Do Female Northern Pintails, *Anas acuta*, Initiate Rapid Follicular Growth During Spring Migration?

PASCALE DOMBROWSKI[1], JEAN-CLAUDE BOURGEOIS[1], RICHARD COUTURE[2], and CHRISTIAN LINARD[3]

[1] Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec G9A 5H7 Canada, and Société de la faune et des parcs du Québec, Direction de l'aménagement de la faune, 5575, Saint-Joseph, Trois-Rivières-Ouest, Québec G8Z 4L7 Canada
[2] Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec G9A 5H7 Canada
[3] Département de Chiropratique, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec G9A 5H7 Canada

Dombrowski, Pascale, Jean-Claude Bourgeois, Richard Couture, and Christian Linard. 000. Do female Northern Pintails, *Anas acuta,* initiate rapid follicular growth during spring migration? Canadian Field-Naturalist 117(4): 605-610.

We describe the reproductive status of female Northern Pintails (*Anas acuta*) staging on a flooded plain along the St. Lawrence River (Quebec, Canada) during the spring of 1997. Nine of the 27 female pintails we collected had ovarian follicles showing Rapid Follicular Growth (RFG). In RFG females, total blood calcium and ash mass increased with follicular development. They had greater muscle and bone mass, and higher blood calcium levels, compared to pre-RFG birds. However, carcass fat mass and sex hormone levels (estradiol and progesterone) did not differ between the two groups. Our results indicate that at least some Northern Pintails initiate egg formation processes prior to arrival at nesting areas, which is consistent with early nesting. The nutrients and energy required for this early egg formation must come from reserves stored during winter, foods consumed in staging areas, or both.

Key Words: Northern Pintail, *Anas acuta*, staging, reproduction, ovarian follicle, growth, Quebec.

Northern Pintails (*Anas acuta*, hereafter called pintails) migrate and nest early (Fredrickson and Heitmeyer 1991; Austin and Miller 1995), and reach staging areas late March to early April (Austin and Miller 1995). Nesting season begins with ovarian follicle maturation in females, which must occur prior to egg laying. During Rapid Follicular Growth (RFG) and until ovulation, the ovarian follicles that will become eggs enlarge to form yolk from deposition of several lipid layers (Alisauskas and Ankney 1992; Joyner 1994). The diameter of the largest follicle distinguishes pre-RFG and RFG female ducks. In pintails, RFG begins when the diameter of this follicle reaches 8.2 mm and rapidly increases to 32.9 mm over about 4.2 days (Esler 1994). Serum sex hormone levels or blood concentration of calcium, phosphorus, and lipids, all of which increase during reproduction, might also be indirect indicators of reproductive status (Hannon 1979; Johnson 1986).

In female pintails, endogenous energy reserves for egg laying and incubation are important because incubating ducks may feed infrequently in contrast other nesting dabbling ducks generally do feed during incubation (Derrickson 1978; Ankney et al. 1991; Mann and Sedinger 1993). The accumulation of nutrient reserves (lipids, proteins, and calcium) to support reproduction thus occurs before egg laying (Mann and Sedinger 1993; Esler and Grand 1994). The only available information on pintail body composition relative to reproductive status is based on breeding females nesting in North Dakota (Krapu 1974) and Alaska (Mann and Sedinger 1993; Esler and Grand 1994; Flint and Grand 1996). Little is known about nutrient reserves and reproductive status of female pintails during spring migration.

In this study, we tested the hypothesis that female pintails prepare for egg laying during spring migration, a trait consistent with early nesting. First, we quantified the nutrient reserves and categorized reproductive status of collected female pintails on a spring staging site in the Lake St. Pierre region, Quebec. Second, we evaluated some of the physiological changes (ovary development and hormones) in the females during the pre-laying period. Finally, we examined the changes in different body components involved in reproduction.

Study Area and Methods

Sampling and specimen gathering

The study took place from 14 April to 9 May 1997 at the St. Barthelemy staging ground, located in the province of Quebec, Canada (46º 11' N, 73º 08' W). This staging site is in the Lake St. Pierre region, a Ramsar wetland site as well as a UNESCO Biosphere Reserve. Spring flood waters from the St. Lawrence River submerge this flood plain (5.0 to 6.0 m above sea level) for 5 to 6 weeks, beginning early April. The study site consists of 80 agricultural fields, for a total area of 246 ha. It is crossed by five drainage ditches and forms a deep basin. The staging area is managed to maintain water levels during spring waterfowl migration. The flooded fields of the study site are heavily used by pintails, which represent approximately 80% of the 10 000 dabbling ducks found in the area during 4 to 5 weeks. It is the second most important spring staging ground along the St. Lawrence River for dabbling ducks.

