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Seasonal patterns of LH, testosterone and semen quality in the Northern pintail duck (*Anas acuta*)

L. M. Penfold[A], *D. E. Wildt, T. L. Herzog, W. Lynch, L. Ware, S. E. Derrickson and S. L. Monfort*

Department of Reproductive Sciences, Conservation Research Center, National Zoological Park, Smithsonian Institution, Front Royal, Virginia 22630, USA.
[A]To whom correspondence should be addressed at White Oak Conservation Center, 3823 Owens Road, Yulee, FL 32097, USA. Email: LindaP@wogilman.com

Abstract. This study characterized seasonal changes in circulating LH and testosterone and in semen production and quality in the Northern pintail duck. Plasma LH and testosterone were measured in blood samples collected weekly throughout the year from eight males exposed to natural fluctuations in day length and temperature. Semen quality was evaluated weekly in these same males from April–June, the months when spermatozoa were produced. Semen quality (based on sperm concentration and normal morphology) peaked 0–2 weeks after sperm production onset and decreased sharply before sperm production cessation in late June. Nadir LH concentrations were measured in July and August with peak LH observed in May and November. There were clear seasonal patterns in circulating testosterone with July–September values being less ($P<0.05$) than October–December which, in turn, were less ($P<0.05$) than January–March. Maximal circulating testosterone ($P<0.05$) occurred during April–June, coincident with semen production. Weekly circulating LH during the breeding season was directly related to testosterone concentrations ($P<0.01$), but was not correlated to any specific semen or sperm trait ($P>0.05$). Testosterone concentrations throughout the breeding season were correlated ($P<0.05$) to total numbers of spermatozoa produced (volume × cell concentration) and percent normal sperm morphology. In summary, the Northern pintail experiences seasonal hormone fluctuations, with maximum circulating testosterone coinciding with peak ejaculate quality reflected by the production of high numbers of morphologically normal spermatozoa.

Introduction

A Conservation and Assessment Management Plan Workshop for waterfowl (Ellis-Joseph *et al.* 1992) assigned 50 of the 154 extant species (39%) to one of three categories of extinction threat based on the Mace-Lande criteria (Mace and Lande 1991). Little is known about the general biology of most of these species, including the fundamentals of reproductive physiology. This is true for the Northern pintail (*Anas acuta*), a dabbling duck that is widely distributed throughout the Northern hemisphere. Although Northern pintails are considered common, recent surveys have revealed a disturbing decline in the wild population, likely related to floods, droughts and habitat loss (US Fish & Wildlife Service 1999).

Captive propagation is one approach for helping manage a species in decline, guarding against extinction and allowing basic research, which may be applicable to wild counterparts. Many wild birds fail to reproduce in captivity, often because of subtle behavioural problems associated with confinement (Hediger 1965). High disease rates in captivity also can prohibit moving animals between populations, thereby complicating efforts to keep populations genetically heterozygous. Artificial insemination could be useful for ensuring that all birds maintained in captivity reproduce, while reducing the risk of disease transfer. However, a prerequisite to the successful use of assisted reproductive technology, such as artificial insemination (AI), for any species is to understand fundamental reproductive biology, including basic endocrine–gonadal interrelationships. This enables semen to be collected during predetermined peak production periods and establishes a standard of normative semen and sperm parameters for determining semen quality. Seasonal profiles for circulating luteinizing hormone (LH) and testosterone and their relatedness to testicular reactivation have been characterized for an array of avian species (for reviews see Farner and Follet 1979; Farner and Wingfield 1980; Follet and Robinson 1980), including the duck (Paulke and Haase 1978; Silver *et al.* 1979; Haase 1983). A histological study of the annual testicular cycle of mallards revealed that sperm production is restricted to March, April and May (Johnson 1966). However, with the exception of one study investigating yearling and adult captive mallard ducks (Stunden *et al.* 1998), essentially no information is available for avian species that simultaneously relates sperm production and quality to circulating reproductive hormones. Therefore, the objective of the present study was to characterize endocrine changes throughout the year and measure sperm production and quality throughout the breeding season of the Northern pintail.

