, 1011 E. Tudor Road, Anchorage, Alaska, 99503, USA*
[3]*Centre for Wildlife Ecology, Simon Fraser University, 5421 Robertson Road, Delta, British Columbia, V5K 3N2, Canada*

Received 3 July 2006; accepted 29 September 2006

ABSTRACT. Studies of female waterfowl nutrient reserve use during egg production require a precise understanding of ovarian follicle dynamics to correctly interpret breeding status, and, therefore, derive proper inference. Concerns over numerical declines of North American scaup have increased the need to better understand the role of female condition in reproductive performance. We quantified ovarian follicle dynamics of female Greater Scaup (*Aythya marila*) breeding on the Yukon–Kuskokwim Delta, Alaska, using a method that accounts for within day variation in follicle size. We considered several models for describing changes in follicle growth with the best supported model estimating the duration of rapid follicle growth (RFG) to be 5.20 ± 0.52 days (±95% confidence intervals) for each developing follicle. Average diameter and dry mass of preovulatory follicles were estimated to be 9.36 mm and 0.26 g, respectively, at the onset of RFG, and these follicle characteristics were 41.47 mm and 15.57 g, respectively, at ovulation. The average diameter of postovulatory follicles immediately following ovulation was estimated to be 17.35 mm, regressing quickly over several days. In addition, we derived predictive equations using diameter and dry mass to estimate the number of days before, and after, ovulation for pre- and postovulatory follicles, as well as an equation to estimate dry mass of damaged follicles. Our results allow precise definition of RFG and nest initiation dates, clutch size, and the daily energetic and nutritional demands of egg production at the individual level. This study provides the necessary foundation for additional work on Greater Scaup reproductive energetics and physiology, and offers an approach for quantifying ovarian follicle dynamics in other species.

SINOPSIS. **Dinámica de los folículos en el ovario de *A th a marila* durante la producción huevos**

Los estudios sobre las reservas de nutrientes utilizadas por hembras de patos durante la producción de huevos requiere un entendimiento claro sobre la dinámica de los folículos en los ovarios para interpretar el estatus´
cho necesario el tratar de ententer el rol de la condición de la hembra en su ejecución
dinámica de folículos en aves reproduciéndose en el delta del Yukon-Kuskokwin,
ue permite determinar variación o cambios en los folículos de un dia para otro.
os para describir los cambios en el crecimiento de los folículos. El mejor modelo
n de crecimiento rápido del folículo (CRF) que fue de 5.20±0.52 dias (±95%
cada folículo que se estaba desarrollando. El diámetro promedio y masa seca de
en 9.36 mm y 0.26 g, respectivamente, en la vispera de CRF, y el tamaño y peso de
m y 15.57 g, respectivamente, al momento de la ovulación. El diámetro promedio
ediatamente luego de la ovulación, fue de 17.35 mm, con una regresión en tamaño
as. Pudimos además derivar ecuaciones de predicción utilizando el diámetro de los
para determinar el número de dias previos y después de la ovulación, para pre y post
(y)-21ecua8.12sn6 [illegible] y ofrece
inámica folicular de otras especies de patos.

Greater Scaup, ovarian follicles, rapid follicle growth, Yukon–Kuskokwim Delta

[4]Corresponding author. Email: kgorman@alumni.sfu.ca

Damaged follicles were removed and discarded. The diameter of dissected follicles and all postovulatory follicles were measured with digital calipers in the plane of the stigma (± 0.01 mm). All dissected follicles, as well as the remaining ovary, were dried to a constant mass (± 0.01 g) in a drying oven at 60°C to obtain estimates of dry mass.