We used a .22 rifle to collect 27 female pintails. We weighed pintails (± 0.5 g) and took blood samples by intra-cardiac puncture (Donham 1979; Hannon 1979) into glass tubes (Vacutainer®, Becton-Dickinson, Rutherford, New Jersey, 07070 USA). We retained all samples on ice and in the dark until their arrival at the laboratory. At the lab, we centrifuged the blood samples, drew off the serum, and froze it at -20°C pending analysis.

We removed the ovarian follicles, esophagus, and gizzard from each carcass, and set the esophagi and gizzards aside pending food habits analysis. We measured the diameter (∅) of the largest follicle of each female with a calliper (±0.1 mm), and obtained follicular mass with a portable electronic balance (±0.1 g). We did not age the hens since both male and female pintails can breed at 1 year of age (Austin and Miller 1995). We divided the pintails into pre-RFG ducks, in which the diameter of the largest follicle was < 8.2 mm, and RFG ducks, in which diameters were ≥ 8.2 mm (Esler 1994). We stored carcasses at -30°C in sealed plastic bags.

Specimen analysis

We shaved frozen carcasses, then weighed them again (carcass weight), and cut them into pieces. We then ground the pieces twice in a meat grinder (Hobart®) to homogenize the components. We dried a 100-g sample of the homogenate to constant mass in a forced-air oven (60°C). We estimated percentage water for the sample and multiplied this by the frozen mass to determine carcass water mass and dry carcass mass (frozen mass – water mass). We ground the lyophilized sample in a high-speed grinder and took 1-g duplicates for lipid extraction (Gauthier et al. 1992). Carcass fat is percent fat in sample × dry carcass mass, hereafter called "fat".

We determined the mineral content of the carcass ("ash") by weighing the ash obtained by incinerating 2-3 g of lyophilized samples at 550°C for 12 hours. This value was then used to determine the ash weight of the dehydrated carcass. The protein content ("protein") was calculated using the following formula:

Protein mass = dehydrated carcass mass – (fat mass + ash mass) (Miller 1989; Dabbert et al. 1997).

Biochemical analyses

We considered many blood biochemical parameters which could indicate the reproductive status of female pintails. Previous studies showed that an increase in calcium can be observed in ovulating hens (Hochtleitner 1994), and a physiological hypercalcemia occurs in birds during egg laying (Amand 1986). Estrogen and progesterone are hormones involved in ovulation. Seasonal hypertrophy of the oviduct in free-ranging birds is dependent on estrogen, and progesterone interacts with estrogen in stimulating oviduct growth and secretory activity (Joyner 1994). On the basis of these studies and some preliminary work we did on specimens collected in the spring of 1996, we chose to quantify total calcium (Ca), estradiol (the main ovarian estrogen), and progesterone.

We used a Hitachi 704 automatic chemical analyzer (Boehringer Mannheim GmbH, Mannheim, Germany) to determine total calcium, with procedures and reagents supplied by the company (Gindler and King 1972). We measured estradiol and progesterone with a solid phase I^{125} radioimmunoassay using Coat-a-Count® kits (Diagnostic Products Corporation, Los Angeles, California, 90045-5597 USA), following the manufacturer's protocol. Because of the small size of serum samples available (1-2 mL), we did not analyze duplicates.

Statistical analyses

We tested all variables for normality, and even with transformed data, the conditions necessary for use of parametric tests were not met (i.e., normality was not achieved), so we used non-parametric tests. We used Spearman rank correlations (r_s) to analyze relationships among diameter of the largest follicle, total blood calcium, and ash mass (Bart and Notz 1994). We used the non-parametric Mann-Whitney comparison test to detect differences (between pre-RFG and RFG females) in body components, follicular mass, largest-follicle diameters, and blood biochemical parameters. We used SYSTAT™ for all analyses (White and Clark 1994), and we report all values as means +/- SE.

