

Reproductive Endocrinology of a Free-Living, Opportunistically Breeding Passerine (White-Winged Crossbill, *Loxia leucoptera*)

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The aim of the study was to investigate, in a free-living opportunistic breeder, the White-winged Crossbill (*Loxia leucoptera*), the degree to which annual changes in photoperiod and differences in the timing of breeding in different years correlate with the secretion of reproductive hormones. Seasonal changes in concentrations of luteinizing hormone (LH) and prolactin were compared in White-winged Crossbills in interior Alaska (64°50'N, 147°50'W) in plasma samples taken in May–December 1994 and July 1998–August 1999. The birds were in breeding condition in May–July 1994 and in February–April 1999. Additional observations were made on plasma testosterone (T) in males in 1998/1999. In 1998, in adult males, plasma LH and T were low in September–October (LH: <0.4 ng/ml; T: undetectable) and increased significantly in November (LH: 3.65 ± 2.20 ng/ml) and December (T: 0.69 ± 0.88 ng/ml). Thereafter, plasma T increased to 2.35 ± 2.03 ng/ml in March/April and returned to basal levels by June/July. In 1998/1999, in females, plasma LH did not change seasonally. In males in 1994, plasma LH was higher in June (2.15 ± 1.22 ng/ml) and July (2.86 ± 0.69 ng/ml) than in the same period in 1999 (June: 1.20 ± 1.82 ng/ml; July: 1.37 ± 1.03 ng/ml), which is consistent with the later breeding season in 1994. In 1994 and 1998/1999 in both sexes, concentrations of plasma prolactin were elevated when day lengths exceeded about 12 h, being high during late spring to

early fall (males: 40–130 ng/ml; females: 40–140 ng/ml) and low during winter (< 15 ng/ml in both sexes). In 1994 and 1998, molt occurred during the fall, at a time when breeding did not occur, although conifer seeds were sufficiently abundant to support a large bird population. These data are consistent with the view that White-winged Crossbills have the potential to breed at all times of the year except when molting in the fall. It is likely that a photoinduced increase in prolactin secretion is a factor that induces molt. Crossbills may not breed when they are molting because of increased metabolic demands for feather formation and for the maintenance of basal metabolism at a time of year when ambient temperatures fall well below 0°. Alternatively or in addition, the birds may become reproductively photorefractory. © 2001 Academic Press

Key Words: crossbill; photoperiodism; molt; luteinizing hormone; testosterone; prolactin; breeding opportunism; seasonal breeding; reproduction.

Crossbills (genus *Loxia*) are of particular interest for studies on the interaction between photoperiodic and other environmental cues in the control of reproduction and the secretion of reproductive hormones. These birds feed primarily on conifer seeds (Newton, 1972; Benkman, 1990, 1992), which in a given region are produced in varying and unpredictable quantities from one year to the next. Crossbills have adapted to this unpredictability by breeding opportunistically, including, in some areas, during winter, in response to adequate food supply

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(Bent, 1968; Newton, 1972; Godfrey, 1986). However, reproductive function in these birds is partly dependent on photoperiod as demonstrated by several findings in male Red Crossbills (*L. curvirostra*; Tordoff and Dawson, 1965; Hahn, 1995, 1998). When captive Red Crossbills are held on long days their gonads are larger, although not maximally developed, than those in birds held on short days. Further, in captive Red Crossbills fed *ad libitum* and exposed to natural photoperiods (Hahn, 1995), and in free-living conspecific birds (Hahn, 1998), plasma luteinizing hormone (LH) and gonadal size are lower during the fall than at other times of the year.

White-winged Crossbills (*L. leucoptera*) live at higher latitudes than Red Crossbills, although the distributions of the two species overlap (Cramp and Perrins, 1994; Kaufman, 2000). White-winged Crossbills also display breeding opportunism in that they can initiate a reproductive cycle in a given region any time between late winter and summer provided that sufficient food is available to raise young (Benkman, 1990). Free-living White-winged Crossbills in interior Alaska have, however, not been observed to breed in fall or early winter, even in the presence of abundant food (Deviche, 1997).

The failure of White-winged Crossbills to breed in fall or early winter even when food is abundant may be because the birds are molting (Tordoff and Dawson, 1965; Deviche 2000). The induction of molt at this time of year is likely to be a consequence of photoinduced prolactin secretion, as has been demonstrated in the European Starling, *Sturnus vulgaris* (Dawson and Sharp, 1998). No observations on seasonal changes in plasma prolactin have been reported in Crossbills to explore this possibility.

The present study determined seasonal changes in plasma LH, testosterone (T) (males only), and prolactin and correlated these with several indicators of breeding activity in a free-living population of White-winged Crossbills. Comparisons of seasonal changes in plasma LH and prolactin were made between years when breeding occurred either early or late.