Statistical methods: continuous models of ovarian follicle growth. Statistical analyses were performed using either the Statistical Analysis System (SAS Institute 1999) or SigmaPlot (SPSS Inc. 2000). Ovaries from females that had ovulated at least one follicle were used ($N = 25$) because they had follicle hierarchies that could be assigned a particular number of days before ovulation, assuming a laying rate of one egg per day (Alisauskas and Ankney 1992, Esler 1994, 1999). Ovaries with damaged follicles were used if the position of the damaged follicle in the hierarchy was known. However, damaged follicles, as well as entire ovaries if the largest follicle was damaged, were excluded. Only developing follicles in a consistent hierarchy from ovaries of females late in their laying cycle were included. Females late in the laying cycle were identified by a large gap in size between nondeveloped and developing follicles.

To quantify preovulatory follicle dynamics, individual preovulatory follicles were first assigned a number of days before ovulation ($N = 249$), with the largest follicle within an ovary assigned DAY 1 and the remaining follicles assigned DAY 2–DAY 12 on the basis of their position in the follicle hierarchy as determined by diameter data. More precise estimates of the time before ovulation for each follicle were derived based on methods described by Esler (1994) that account for within day variation in follicle size. Correcting for this variation allows derivation of continuous models of follicle growth, rather than estimating a mean follicle size for each day. A correction value for the time before ovulation (*PreovCorr*) for the DAY 1 follicle of each ovary sample was calculated as:

PreovCorr for DAY 1 follicle = ([estimated dry mass of an individual's follicle at ovulation – dry mass of an individual's DAY 1 follicles] / [estimated dry mass of an individual's follicle at ovulation – dry mass of the smallest DAY 1 follicle of the data set]).

Dry mass estimates of an individual's follicle at ovulation 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 when necessary ($N = 31$). Therefore, values of *PreovCorr* for DAY 1 follicles ranged from 0 for follicles just prior to ovulation to 1 for follicles 1 day from ovulation. *PreovCorr* estimates for the remaining follicles within each ovary were calculated as:

PreovCorr for DAY 2 – 12 follicles = (the follicle's original DAY assignment – 1 (+ the *PreovCorr* correction value from the DAY 1 preovulatory follicle of that ovary)).

For example, if a DAY 1 follicle's *PreovCorr* value was estimated to be 0.25 days, DAY 2 follicle's *PreovCorr* value would be 1.25.

Similar to the methods described above, postovulatory follicles were assigned a number of days after ovulation ($N = 108$) based on their position in the postovulatory follicle hierarchy as determined by diameter data. Following methods described by Esler (1994), a correction value for the number of days after ovulation (*PostovCorr*) for the largest postovulatory follicle (DAY 1) was calculated as:

PostovCorr for DAY 1 postovulatory follicles = (1 – *PreovCorr* of the DAY 1 follicle of that ovary).

PostovCorr estimates for any remaining postovulatory follicles were calculated as:

PostovCorr for DAY 2+ postovulatory follicles = (the postovulatory follicle's original DAY assignment – 1 (+ the *PostovCorr* correction value from the DAY 1 postovulatory follicle of that ovary)).

For example, an ovary sample with a *PreovCorr* value of 0.25 would be estimated as having a *PostovCorr* value of 0.75 for the DAY 1 postovulatory follicle, and 1.75 for the DAY 2 postovulatory follicle.

Statistical methods: defining RFG initiation. To quantify the onset and duration of RFG (i.e., *PreovCorr* value at which follicles begin to grow rapidly), we considered six models including a joint-linear, quadratic polynomial, 2- and 3-parameter exponential decay, plus 2- and 3-parameter hyperbolic decay models using diameter and dry mass data sets including pre-RFG and RFG follicles ($N = 249$ for both data sets) measured from laying female ovaries. Joint-linear models were determined by iteratively assigning diameter and dry mass data to either pre-RFG or RFG groups based on *PreovCorr* values that putatively corresponded to RFG initiation. Joint-linear models that maximized the

r^2 value and minimized the sum of squares error value were used.