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Materials and methods

Animals and location

Eight adult (2–6 years), male Northern pintails (~850 g bodyweight) were fitted with a leg ring, allowing individual bird identification. Each was housed in a bachelor group with five other males (not included in the study) in individual outdoor (24 × 84 m) pens, each containing a pond. Sinking Duck Diet (Zeigler Brothers, Inc., Gardners, PA, USA) was provided ad libitum and removed from the pen approximately 12 h before semen collection. All males had vocal and visual cue exchange with conspecific, adult females that were housed in groups of four in adjacent pens separated by chain link fencing. The study was conducted at the Smithsonian's Conservation & Research Center located in Front Royal, VA (38° 55′ N 78° 11′ W). The Conservation & Research Center's Institutional Animal Care and Use Committee approved the study plan.

Semen collection and processing

Attempts were made to collect semen from each of the eight males once weekly, starting 30 March and concluding 30 June, encompassing the natural breeding season previously reported for the species in the Northern hemisphere (Austin and Miller 1995). Males were caught (using a long-handled net) and restrained in a supine position. Semen was collected by massaging the ventral aspect of the abdomen towards the cloaca (Gee 1983). Further massage around the cloacal base caused semen to pool at the cloacal opening. Care was taken to avoid everting the copulatory organ, which would result in semen spreading into the numerous grooves and crypts. In general, semen was collected within 20 s of massage onset. Unresponsive birds were released within 1 min of attempting semen collection to minimize handling stress. Semen was collected in a tom-cat catheter (Sherwood Medical, St. Louis, MO, USA), which had been trimmed to 4 cm in length and attached to a 1-mL tuberculin syringe. After semen collection, the catheter and syringe were placed within a 5-mm glass tube on crushed ice for transport to the laboratory.

Ejaculate and sperm assessments

All laboratory semen assessments were performed within 40 min of collection. Semen volume was determined by aspiration into a calibrated Eppendorf pipette (Brinkmann Instruments, Inc., Westbury, NY, USA). Sperm concentration was assessed by diluting semen 1 : 800 with 0.2% glutaraldehyde in phosphate-buffered saline and counting the number of sperm in 10 μL using a haemocytometer. Total sperm produced per ejaculate was calculated by multiplying volume by concentration.

Percent sperm motility and motility rate (scale 0–5, with 0 = non-motile and 5 = highly motile; Howard *et al.* 1991) were assessed subjectively within 1 min of diluting raw semen 1 : 100 with Beltsville Poultry Semen Extender (BPSE; Sexton 1977). Aliquots (5 μL) were placed on a pre-warmed (38°C) microscope slide, covered with a 22 × 22 mm cover slip and examined under a Leitz Diaplan microscope (×20; Leica, 6330 Wetzlar, Germany). Sperm parameters were averaged from three fields taken for each slide examined. Sperm morphology was assessed by incubating 2 μL of raw semen in 20 μL of eosin/nigrosin solution (Burrows and Quinn 1939) for 2 min. Smears made on microscope slides were air dried, and 100 sperm per ejaculate were assessed (×40) for normal or malformed structure (Gee and Temple 1978). A record was made of the proportions of sperm with the following pleiomorphisms: microcephaly, bent head, bent midpiece, bent flagellum, giant cell or head encased in a cytoplasmic droplet. Viability was assessed by incubating 200 μL of semen diluted 2 : 1 with BPSE with live/dead stain (Molecular Probes, Inc., Eugene, OR, USA; Garner et al. 1994; Kasai et al. 1996). For the latter, 5 μL component A stock solution (SYBR-14) was diluted in 95 μL of BPSE (working solution). Diluted semen (200 μL) was incubated for 10 min with 2 μL of component A working solution and 2 μL of stock propidium iodide (component B). Spermatozoa that stained green were considered viable, whereas those that stained red or partially red were deemed non-viable (Garner et al. 1994; Kasai et al. 1996).