MATERIALS AND METHODS

White-winged Crossbills were caught at a site located in a mixed spruce (*Picea glauca* and *P. mariana*)

and birch (*Betula papyrifera*) forest in Fairbanks, Alaska (64°50'N, 147°50'W) in 1994 (May to December, $n = 178$) and between July 1998 and August 1999 ($n = 661$). Birds were attracted into a mist net by the placement of one or two live male decoys next to the net and most captures took place between 07:30 AM and noon (median capture time: 09:00 AM). Within minutes of capture, birds were removed from the net and blood samples (max. 700 μ l) were collected from a wing vein into heparinized microhematocrit tubes. Plasma was harvested and stored at -20 or -80° until assayed for hormones. Whenever possible, birds were sexed and aged by plumage characteristics and were classified as juveniles (first year) or adults (Pyle, 1997; Deviche, 2000). We defined juvenile birds as those individuals that were either hatched during the capture year or were in their second calendar year and in this case were caught between January and early fall, i.e., prior to the second prebasic molt. Adults included birds caught between early fall and winter that were at least in their second calendar year and all older birds (see Pyle, 1997 for nomenclature). The sex of juvenile crossbills can generally not be determined externally until they start acquiring their first adult plumage between September and December (Deviche, 2000). Nine percent of the birds caught during this period could not be sexed, but the sex of all individuals hatched in 1998 and caught in 1999 was determined.

We examined each bird for the presence of contour or flight feather molt, and females were examined for the presence of an incubation patch. For each individual, we measured the following morphological parameters: wing chord (an estimate of body size; measured to the nearest 1 mm), body mass (measured to the nearest 0.1 g), and body fat reserves (scale ranging from 0 to 5 and estimated as the amount of fat visible in the furcular space; Rogers, 1991). After data collection, birds received a National Biological Survey numbered aluminum leg band and were released. Four juveniles and 12 adults (1.9% of the total number of captures) were recaptured once during the time course of the study. Intervals between first and second captures ranged from 6 days to 6 months. There was no indication that the first capture influenced any morphological or physiological variable examined following the second capture and data from recaptured individuals were, therefore, retained in the analyses.

Data on local changes in photoperiod (not including twilight) were obtained from the U.S. Naval Observatory, Washington, DC. Data on air temperature were obtained from the Geophysical Institute, The University of Alaska Fairbanks.

Hormone Assays

We used radioimmunoassays (RIAs) to measure circulating concentrations of LH, prolactin (both sexes), and testosterone (males only). Testosterone was not measured in samples collected in 1994. For each hormone, samples were assayed in duplicate and in a single assay.

Luteinizing hormone. LH concentrations were measured with a micromodification of the chicken LH assay described by Sharp (1987) with 20 μl /assay tube. A dilution curve generated by dilution of a crossbill plasma pool was parallel to the standard curve. The assay sensitivity was 2 pg/tube and the intraassay coefficient of variation was 12.1%.

Testosterone. Testosterone concentrations were determined with a commercial solid-phase RIA system (Diagnostic Products Co., Los Angeles, CA; Deviche *et al.*, 2000) with 35 μl plasma/assay tube. The antibody used in the assay has low (<5%) cross-reactivity with corticosterone, estradiol, progesterone, and 5 α -dihydrotestosterone. The standard curve and a curve generated by serial dilution of a plasma pool from adult males were parallel (slopes of the linear regression lines: standard curve: -1.78 ; crossbill plasma: -1.67), and the recovery of T added to a plasma pool was proportional to the amount added. The assay sensitivity was 10 pg/tube and the intraassay coefficient of variation was 9.99%.

Prolactin. Prolactin concentrations were determined with a recombinant-derived starling prolactin RIA (Bentley *et al.*, 1997). Recombinant-derived starling prolactin was used to produce ^{125}I -labeled tracer and assay standards. A dilution curve generated by dilution of a crossbill plasma pool was parallel to the standard curve. Slopes of the linear regression lines were -1.86 (standard curve) and -1.74 (crossbill plasma). The assay sensitivity was 91 pg/tube and the intraassay coefficient of variation was 8.6%. All plasma samples (20 μl) were on the linear portion of the standard curve.

Statistical Analyses

Whenever possible, data were grouped by capture month and analyzed as such. In some cases, small sample sizes required the combining of data for two or three consecutive months into one group. Multiple group comparisons were made with one- and two-way analyses of variance (ANOVA) followed with Student–Newman–Keuls (SNK) multiple pairwise comparison tests when appropriate (STATISTICA, version 5.5). Many data sets did not comply with normality and/or variance equality criteria for ANOVA and required transformation prior to analysis. For this reason and to standardize analytical procedures, we transformed all data sets into ranks prior to applying statistical tests as recommended by Conover and Iman (1981). In most cases, wing chord was linearly related to body mass (Pearson product moment coefficients: $P < 0.05$). Before ANOVA, body mass data were adjusted to wing chord with slope values from linear regressions, thus eliminating the contribution of individual differences in body size to body mass. Only adjusted body mass values are presented. All results are shown as means \pm standard deviations and statistical significance level of all tests was set at $\alpha = 0.05$.