We employed information-theoretic methods to direct model selection (Burnham and Anderson 2002). For all six models, 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 (i.e., the most parsimonious model) within the candidate model set. In addition, AICc weight (AICcW) values were used to consider the likelihood that a particular model was the best supported model (Burnham and Anderson 2002). Finally, best supported models, as determined above, were subsequently compared with the same model structure including a YEAR term, where 2003 was set as the reference value, to assess interannual variation in ovarian follicle growth. Best supported models for both follicle diameter and dry mass data sets were used to determine the onset and duration of RFG. Where joint-linear models were best supported, the demarcation point for the model was considered the best estimate of RFG initiation (Esler 1994). If nonlinear models were best supported, the point that maximized the second derivative would be used to define the onset of RFG. We calculated 95% confidence intervals (CI) for our estimate of the onset and duration of RFG (Sokal and Rohlf 1981).

Statistical methods: defining RFG functions and predictive models. Information-theoretic methods also were used to determine relationships between *PreovCorr* and preovulatory follicle diameter, as well as preovulatory dry mass for follicles in RFG ($N = 114$) from laying female ovaries. This approach was used to define the growth function of RFG follicles and to estimate average follicle diameter and dry mass at the onset of RFG and at ovulation. In addition, these same methods were used to determine the relationship between *PostovCorr* and postovulatory follicle diameter ($N = 108$) for ovaries from laying females. The candidate model set for each analysis included linear, quadratic polynomial, 2- and 3-parameter exponential decay, as well as 2- and 3-parameter hyperbolic decay models.

Predictive models of *PreovCorr* from preovulatory follicle diameter and dry mass, as well as a predictive model of *PostovCorr* from postovulatory follicle diameter, were derived based on best supported model structures, as determined above by analyses of RFG follicles only. Estimates of damaged follicle dry mass 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 ($N = 81$).

RESULTS

Defining RFG initiation. A joint-linear model was best supported for describing the relationship between *PreovCorr* and preovulatory follicle diameter for the data set including pre-RFG and RFG ovarian follicles (Table 1). When this model was compared to the same model including the YEAR term, the joint-linear model without YEAR received substantially more support (Table 1). The best supported model estimated the onset and duration (±CI) of RFG to be 5.20 ± 0.52 days.

The best supported model for describing the relationship between *PreovCorr* and follicle dry mass for the data set including pre-RFG and RFG ovarian follicles was joint-linear (Table 1). Compared to the same model including the YEAR term, the model including YEAR received substantially more support (Table 1), with a parameter estimate indicating that 2002 follicles weighed an average of 0.15 g less than 2003 follicles for any given *PreovCorr* value. This model estimated the onset and duration (±CI) of RFG to be 3.2 ± 0.47 days. Given that our analyses of the onset and duration of RFG, based on diameter and dry mass data sets, resulted in different estimates of RFG, we used the estimate of RFG based on diameter data as the final estimate defining RFG initiation in subsequent analyses (Tables 2 and 3; Figs. 1 and 2; see Discussion).

Defining RFG functions. The best supported relationship between *PreovCorr* and follicle diameter for follicles in RFG was linear (Table 2), and estimated average follicle diameter at the onset of RFG and at ovulation to be 9.36 and 41.47 mm, respectively (Fig. 1). The relationship between *PreovCorr* and follicle dry mass for follicles in RFG was best supported

Table 1. Models describing the relationship between *PreovCorr*[1] (a) preovulatory follicle diameter, and (c) preovulatory follicle dry mass, for pre-RFG and RFG ovarian follicles measured. Models receiving ΔAICc[2] values ≤2 are presented. (b, d) Comparison of best supported models with the same model including the YEAR term to assess interannual variation in ovarian follicle growth.