Results

Nine of the 27 female pintails we collected had ovarian follicles showing RFG (Figure 1). These 9 females had a mean follicle diameter of 12.0 ± 0.9 mm. Mean diameter for pre-RFG females was 5.7 ± 0.3 mm. The earliest date we collected RFG females was 21 April; none of the females collected between 14 and 20 April showed RFG, but all those collected after 2 May did (Figure 1a). For all females, diameter of the largest ovarian follicle increased as collection date advanced ($r_s = 0.71$, $P < 0.001$). Likewise, for RFG females ($n = 9$), total calcium levels ($r_s = 0.75$, $P < 0.05$, Figure 1b) and ash mass ($r_s = 0.85$, $P < 0.01$, Figure 1c) increased with increasing diameter of the largest follicle. Body mass (fresh and carcass), protein mass, ash mass, and total blood calcium of RFG females exceeded those of pre-RFG females (Mann-Whitney test, Table 1). However, carcass fat mass and sex hormone levels in the blood did not differ between pre-RFG and RFG groups (Table 1).

Discussion

Our results show that the pintails' staging period in St. Barthelemy coincides with the beginning of ovarian follicle maturation for some birds. At the end of the sampling period, all females we examined showed RFG, suggesting these female pintails were preparing for egg laying. This is consistent with the fact that

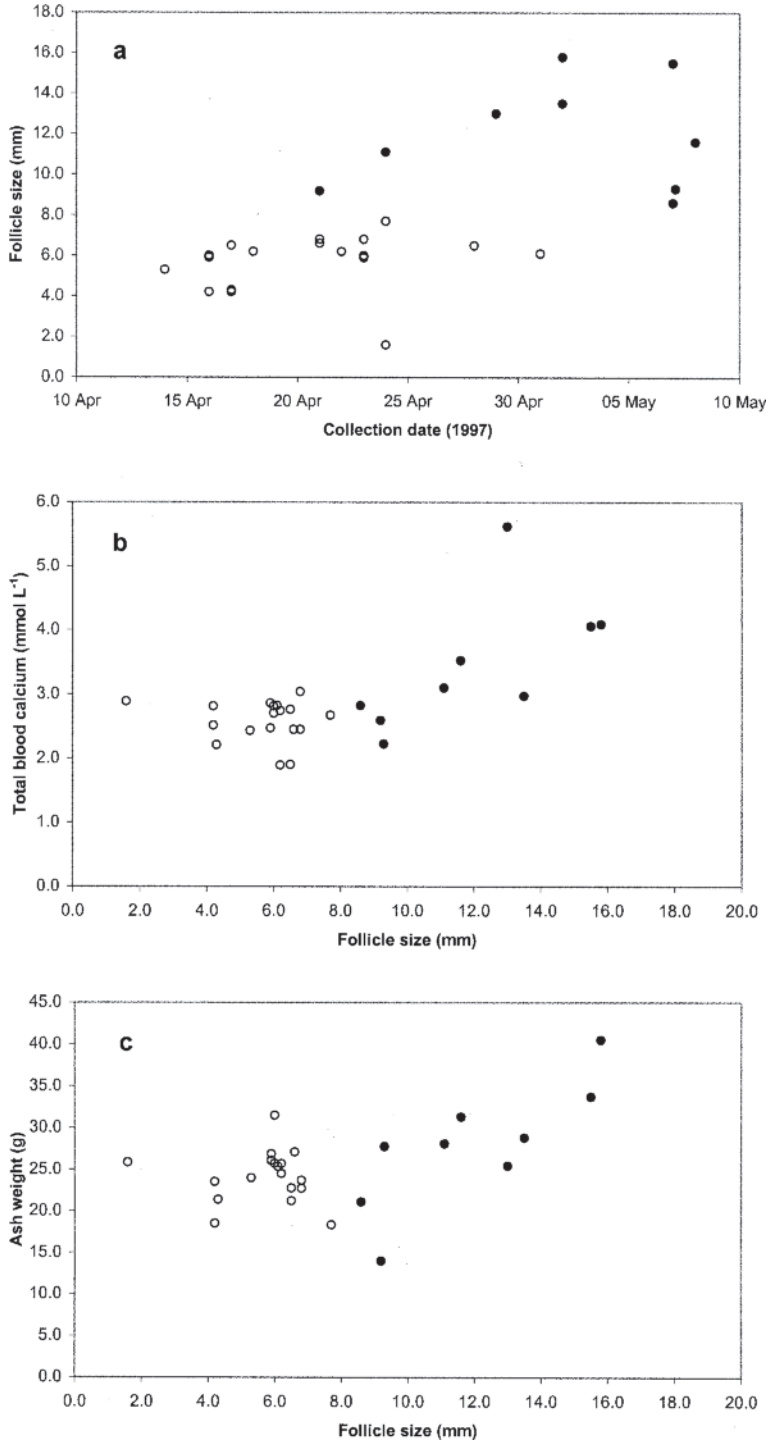

FIGURE 1. (A) Diameters of the largest ovarian follicle in the 27 female Northern Pintails sampled at staging ground in spring 1997 according to sample date. (B) Total blood calcium in the 27 female pintails sampled at staging ground in spring 1997 versus the largest ovarian follicle diameter. (C) Ash weight in the 27 female pintails sampled at staging ground in spring 1997 versus the largest ovarian follicle diameter. Solid circles represent the nine females in RFG stage (∅ > 8.2 mm, Esler 1994).