RESULTS

1998–1999 Annual Cycle

Ambient conditions. Photoperiod at the study site varies between 4 h (winter solstice) and 22 h (summer solstice). During the 1998/1999 study period, monthly average temperatures were below freezing between October (-3.3°) and March (-13.7°) and were highest in June and July (16 – 17° ; Fig. 1).

Breeding phenology. The males started singing in mid-November and were most frequently heard in January and February when daytime temperature was around -35° . Singing declined in March and was rarely heard after April. Fledglings begging food from accompanying adults were most frequently observed in May. Until the end of April, the birds fed mostly on White Spruce seeds obtained from cones hanging in trees. By May, few seeds remained in hanging cones

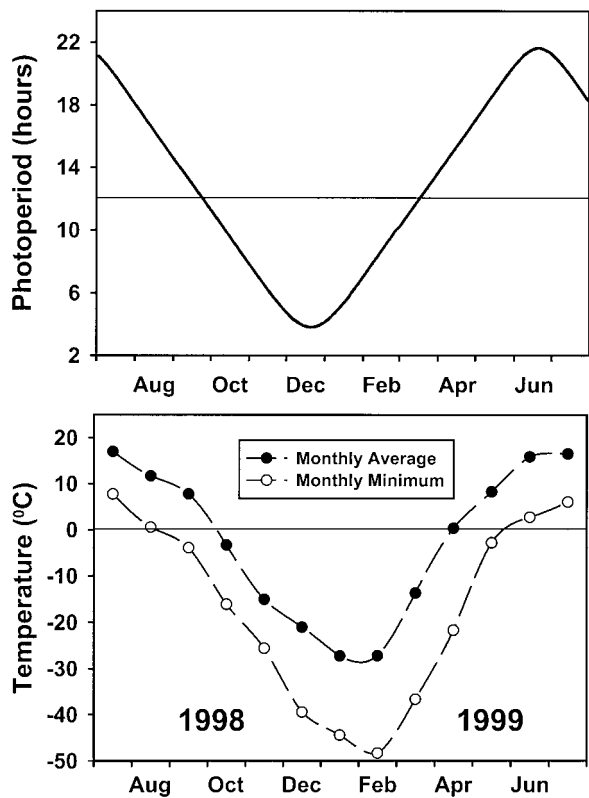


FIG. 1. Seasonal changes in photoperiod and ambient temperatures in Fairbanks, Alaska (64°50'N, 147°50'W).

and juveniles and adults often fed on the ground, extracting seeds from fallen cones. In June the birds resumed feeding in trees, including birch, where they may have taken insects, as new spruce cones were present only in small numbers and were not producing seeds. By July 1999, most birds had left the study area.

In 1998 some birds started molting in August, and all were molting in September (Fig. 2A). By December, molt was complete in most birds. The time course of molt was similar in both sexes. Females with an incubation patch were first caught in April, and all females had an incubation patch in May (Fig. 2B). Hatching-year birds were first caught in April, when they represented a small proportion (5.7%) of the sampled individuals (Fig. 2C). The proportion of hatching-year birds increased in May and June and reached more than 80% in July (Fig. 2C).

Body masses. Body masses varied seasonally in both sexes (males: $F_{7,172} = 10.05$, $P < 0.001$; females: $F_{4,133} = 4.91$, $P = 0.001$). Figure 3 presents data for

ages combined as masses were not age related and there was no age \times month interaction in either sex. Masses of males increased significantly between September and October, then did not change until January/February, and decreased significantly in March/April. Males had similar masses in May and June and