Blood sampling

Blood samples were collected once weekly (on days when semen was not collected). Males were caught (as before) and manually restrained. A 3-mL blood sample was obtained from the jugular vein using a heparinized 6-mL syringe and 25-gauge needle. Capture and blood sampling were completed within 3 min of entering the pen to minimize potential animal handling (i.e. stress effects) on circulating hormones (Gratto-Trevor et al. 1991). Blood samples were centrifuged (2500*g*, 20 min), and plasma was stored frozen (–20°C) until analysis.

Radioimmunoassays

Plasma LH was measured using a radioimmunoassay (RIA) previously validated for the Northern pintail (Sorenson *et al.* 1997). In brief, plasma LH was measured using a heterologous ^{125}I-double antibody RIA with a rabbit anti-chicken antiserum (chLH 3/3, 1 : 35 000, provided by P. Sharp, Roslin Institute, Midlothian, UK) as the first antibody, sheep anti-rabbit gamma globulin as the second antibody, USDA-cLH-K-3 as standard and iodinated USDA-cLH-I-3 as tracer (provided by Dr J. Proudman, National Hormone Programme, USDA, Beltsville, MD, USA). Standards (100 μL) or unknowns (25 μL undiluted plasma) were combined with antiserum (100 μL, 1 : 35 000) and incubated for 24 h at 20°C. Iodinated cLH (100 μL) was added and the assay incubated for an additional 24 h. After adding the second antibody (1 mL; 1 : 300) and further incubating for 1 h, the assay was centrifuged (3500*g* for 25 min, 4°C) and the supernatant decanted. Unknowns were assayed in duplicate and analysed in a single assay. The minimal detectable concentration was approximately 0.02 ng mL^{-1}.

Testosterone concentrations in unextracted plasma samples were analysed using a double-antibody [^{125}I] RIA (ICN, Costa Mesa, CA, USA) according to the instructions except that all reagent volumes were halved. Plasma samples were assayed neat or undiluted 1 : 2 (25 μL) in duplicate. This antiserum cross-reacts 100% with testosterone, 3.4% with 5α dihydrotestosterone, 2.2% with 5α-androstane-3β-17β-diol, 2.0% with 11-oxotestosterone and less than 1% with all other steroids tested. Parallel displacement curves were obtained by comparing serial dilutions (undiluted, 1 : 8) of pooled duck plasma and testosterone standard preparations. Inter-assay coefficients of variation for two separate internal controls were 9.4% (n = 3) and 7.5% (n = 3). Intra-assay coefficients of variation were less than 10%, and assay sensitivity was 0.1 ng mL^{-1}. Recovery of known amounts of unlabelled testosterone (range, 0.1–5.0 pg tube^{-1}) added to a pool of diluted duck plasma was 95.9 × 4.2% ($y = -0.08 + 1.06x$, $r^2 = 0.99$).

Statistical analysis

Semen and sperm trait changes throughout the breeding season were assessed using SigmaStat one-way ANOVA. Data that were not normally distributed were arcsin transformed, and where appropriate (n>2), multiple comparisons were examined using Tukey's test. Correlation of LH and testosterone concentrations with each other and with semen or sperm traits were determined using Pearson product moment correlation. One-way ANOVA was performed on LH and testosterone monthly means. Multiple comparisons were made using Tukey's test. Values of P<0.05 were considered statistically different.

Results

Seasonal changes in semen and sperm traits

Onset and cessation of sperm production within individual birds were abrupt. Among all eight males, spermic ejaculates were first produced from 6 to 27 April, whereas the last

spermic ejaculates were produced from 18 to 23 June. Peak sperm quality for individual birds occurred within 0–14 days after the first spermic ejaculate was collected. Sperm concentration and percent normal sperm morphology for three representative males are depicted in Fig. 1. Generally, sperm concentration increased rapidly after the first spermic ejaculate and reached maximal concentrations 1–2 weeks later (Fig. 1*a*,*b*). However, one male was asspermic early in the breeding season, but produced sperm at near maximal capacity within 7 days (Fig. 1*c*) of first sperm production. Although sperm numbers fluctuated among and within males during the succeeding 4–6-week interval (Fig. 1*a*–*c*),