TABLE 1. Characteristics of pre-RFG and RFG female Northern Pintails (mean ± SE) during the spring migration stop of 1997. The last column gives the significance level when the two groups were compared using the Mann-Whitney test (ns: not significant, $P > 0.05$).

Characteristic	Pre-RFG (n = 18)	RFG (n = 9)	P
Carcass weight (g)	937.0 ± 16.8	1038.0 ± 25.2	< 0.01
Eviscerated weight (g)	825.5 ± 16.6	900.5 ± 22.3	< 0.05
Fat (g)	146.0 ± 9.6	168.5 ± 20.2	ns
Protein (g)	212.5 ± 14.2	258.0 ± 28.2	< 0.05
Ash (g)	24.0 ± 0.8	28.0 ± 2.5	< 0.05
Follicular mass (g)	1.3 ± 0.1	2.7 ± 0.4	< 0.001
Follicular diameter (mm)	5.7 ± 0.3	12.0 ± 0.9	< 0.001
Total calcium (mmol L^{-1})	2.6 ± 0.1	3.4 ± 0.3	< 0.01
Estradiol (pg mL^{-1})	52.4 ± 8.0 [a]	61.7 ± 15.1	ns
Progesterone (mmol L^{-1})	0.7 ± 0.2 [a]	0.6 ± 0.2	ns

[a] n = 16

pintails nest early (Austin and Miller 1995). No pre-RFG females were found after 2 May, which indicates that some females nest later, in more northern sites, and that they had already left. Because northern nesting sites are used at the end of May and early June, once the snow is melted, this might suggest that intermediary staging areas are used between these northern sites and St. Barthelemy.

The increase in blood calcium which accompanied increasing diameter of the largest follicles in RFG females suggested ducks obtained dietary calcium during ovarian follicle growth (Hohman 1986; Hochleitner 1994). The increased bone mass (ash) we found in females during RFG confirmed that calcium is stored in the medullary bones of female pintails. Medullary bone formation occurs during the reproductive period in some nesting female dabbling ducks (Johnson 1986; Kenny 1986), and is a source of calcium that can be rapidly mobilized during eggshell formation if dietary calcium is deficient (Sturkie 1986).

A difference in ash amounts between pre-RFG and RFG females has previously been reported for Wood Ducks (*Aix sponsa*) (Drobney 1982), Lesser Snow Geese (*Chen caerulescens caerulescens*) (Ankney and MacInnes 1978), and Ring-necked Ducks (*Aythya collaris*) (Hohman 1986). Increases in ash amounts and total calcium related to the diameter of the largest follicle have been reported in female Canvasbacks (*Aythya valisineria*) in Manitoba (Barzen and Serie 1990), domestic female Mallards (Fairbrother et al. 1990), and now in pintails. On the other hand, Mann and Sedinger (1993) saw no variations in ash amounts between pre-RFG and RFG pintails in Alaska during the pre-laying period. This may be a phenomenon associated with spring staging areas, where ducks from several different wintering areas and headed to different nesting regions, converge to forage and prepare for nesting on different schedules.

Like calcium, the proteins required for reproduction in females can be obtained in part from carcass reserves, as shown for Lesser Snow Geese (Ankney and MacInnes 1978), Greater Snow Geese (*Chen caerulescens atlantica*) (Choinière and Gauthier 1995), Common Eiders (*Somateria mollissima*) (Milne 1976), Canvasbacks (Barzen and Serie 1990), Gadwalls (*Anas strepera*) (Ankney and Alisauskas 1991), and pintails in Alaska (Mann and Sedinger 1993). For example, endogenous proteins provided 21-62% of the proteins necessary for egg production in Alaska (Mann and Sedinger 1993). In our study, female pintails in RFG contained more protein than did pre-RFG females. This suggested that, like Canvasbacks (Barzen and Serie 1990) and Gadwalls (Ankney and Alisauskas 1991), follicular growth does not occur until females accumulate a certain amount of protein or markedly increase protein in the diet. Observed differences in body protein between pre-RFG and RFG pintails (as was observed for blood calcium and ash) might also relate to diversity in wintering origins, migration strategies, and ultimate destinations, all of which are unknown for any collected bird. Further investigations using radio telemetry or stable-isotope studies might provide some suggestions on this.