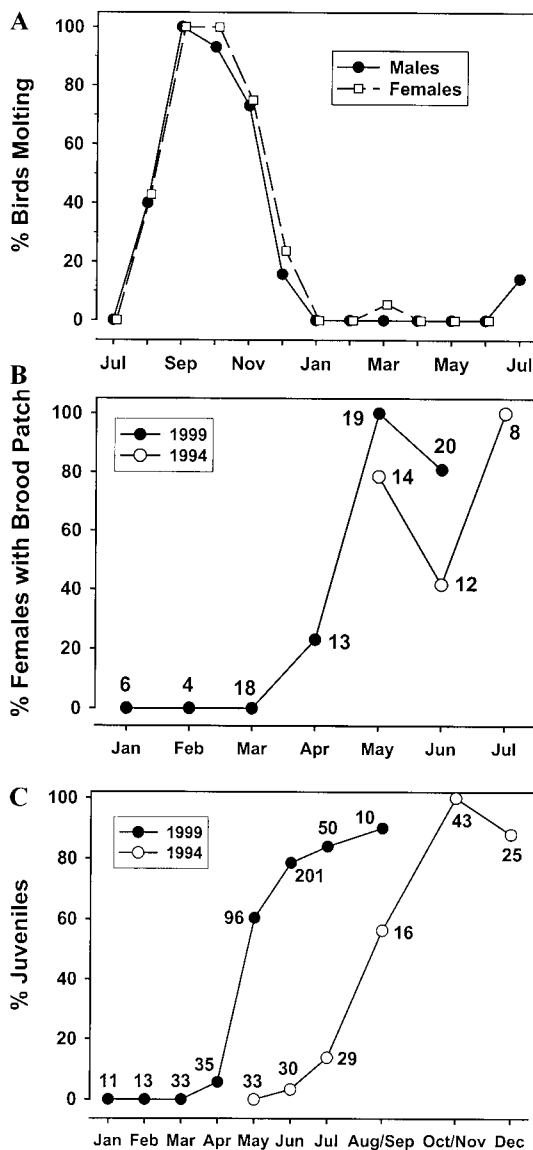


FIG. 2. (A) Seasonal changes in the proportion of males ($n = 5-29/\text{point}$) and females ($n = 4-21/\text{point}$) exhibiting prebasic molt in a free-living population of White-winged Crossbills sampled at a single location in Fairbanks, Alaska (64°50'N, 147°50'W) in 1998–1999. (B) Proportion of adult females with an incubation patch in 1994 and 1999. (C) Proportion of juvenile birds in the sampled population in 1994 and 1999. Numbers next to symbols indicate sample sizes.

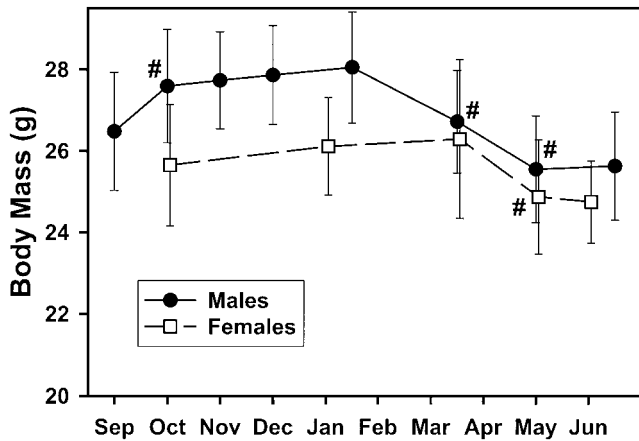


FIG. 3. Seasonal changes in body masses (means \pm standard deviations) of male ($n = 14$ – 33 /point) and female ($n = 20$ – 44 /point) White-winged Crossbills of the sampled population in 1998–1999. #Indicates a significant difference ($P < 0.05$, Student–Newman–Keuls pairwise comparison test following ANOVA on ranked data) compared to the previous measurement for the same age group. Data for hatching-year birds caught in April–June 1999 are not included.

weighed significantly less during these months than in September of the previous year ($P \leq 0.03$). Body masses of females did not change between October and March/April and then decreased significantly in May (Fig. 3).

Plasma testosterone, LH, and prolactin concentrations in males. Plasma T in adult males ($n = 12$) in July and August 1998 was 1.63 ± 2.67 ng/ml. Juveniles ($n = 13$) caught during these months could not be sexed (see Materials and Methods), so data collected for this period were not used to analyze age-related seasonal changes in hormone levels. Data for August 1999 were not included in this analysis either because only one adult male was caught during this month. Between September 1998 and July 1999 plasma T changed seasonally ($F_{8,130} = 7.47$, $P < 0.001$), was age-dependent ($F_{1,130} = 9.41$, $P = 0.003$), and showed an age \times month interaction ($F_{8,130} = 2.40$, $P = 0.019$). In adults, plasma T was undetectable in September/October, started to increase in December, when it was higher than that in juveniles, peaked in March, and decreased to basal levels by June/July (Fig. 4). In juveniles, plasma T concentrations were maximal in March. During this month they were significantly higher than during the September to December pe-

riod, but not different from concentrations measured in January/February or in June/July (Fig. 4).

Plasma LH varied seasonally ($F_{7,112} = 7.79$, $P < 0.001$) and was age dependent ($F_{1,112} = 12.96$, $P < 0.001$). There also was a significant age \times month interaction ($F_{7,112} = 2.30$, $P = 0.032$). In adults, LH concentrations were lowest in September and October and then increased in November, the only time when they differed from those in juveniles (Fig. 5). In juvenile males, LH did not change significantly across the study period, although the highest mean values occurred in March/April (Fig. 5).

Concentrations of plasma prolactin varied seasonally ($F_{7,112} = 21.50$, $P < 0.001$). There was no overall age difference, but there was an age \times month interaction ($F_{7,112} = 2.54$, $P = 0.018$). In September plasma prolactin was higher in adults than in juveniles (Fig. 5). Thereafter, in juvenile and adult males, concentrations of plasma prolactin were uniformly low until March/April when they increased significantly (Fig. 5). Subsequently a decrease was observed in June/July in adult males but not in juveniles.