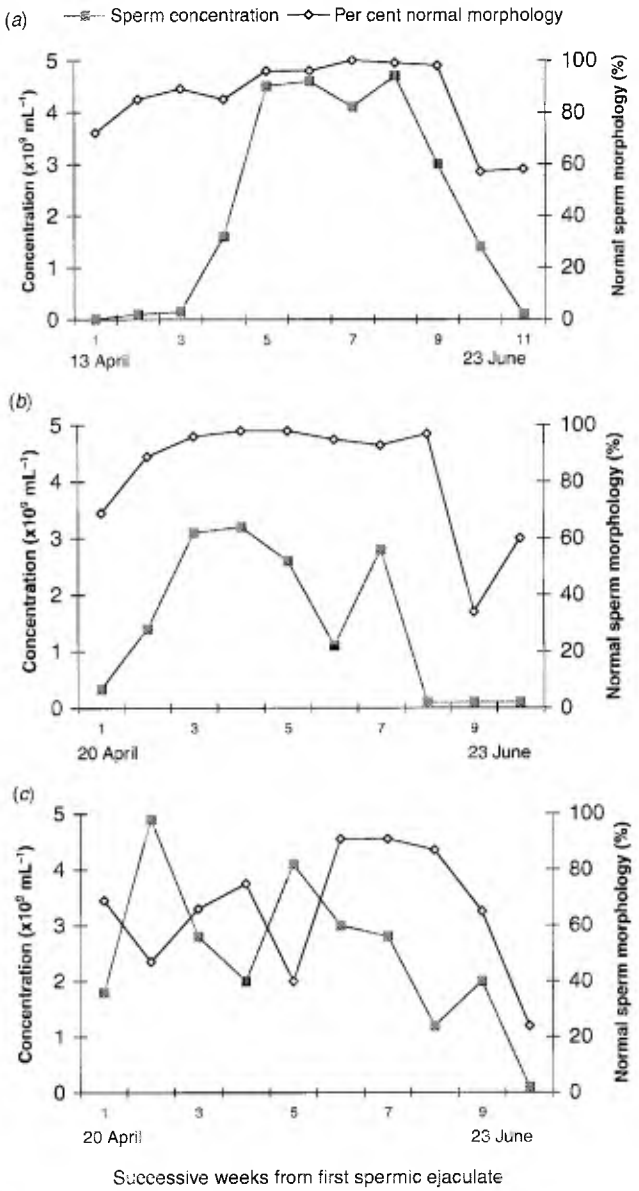

Fig. 1. Sperm concentration and percent normal sperm morphology of ejaculates from three representative Northern pintails throughout the breeding season.

sperm concentration always exceeded 1×10^9 mL^{-1} (range, $1–4 \times 10^9$ mL^{-1}). Peak sperm production (4–6-week interval) was typically followed by a rapid decline in sperm concentration during the last 1–2 weeks of the breeding season (Fig. 1*a*–*c*). Six types of sperm abnormalities were observed (microcephalic, bent head, bent midpiece, bent flagellum, giant cell or head encased in a cytoplasmic droplet). Percent normal sperm morphology generally increased after the first spermic ejaculate (Fig. 1*a*,*b*), although the proportion of normal sperm ejaculated fluctuated in some birds (Fig. 1*c*). The proportion of morphologically normal spermatozoa declined by 13–67% during the last 1–2 weeks of the season (Fig. 1*a*–*c*).