Alisauskas and Ankney (1992) suggested waterfowl store nutrients (lipids, proteins, and minerals) before laying if, on average, they are unable to meet the daily cost of converting exogenous nutrients to egg nutrients simultaneously with egg production. Lipids are most often stored to take advantage of their high energy content (Alisauskas and Ankney 1992). Our study revealed no difference in fat content between pre-RFG and RFG females. These results may indicate that females do not store or accumulate fat between the two reproductive stages. They may also indicate the sample size was small, considering it was taken from a heterogeneous population on different reproductive schedules and strategies.

In female pintails from our study, progesterone and estradiol did not differ between pre-RFG and RFG stages. It only takes one bird in a small sample (9) to

cause statistical problems, but Donham's (1979) result regarding progesterone in female Mallards agrees with ours. However, that author reported higher estrone and estradiol 17-ß levels during nesting period. This indicates that estrone may be more appropriate for monitoring the reproductive status in birds. For example, in the Eastern Wild Turkey (*Meleagris gallopavo silvestris*), estrone was observed to be the predominant plasma estrogen and exhibited greater fluctuations than estradiol during breeding season (Martin et al. 1981).

The physiological changes we documented in pintail reproductive organs show that the St. Barthelemy staging site is used by females at various reproductive stages. Some females in RFG at the study site could have nested nearby, and not continue migrating. Indeed, pintail commonly nest in the Lake St. Pierre floodplain (Bélanger and Couture 1989). Our study therefore suggests the nutrients and energy required for early egg formation must come from reserves stored during winter, foods consumed in staging areas, or both.

Acknowledgments

This study was made possible thanks to the financial support of La Fondation Héritage Faune (Fédération québécoise de la faune) and La Fondation de la faune du Québec, and grants from La Fondation Universitaire du Centre du Québec and l'Université du Québec à Trois-Rivières (UQTR). We thank the staff of La Société de la faune et des parcs du Québec for their technical support and Gilles Gauthier (Université Laval) for allowing us to use his equipment for carcass analysis. We appreciated the helpful comments of Antoine Aubin (UQTR), Hélène Glémet (UQTR), and Gilles Gauthier. We also thank Laure Devine and Brigitte Duval for reviewing this manuscript.

Literature Cited

Alisauskas, R. T., and **C. D. Ankney.** 1992. The cost of egg laying and its relationship to nutrient reserves in waterfowl. Pages 30-61 *in* Ecology and management of breeding waterfowl. *Edited by* B. D. J. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H. Johnson, J. A. Kadlec, and G. L. Krapu. University of Minnesota Press, Minneapolis, Minnesota.

Amand, W. B. 1986. Avian clinical hematology and blood chemistry. Pages 264-276 *in* Zoo and wild animal medicine, 2nd edition. *Edited by* M. E. Fowler. W. B. Saunders, Philadelphia, Pennsylvania.

Ankney, C. D., and **R. T. Alisauskas.** 1991. Nutrient-reserve dynamics and diet of breeding female gadwalls. Condor 93: 799–810.

Ankney, C. D., and **C. D. MacInnes.** 1978. Nutrient reserves and reproductive performance of female lesser snow geese. Auk 95: 459–471.

Ankney, C. D., A. D. Afton, and **R. T. Alisauskas.** 1991. The role of nutrient reserves in limiting waterfowl reproduction. Condor 93: 1029–1032.

Austin, J. E., and **M. R. Miller.** 1995. Northern Pintail (*Anas acuta*). *In* The birds of North America, number 163. *Edited by* A. Poole and F. Gill. The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D.C.

Bart, J., and **W. Notz.** 1994. Analysis of data. Pages 24–74 *in* Research and management techniques for wildlife and habitats, 5th edition. *Edited by* T. A. Bookhout. The Wildlife Society, Bethesda.

Barzen, J. A., and **J. R. Serie.** 1990. Nutrient reserve dynamics of breeding canvasbacks. Auk 107: 75–85.

Bélanger, L., and **R. Couture.** 1989. Pintail, *Anas acuta*, use of man-made ponds in Quebec. Canadian Field-Naturalist 103: 547–550.