Plasma LH and prolactin concentrations in females. LH levels in adult females sampled between July 1998 and June 1999 did not change seasonally

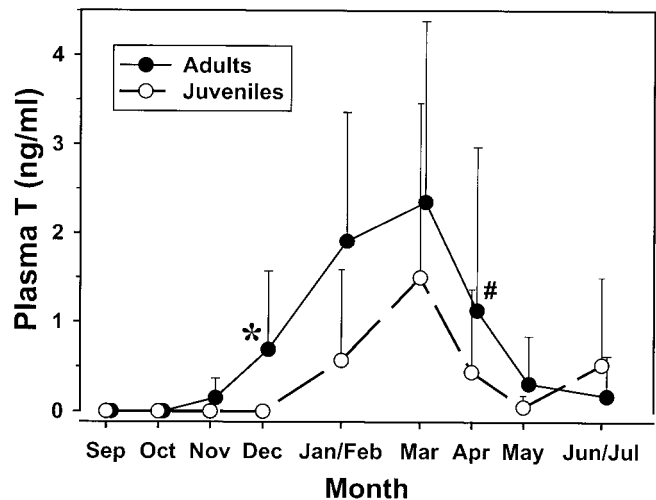


FIG. 4. Plasma concentrations of testosterone (T; means \pm standard deviations) of young ($n = 6$ – 14 /point) and adult ($n = 4$ – 10 /point) male White-winged Crossbills in 1998–1999. #Indicates a significant difference compared to the previous measurement for the same age group. *Indicates a significant age difference for the corresponding time point ($P < 0.05$; Student–Newman–Keuls pairwise comparison tests following ANOVA on ranked data).

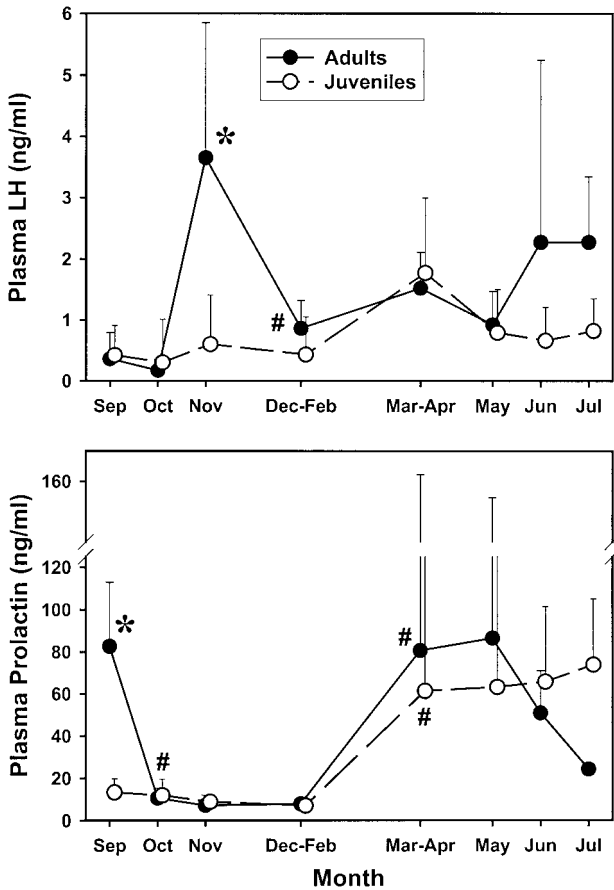


FIG. 5. Plasma concentrations of luteinizing hormone (LH) and prolactin (means + standard deviations) of juvenile ($n = 4-17/\text{point}$) and adult ($n = 3-13/\text{point}$) male White-winged Crossbills in 1998–1999. #Indicates a significant difference compared to the previous measurement for the same age group. *Indicates a significant age difference at the same time point ($P < 0.05$; Student-Newman-Keuls pairwise comparison tests following ANOVA on ranked data).

(one-way ANOVA: $F_{6,36} = 2.23$, n.s.; Fig. 6). Juveniles caught before September could not be sexed so that only data collected between this month and June 1999 were used to analyze age-related differences in hormone levels. Between September–November 1998 and June 1999 LH did not change seasonally and was similar in adults and juveniles. There also was no age \times month interaction (Fig. 6).

In adult females prolactin was elevated in July and August 1998, declined to a minimum in December–February, and then increased significantly in May. Between September–November 1998 and June 1999 plasma prolactin concentrations varied significantly

($F_{4,97} = 32.45$, $P < 0.001$). There was a significant effect of age ($F_{1,97} = 6.64$, $P = 0.011$) but no age \times month interaction. Between March/April and June concentrations of plasma prolactin in adults did not differ from levels in July in the previous year. In juveniles, plasma prolactin increased in May as in the adults (Fig. 6).