Weekly mean semen quality data (6 April–23 June) aligned by weeks are presented in Table 1. The onset of sperm production was asynchronous (not all males were producing spermic ejaculates until 27 April), but sperm production continued until approximately 18 June (Table 1). No spermic ejaculates were able to be collected from any of the eight males by 1 July. Because of variations in the onset of sperm production and inter-male variation, statistical analysis of mean semen and sperm traits (Table 1) indicated no differences ($P>0.05$) throughout the breeding season. However, comparing semen and sperm traits from each male's first, mid-season and last spermic ejaculates revealed differences over the course of the breeding season (Table 2). Using this approach, mean sperm concentration was less ($P<0.05$) in the first and last ejaculate compared with the mid-season ejaculate (Table 2). No differences ($P>0.05$) in semen volume and sperm motility rate, percent motility, normal sperm morphology or viability were observed between the first and mid-season ejaculates. In contrast, each of these traits declined ($P<0.05$) in the last ejaculate compared with the mid-season spermic ejaculate (Table 2).

Seasonal changes in plasma LH and testosterone

Mean weekly (Fig. 2*a*) and monthly LH (Fig. 3) concentrations are presented. Analysing LH data on the basis of monthly means (Fig. 3) revealed higher ($P<0.001$) LH concentrations in May (peak breeding season) than July and August. LH concentrations (weekly and monthly) began to increase in autumn (September–October) and by November were higher ($P<0.001$) than July/August values. LH (and subsequently testosterone) concentrations started to rise in mid-April (14 h daylight). Decreased LH and testosterone concentrations were measured in June, during which birds were exposed to day lengths longer (14.6 h daylight) than those that stimulated gonadal recrudescence, indicating the development of photorefractoriness.

Annual variations in circulating testosterone showed clear seasonal trends (Fig. 2*b*) with four distinctive periods identified: summer nadir concentrations followed by a modest autumn elevation followed by a January–March increase followed by peak testosterone production during semen

Table 1. Weekly mean (± SEM) testosterone concentrations and sperm traits throughout the Northern pintail's breeding season

Date	No. birds	Total sperm ($\times 10^9$)	Motility rate 0–5	Motility (%)	Morphology (%)	Viability (%)	Testosterone[A] (ng mL^{-1})
6 April	1	0.05	1.0	10	72		0.24 ± 0.19
13 April	2	0.03	1.8	40	80	79	0.31 ± 0.14
20 April	6	0.52 ± 0.50	3.0 ± 0.6	68 ± 12	81 ± 4.5	64 ± 13	0.90 ± 0.35
27 April	8	0.98 ± 0.50	3.8 ± 0.1	72 ± 8.7	88 ± 6.1	72 ± 8.7	1.07 ± 0.71
4 May	6[B]	0.25 ± 0.10	3.7 ± 0.3	72 ± 8.6	88 ± 5.8	72 ± 8.6	0.92 ± 0.47
11 May	8	0.22 ± 0.10	3.6 ± 0.3	71 ± 9.9	91 ± 4.2	71 ± 9.9	6.20 ± 0.54
18 May	8	0.44 ± 0.10	4.1 ± 0.2	79 ± 6.4	89 ± 7.0	79 ± 6.4	1.14 ± 0.51
25 May	6[B]	0.67 ± 0.40	3.8 ± 0.4	70 ± 13.0	88 ± 12	70 ± 13	1.76 ± 0.65
1 June	8	0.46 ± 0.30	3.2 ± 0.5	49 ± 12.4	89 ± 5.8	49 ± 12	1.29 ± 0.34
9 June	8	0.17 ± 0.04	3.7 ± 0.2	76 ± 7.3	94 ± 2.7	76 ± 7.3	0.52 ± 0.29
18 June	8	0.27 ± 0.20	1.9 ± 0.6	33 ± 13.5	65 ± 7.8	33 ± 14	0.20 ± 0.15
23 June	5	0.15 ± 0.11	0.01 ± 0.0	1.1 ± 0.5	9 ± 5.6	50 ± 6.9	0.02 ± 0.56

[A]Mean testosterone concentration from all eight Northern pintails. [B]Two ejaculates omitted because of faecal contamination.