Choinière, L., and **G. Gauthier.** 1995. Energetics of reproduction in female and male greater snow geese. Oecologia 103: 379–389.

Dabbert, C. B., T. E. Martin, and **K. C. Powell.** 1997. Use of body measurements and serum metabolites to estimate the nutritional status of mallards wintering in the Mississippi Alluvial Valley, USA. Journal of Wildlife Disease 33: 57–63.

Derrickson, S. R. 1978. The mobility of breeding Pintails. Auk 95: 104–114.

Donham, R. S. 1979. Annual cycle of plasma luteinizing hormone and sex hormones in male and female Mallards (*Anas platyrhynchos*). Biology of Reproduction 21: 1273–1285.

Drobney, R. D. 1982. Body weight and composition changes and adaptations for breeding in wood ducks. Condor 84: 300–305.

Esler, D. 1994. Dynamics of ovarian follicles in breeding ducks. Wilson Bulletin 106: 679–688.

Esler, D., and **J. B. Grand.** 1994. The role of nutrient reserves for clutch formation by Northern Pintails in Alaska. Condor 96: 422–432.

Fairbrother, A., M. A. Craig, K. Walker, and **D. O'Loughlin.** 1990. Changes in mallard (*Anas platyrhynchos*) serum chemistry due to age, sex, and reproductive condition. Journal of Wildlife Disease 26: 66–77.

Flint, P. L., and **J. B. Grand.** 1996. Variation in egg size of the Northern Pintail. Condor 98: 162–165.

Fredrickson, L. H., and **E. Heitmeyer.** 1991. Life history strategies and habitat needs of the northern pintail. U. S. Fish and Wildlife Service, Fish and Wildlife Leaflet number 13.1.3, 8 p.

Gauthier, G., J.-F. Giroux, and **J. Bédard.** 1992. Dynamics of fat and protein reserves during winter and spring migration in greater snow geese. Canadian Journal of Zoology 70: 2077–2087.

Gindler, E. M., and **J. D. King.** 1972. Rapid colorimetric determination of calcium in biological fluids with methylthymol blue. American Journal of Clinical Pathology 58: 376–382.

Hannon, S. J. 1979. Plasma calcium as an indicator of reproductive condition in female blue grouse. Journal of Wildlife Management 57: 463–465.

Hochleitner, M. 1994. Biochemistries. Pages 223–245 *in* Avian medicine: principles and application. *Edited by* B. W. Ritchie, G. J. Harrison, and L. R. Harrison. Wingers Publishing, Lake Worth.

Hohman, W. L. 1986. Changes in body weight and body composition of breeding ring-necked ducks (*Aythya collaris*). Auk 103: 181–188.

Johnson, A. L. 1986. Reproduction in the female. Pages 403–431 *in* Avian physiology, 4th edition. *Edited by* P. D. Sturkie. Springer-Verlag, New York.

Joyner, K. L. 1994. Theriogenology. Pages 748–804 *in* Avian medicine: principles and application. *Edited by* B. W. Ritchie, G. J. Harrison, and L. R. Harrison. Wingers Publishing, Lake Worth.

Kenny, A. D. 1986. Parathyroid and ultimobranchial glands. Pages 466–478 *in* Avian physiology, 4th edition. *Edited by* P. D. Sturkie. Springer-Verlag, New York.

Krapu, G. L. 1974. Feeding ecology of Pintail hens during reproduction. Auk 91: 278–290.

Mann, F. E., and **J. S. Sedinger.** 1993. Nutrient-reserve dynamics and control of clutch size in Northern Pintails breeding in Alaska. Auk 110: 264–278.

Martin, R. M., M. E. Lisano, and **J. E. Kennamer.** 1981. Plasma estrogens, total protein, and cholesterol in the female eastern wild turkey. Journal of Wildlife Management 45: 798–802.

Miller, M. R. 1989. Estimating carcass fat and protein in Northern Pintails during the nonbreeding season. Journal of Wildlife Management 53: 123–129.

Milne, H. 1976. Body weights and carcass composition of the common eider. Wildfowl 27: 115–122.

Sturkie, P. D. 1986. Avian physiology. Fourth edition. Springer-Verlag, New York, New York.

White, G. C., and **W. R. Clark.** 1994. Microcomputer applications in wildlife management and research. Pages 75–93 *in* Research and management techniques for wildlife and habitats, 5th edition. *Edited by* T. A. Bookhout. The Wildlife Society, Bethesda.

Received 4 December 2002
Accepted 15 April 2004