Comparisons of Breeding Birds in 1994 and 1999

Breeding phenology. Crossbills in the study area bred earlier in 1999 than in 1994. In 1999 two hatching-year individuals were caught in April and the proportion of these birds in the sampled population rapidly

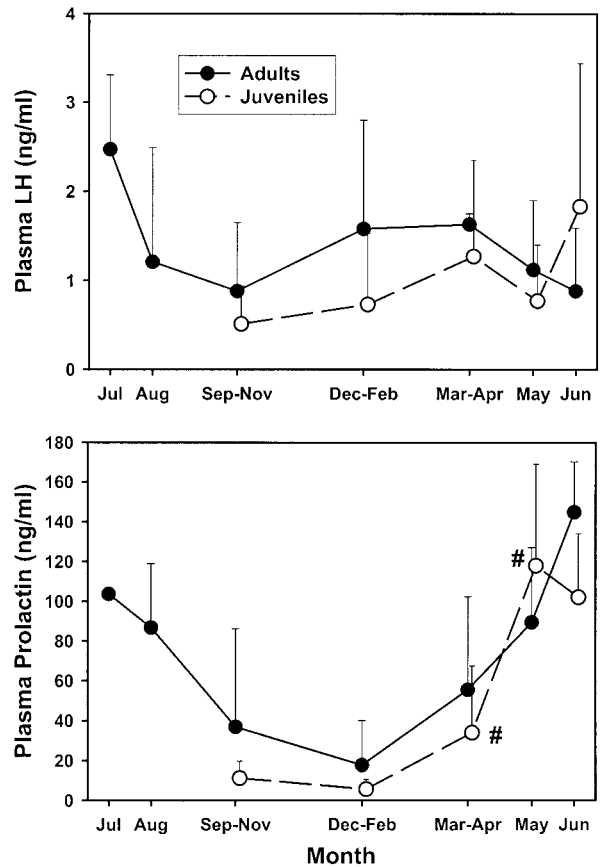


FIG. 6. Plasma concentrations of luteinizing hormone (LH) and prolactin (means + standard deviations) of juvenile ($n = 5-25/\text{point}$) and adult ($n = 4-10/\text{point}$) female White-winged Crossbills in 1998–1999. #Indicates a significant difference compared to the previous measurement for the same age group ($P < 0.05$; Student-Newman-Keuls pairwise comparison tests following ANOVA on ranked data).

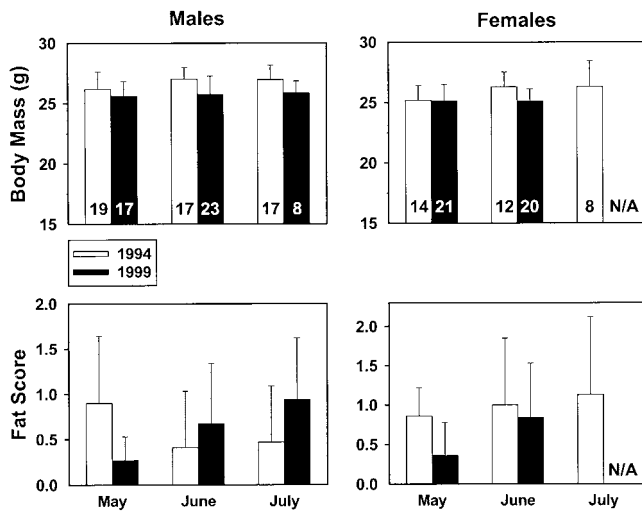


FIG. 7. Body masses and furcular fat scores (means + standard deviations) of adult male and female White-winged Crossbills sampled at a same location in May, June, and July 1994 and 1999. Numbers inside the bars (top) indicate sample sizes, which are similar for body masses and fat scores. N/A, data not available.

increased in May (Fig. 2C). Female White-winged Crossbills incubate for 12–14 days (Ehrlich *et al.*, 1988; Benkman, 1992). Young fledge when approximately 3 weeks old and their mandibles start to cross about 2 weeks after fledging (Benkman, 1992). Juveniles caught in April had partially to completely crossed mandibles and were, therefore, presumably at least 50 days old; i.e., they were hatched from eggs laid at the end of February or earlier. In contrast, no hatching-year crossbill was caught until June in 1994. Further evidence for later breeding in 1994 than in 1999 is provided by the observation that in May and June 1994, 79 and 42%, respectively, of the sampled females had an incubation patch (average for the 2 months combined: 62%; $n = 26$ birds sampled; Fig. 2B). The corresponding May–June percentage in 1999 was 90 ($n = 39$ birds sampled; two-tailed Fisher exact test comparing 1994 and 1999; $P = 0.012$; Fig. 2B). Birds sampled in 1994 were molting between September and November, but neither in August nor December, indicating that the timing of molt was similar to that in 1998 (Fig. 2A).