production in the spring. Weekly values illustrate these trends, including a marked decline in circulating testosterone to baseline in late March immediately before commencement of semen production. Monthly means (data not shown) were used to statistically evaluate seasonal differences in testosterone secretion. Testosterone remained at baseline concentrations (<0.02 ng mL^{-1}) from July through September and increased ($P<0.05$) during October (0.08 × 0.03 ng mL^{-1}), November (0.09 × 0.03 ng mL^{-1}), December (0.14 × 0.02 ng mL^{-1}) and January (0.26 × 0.05 ng mL^{-1}), followed by an additional elevation ($P<0.05$) in February (0.39 × 0.07 ng mL^{-1}). Plasma testosterone peaked ($P<0.05$) in April (0.78 × 0.31 ng ml^{-1}) and May (0.83 × 0.16 ng mL^{-1}) and declined ($P<0.05$) rapidly during June (0.37 × 0.12 ng mL^{-1}).

Circulating LH concentrations were correlated to testosterone (r = 0.78; P = 0.003) during the breeding season (6 April–24 June), but were unrelated ($P>0.05$) to any specific semen or sperm trait. Elevated LH concentrations outside the breeding season (November through January) were not correlated with testosterone ($P>0.05$). Testosterone concentrations during the breeding season were correlated to the mean total number of spermatozoa produced (concentration × volume; $r = 0.70$; $P = 0.01$) and with percent normal sperm morphology (r = 0.66; P = 0.02). On the basis of weekly semen collections, sperm production commenced 2–18 days (mean ± SEM, 9.7 ± 2.0) after the first measured rise in plasma testosterone, with one exception. In one bird, a spermic ejaculate was collected before the breeding season rise in plasma testosterone was measured (observed 4 days later, data not shown).

Discussion

This study showed that the Northern pintail has a rapid onset and discrete interval of sperm production in the spring related to high circulating testosterone concentrations, which in turn, were related to elevated LH. In keeping with other seasonal avian species, the data indicate a hypothalamic–pituitary activation preceding gonadal recrudescence (Farner and Wingfield 1980; Farner and Follett 1979). Although testosterone concentrations were sufficient for testes regeneration and onset of sperm production, there was lag time (0–2 weeks) before high-quality semen (total sperm output and proportions of ejaculated sperm with normal morphology) was produced, which in turn was related to maximal testosterone concentrations during the breeding season. Thus, it is possible that a critical level of circulating testosterone must be attained before adequate numbers of morphologically normal spermatozoa

Table 2. Comparison of mean (± SEM) semen and sperm traits at the beginning, middle and end of the Northern pintail's breeding season

	No. birds	Sperm concentration ($\times 10^9$ mL^{-1})	Semen volume (μL)	Sperm motility rate (0–5)	Sperm motility (%)	Sperm morphology (%)	Sperm viability (%)
First spermic ejaculate	8	1.61 ± 0.6[a]	118.8 ± 47.5[a]	3.1 ± 0.5[a]	52.9 ± 13.0[a]	75.9 ± 4.9[a]	89.1 ± 3.3[a]
Mid-season ejaculate	8	3.58 ± 0.3[b]	121.4 ± 24.9[a]	4.1 ± 0.1[a]	81.3 ± 5.2[a]	82.1 ± 6.0[a]	89.0 ± 7.0[a]
Last spermic ejaculate	8	0.34 ± 0.2[a]	33.8 ± 11.8[b]	0.9 ± 0.3[b]	7.0 ± 3.6[b]	46.4 ± 13.4[b]	52.3 ± 6.5[b]

Column means with different superscripts differ ($P<0.05$).

are produced for optimal fertility. Such fundamental gamete and endocrine information is the first step to formulating a reproductive physiology database for the Northern pintail, currently being used as a model for developing AI to assist in managing and conserving rare waterfowl species.