Response variable	Explanatory variable	Number of parameters	Model	ΔAICc[2]	AICcW[3]	*r*-square
(a) *PreovCorr*[1]	Preovulatory follicle diameter	5	Joint-linear	0.00	1.00	0.99
(b) *PreovCorr*[1]	Preovulatory follicle diameter	5	Joint-linear	0.00	0.69	0.99
PreovCorr[1]	Preovulatory follicle diameter, Year	6	Joint-linear	1.58	0.31	0.99
(c) *PreovCorr*[1]	Preovulatory follicle dry mass	5	Joint-linear	0.00	1.00	0.98
(d) *PreovCorr*[1]	Preovulatory follicle dry mass, Year	6	Joint-linear	0.00	0.71	0.98
PreovCorr[1]	Preovulatory follicle dry mass	5	Joint-linear	1.80	0.29	0.98

[1]*PreovCorr* is the time before ovulation for preovulatory ovarian follicles.
[2]ΔAICc values are the difference between the AICc value for a particular model and the lowest AICc value within the candidate model set (i.e., the best supported model).
[3] AICcWvalues are the likelihood that a particular model is the best supported model.

PostovCorr and postovulatory follicle diameter was best supported by a 3-parameter exponential decay model (Table 2) that estimated average postovulatory follicle diameter at ovulation to be 17.35 mm (Fig. 3).

Predictive models. *PreovCorr* was estimated with a linear model of preovulatory follicle diameter and a quadratic polynomial model of preovulatory follicle dry mass (Table 3). *PostovCorr* was estimated with a 3-parameter exponential decay model of postovulatory follicle diameter (Table 3). These equations can be used to precisely predict the number of days prior to, or following, ovulation for any preovulatory follicle in RFG or any postovulatory follicle. Preovulatory follicle dry mass was estimated with a cubic polynomial model of dry mass of the next smallest follicle in the ovarian follicle hierarchy

Table 2. Models describing the relationship between *PreovCorr*[1] (a) preovulatory folicle diameter, and (b) preovulatory follicle dry mass, for ovarian follicles in rapid follicle growth. (c) Models describing the relationship between *PostovCorr* and postovulatory follicle diameter. Models receiving ΔAICc[2] values≤ 2 are presented.

Response variable	Explanatory variable	Number of parameters	Model	ΔAICc[2]	AICcW[3]	*r*-square
(a) *PreovCorr*[1]	Preovulatory follicle diameter	3	Linear	0.00	0.36	0.97
		4	Quadratic polynomial	0.57	0.27	0.97
		4	3-parameter exponential decay	0.65	0.26	0.97
(b) *PreovCorr*[1]	Preovulatory follicle dry mass	4	Quadratic polynomial	0.00	1.00	0.97
(c) *PostovCorr*[4]	Postovulatory follicle diameter	4	3-parameter exponential decay	0.00	0.45	0.90
		4	Quadratic Polynomial	0.18	0.41	0.90

[1]*PreovCorr* is the time before ovulation for preovulatory ovarian follicles.
[2]ΔAICc values are the difference between the AICc value for a particular model and the lowest AICc value within the candidate model set (i.e., the best supported model).
[3]AICcW values are the likelihood that a particular model is the best supported model.
[4]*PostovCorr* is the time after ovulation for postovulatory ovarian follicles.

Models estimating *PreovCorr*[1] and *PostovCorr*[2] from follicle characte
dry mass from dry mass of the next smallest follicle.