Body masses and fat reserves. In May–July (males) and May–June (females) adults weighed significantly less in 1999 than 1994 (males: $F_{1,93} = 13.27$, $P < 0.001$; females: $F_{1,63} = 4.03$, $P = 0.049$; Fig. 7). Fat reserves in males did not differ consistently between

1994 and 1999, but there was a significant year \times month interaction ($F_{2,95} = 6.86$, $P < 0.002$). Males in 1999, but not in 1994, had significantly higher fat reserves in July than in May (Fig. 7). Fat reserves in females were higher in May/June 1994 than during the same period in 1999 ($F_{1,62} = 5.89$, $P < 0.02$; Fig. 7) and there was no year \times month interaction.

Juveniles were in better body condition in 1994 than in 1999 (Fig. 8). In 1994, the body masses of these birds gradually increased between July, when they were first caught in sufficient numbers to warrant analysis (Fig. 2), and December (one-way ANOVA: $F_{3,74} = 18.096$, $P < 0.001$). During this month juveniles weighed significantly more than in July–September, but not in October/November (Fig. 8). The body

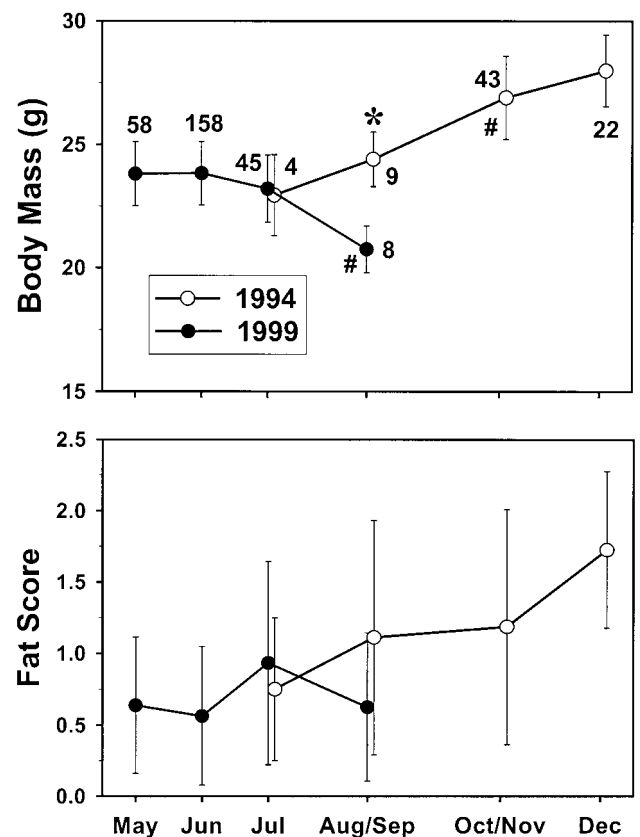


FIG. 8. Body masses and furcular fat scores (means \pm standard deviations) of hatching-year White-winged Crossbills sampled at the same location in 1994 and 1999. Numbers next to symbols at top indicate sample sizes. Sizes are similar for body masses and fat scores. *Indicates a significant difference between years, whereas # indicates a significant difference compared to the previous measurement for the same age group ($P < 0.05$; Student–Newman–Keuls pairwise comparison tests following ANOVA on ranked data).