There are few data on sperm pleiomorphisms in relation to breeding season onset in any bird species. Exceptions are the American kestrel (*Falco sparverius*) and the Sandhill crane (*Grus canadensis*), in which increased numbers of abnormal spermatozoa have been observed early in the breeding season (Bird *et al.* 1976; Gee and Temple 1978). Most of these abnormalities are associated with immature cells (Gee 1983). Thus, the Northern pintail shared a similar reproductive process to these species, in that malformed spermatozoa were measured early in the season, with numbers of morphologically normal spermatozoa rapidly produced as the season progressed. The rapid production of morphologically normal spermatozoa is likely to be important for reproductive success: high numbers of morphologically abnormal spermatozoa have been shown to affect fertility in the fowl (Kamar and Baldredin 1959). The breeding season of wild Northern pintails in North America occurs from April to June (del Hoyo *et al.* 1992) and coincides with the spermatogenic period identified in the captive birds examined in the present study. Not surprisingly, the rapid onset of high-quality semen production in the Northern pintail coincides with the commencement of egg laying. During the breeding season, captive or wild females are known to lay one, or perhaps two, clutches of 6–12 eggs each (del Hoyo *et al.* 1992) and a single mating with good quality semen during the breeding season may be adequate to fertilize several eggs (Smyth 1968, Birkhead and Moller 1992), adequately ensuring an individual male's genetic contribution. This has been demonstrated in a parallel study, whereby a single AI with fresh sperm yielded 3–4 fertilized eggs (Penfold *et al.* 2001).

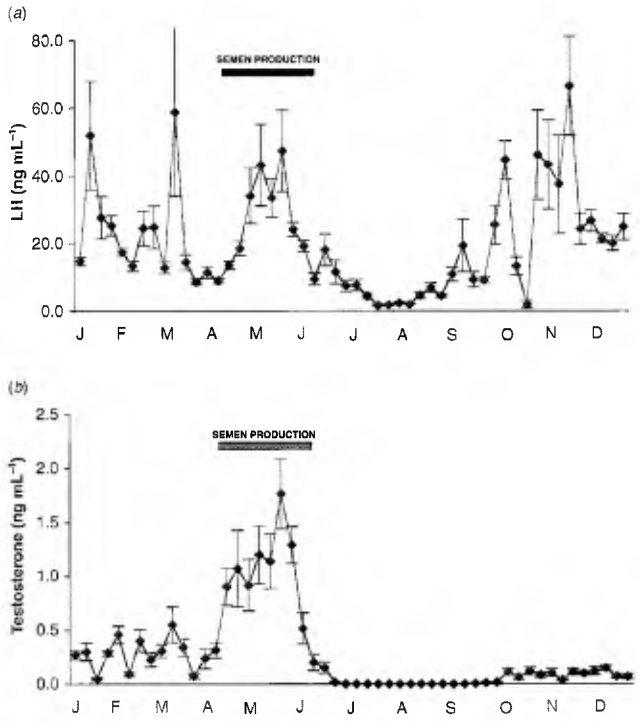

Fig. 2. Mean (± SEM) weekly plasma LH (*a*) and testosterone (*b*) concentrations in the Northern pintail.

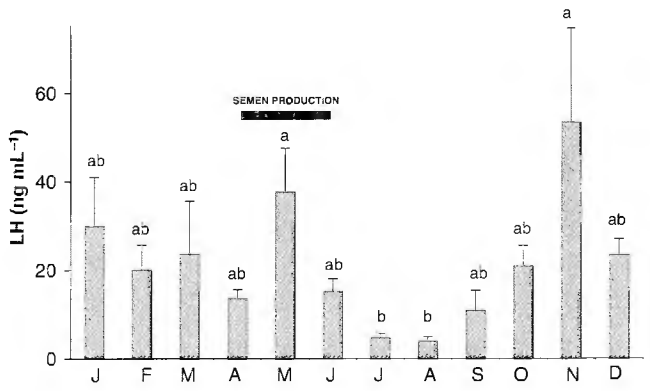

Fig. 3. Mean (±SEM) monthly plasma LH concentrations in the Northern pintail.

Elevated LH concentrations in the spring were clearly related to breeding season peaks in testosterone secretion. Likewise, a marked decline in LH and testosterone, accompanied by cessation of sperm production, at the end of the mating season presumably accompanied testes regression. Although not dealt with in this study, prolactin and FSH also are known to be integrally involved with steroidogenesis control (Follett and Robinson 1980; Sharp *et al.* 1998), and future studies aim to investigate their role in modulating LH and testosterone production in Northern pintails.