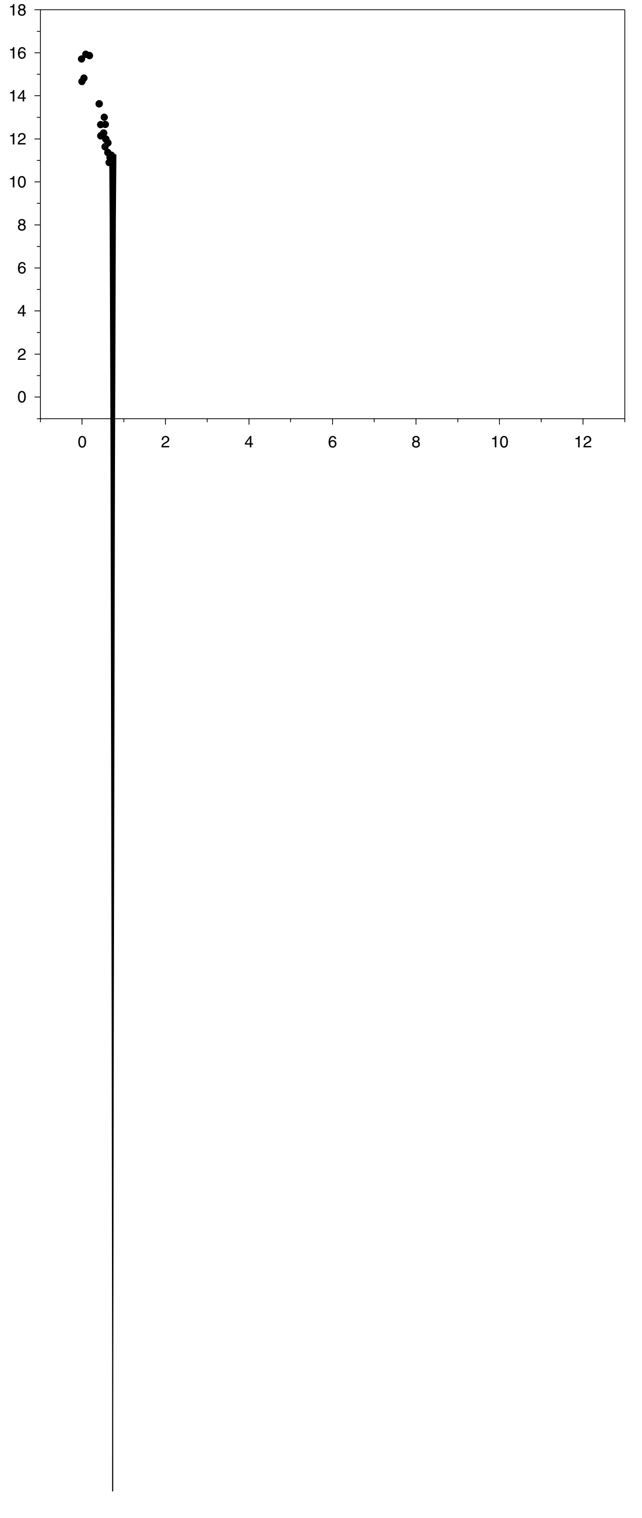
se variable	Explanatory variable	
orr[1]	Preovulatory follicle diameter	Y = 6.60 − 0.1
orr[1]	Preovulatory follicle dry mass	Y = 4.65 − 0.5
Corr[2]	Postovulatory follicle diameter	Y = −0.29 + 9
e dry mass	Next smallest follicle dry mass	Y = 0.2745 + 2 + 0.0129 X^3;

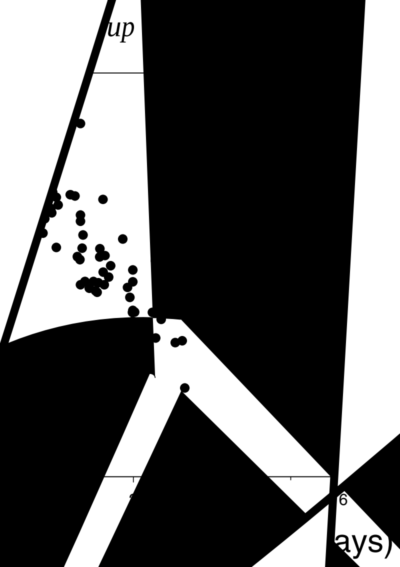
Corr is the time before ovulation for preovulatory ovrian follicles.
vCorr is the time after ovulation for postovulatory ovarian follicles.

e 3). This model permits for appropriate ctions in energetic studies for follicles burst amaged during collection and dissection.

DISCUSSION

Our study expands on previous work that exined ovarian follicle dynamics of ducks in the nera *Aythya* and *Anas* (Esler 1994) by applying nilar methods, but also considering additional odel structures to quantify pre and postovulary follicle dynamics in female Greater Scaup.


18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12



KRAPU, G. L. 1974. Feeding ecology of pintail hens during reproduction. Auk 91: 471–473.
MAC