For photorefractory avian species, photosensitivity to long days is re-acquired after exposure to the shortening and short days of summer and autumn. For the Northern pintails in the present study, the early autumn increase in LH is likely to be indicative of the end of photorefractoriness. However, although LH concentrations were equivalent to those measured in the spring, a concomitant rise in testosterone was not seen. For the first few days of the spring photostimulatory period, LH is released in a rhythmic manner, believed to reflect a daily 'decision' that the day is photostimulatory (Follet and Robinson 1980). The mechanism of LH release in the autumn warrants further investigation in the Northern pintail to determine why testes recrudescence does not occur, particularly in captive birds, where external influencing factors, such as food availability, are not a concern. Elevated LH outside the breeding season has also been observed in the mallard duck, with the lack of a concomitant testosterone rise similar to that observed in spring and speculated to be related to testicular refractivity (Haase *et al.* 1975*a*). The transient decrease in LH measured in mid-October for all

birds is unexplained, but it has been proposed that social cues in waterfowl are associated with elevated LH during the pre-breeding season leading to courtship behaviour and pair-bonding (Raitasua 1964; Paulke and Haase 1978). As previously also suggested for the Khaki Campbell and mallard duck (Haase *et al.* 1987*a*, 1987*b*), LH secretion in the Northern pintail was not tightly regulated by photoperiod only, but also by as yet unknown other mediators, perhaps a circadian mechanism (Murton and Westwood 1974).

Testosterone concentrations gave a clear indication of reproductive seasonality in this duck species. In this context, the Northern pintail produced androgenic profiles similar to those of the mallard (Paulke and Haase 1978; Donham 1979; Stunden *et al.* 1998). However, autumnal elevations (following summer nadirs) in circulating testosterone were more like those observed in game-farmed (Donham 1979) than wild (Paulke and Haase 1978) mallards. The latter tend to produce high circulating testosterone patterns mimicking those measured during the spring breeding season, whereas the former produce a more attenuated profile (Paulke and Haase 1978). Assessment of testosterone secretion in wild birds of this species is required to determine if some variable associated with captivity diminishes an autumnal testosterone rise. There also is a need to investigate the significance of this secondary, nonbreeding testosterone surge and its role in reproductive behaviour, pair bonding or fitness for the forthcoming breeding season.

Within the breeding season and among males, there usually was a good relationship between onset of the testosterone rise and the first spermic ejaculate. In all but one case, plasma testosterone increased before the first spermic ejaculate. However, the exception in which a male produced sperm in the presence of low circulating testosterone is worth noting. In retrospect, this is not surprising because after the bird testis attains maximal growth at breeding season onset, testosterone likely is sequestered by the seminiferous tubules for the early stages of spermatogenesis (Wingfield and Farner 1993). A closer examination of the testosterone profiles of individual Northern pintails revealed a transient androgenic decline immediately before the maximal vernal concentration associated with the breeding season. Although this effect is less dramatic when presented as the mean testosterone for all eight birds (related to individual differences in weeks in which this was measured), this phenomenon was observed in all Northern pintail males. A cautious interpretation is that this transient testosterone decline may have reflected sequestered testosterone by the seminiferous tubules to enhance high quality sperm production. This, in turn, could explain the sudden, rapid production of high numbers of morphologically normal spermatozoa. The speed of onset of the avian photoperiodic response with regard to gonadotropin secretion has previously been discussed (Follett and Robinson 1980), but has not been linked with semen quality.

In summary, a review of the avian literature reveals a paucity of information on gamete biology and virtually no integrative data on hormonal–sperm relationships in any of the 154 extant waterfowl species. Given that 39% of waterfowl species are formally recognized as threatened by extinction, more systematic studies are warranted to generate data that may be useful for enhanced natural or assisted breeding. Understanding the fundamental reproductive processes in a common model species, such as the Northern pintail, will be useful for eventually extrapolating knowledge and reproductive technology to endangered waterfowl requiring *ex situ* breeding.

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