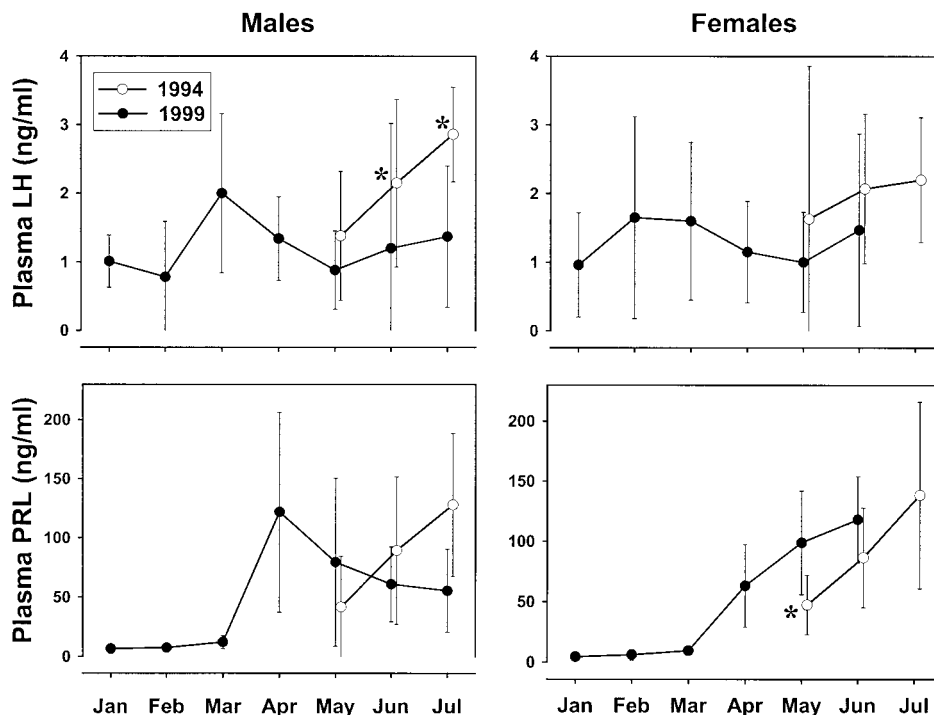


FIG. 9. Plasma concentrations of luteinizing hormone (LH) and prolactin (PRL; means \pm standard deviations) of adult male ($n = 5\text{--}15/\text{point}$) and female ($n = 4\text{--}16/\text{point}$) White-winged Crossbills sampled at a single location in 1994 and 1999. *Indicates a significant year-to-year difference for the considered hormone, sex, and month ($P < 0.05$; Student-Newman-Keuls test on data transformed to ranks before analysis).

masses of juveniles sampled in 1999 were stable between May and July, but in contrast to 1994, declined significantly in August, when birds weighed significantly less than during the previous 3 months (Fig. 8). A two-way ANOVA on data collected for July–September 1994 and 1999 revealed that during this period the body masses of young differed between years ($F_{1,62} = 12.28$, $P < 0.001$), but there was a significant year \times month interaction ($F_{1,62} = 15.38$, $P < 0.001$). Juveniles had similar body masses in July 1998 and 1999, but weighed significantly less in August/September 1999 than in 1994 (Fig. 8). Fat reserves in juveniles in 1994 increased significantly between July and December (one-way ANOVA: $F_{3,74} = 3.95$, $P = 0.011$; Fig. 8). These reserves did not vary between May and August/September 1999 and did not differ in 1994 and 1999 during the July to September period.

Plasma LH and prolactin. In 1999, in males, plasma LH did not change between May and July, whereas in 1994 it increased significantly between these months ($F_{2,60} = 5.18$, $P < 0.0009$; Fig. 9). As a result, plasma LH was higher in June and July 1994

than during these months in 1999. In 1999 and 1994 plasma prolactin in males was equally high between May and July (Fig. 9). In 1999, in females, plasma LH did not change significantly between May and June but was higher in July 1994 than in June 1999 (Fig. 9). In 1999 and 1994, plasma prolactin increased between May and June/July ($F_{1,41} = 5.63$, $P = 0.022$; Fig. 9).

DISCUSSION

This study supports the view that crossbills can breed at anytime of the year except in the fall when they are molting (Red Crossbill: Hahn, 1998; White-winged Crossbill: Benkman, 1992). This conclusion is based on observations on the change in percentage of juveniles caught during each month of the year, showing that breeding occurred in February–April in 1999 and in May–July in 1994. There was no evidence that breeding occurred in the study area during other periods in these years. Early breeding in 1999 was fur-

ther supported by the observations that males sang only during winter 1998 and early spring 1999 and that females with an incubation patch were present in spring and summer, and not at other times, in 1999. Observations during May–June in 1994 and in 1999 demonstrated that differences in the timing of breeding were correlated with differences in body mass and fat scores. When breeding occurred during these months in 1994 the birds tended to be in better body condition than in 1999 when breeding did not occur at this time. The question, therefore, arises of whether photoperiod controls the beginning and end of the long period of the year, from late winter until early fall, when White-winged Crossbills are potentially capable of breeding.

For the majority of birds breeding at temperate-high latitudes, the breeding year begins while day lengths are still short, after the dissipation of reproductive photorefractoriness (Nicholls *et al.*, 1988; Sharp, 1996). At this stage of the annual cycle, the biological clock reads day lengths as short, but it does not transduce any photoperiodic information, either stimulatory or inhibitory, to the gonadotropin-releasing hormone (GnRH-I) neurons. The activity of these neurons in these circumstances may be influenced by nonphotoperiodic environmental factors including ambient temperature, food availability, and presence and physiological condition of mates (Engels and Jenner, 1956; Lewis and Farner, 1973; Wingfield, 1985a,b; Silverin and Viebke, 1994; Silverin and Westin, 1995). A nonphotoperiodic increase in LH secretion is commonly observed in temperate latitude birds, including the Red Crossbill (Hahn, 1995), in the fall and/or winter (e.g., Scanes *et al.*, 1974; Lincoln *et al.*, 1980; Lea *et al.*, 1986; Sharp *et al.*, 1986; Silverin, 1991). The increase in plasma LH observed in free-living male White-winged Crossbills in November, when the photoperiod is on average only 6.5 h long and decreasing, is consistent with this general observation.

The baseline seasonal pattern of LH secretion in the White-winged Crossbill is similar to that in the Ring Dove (*Streptopelia risoria*), with moderately elevated levels during most months, except during early fall when they are depressed (Lea *et al.*, 1986). In the Ring Dove (Balthazart *et al.*, 1981), as in the Red Crossbill (Hahn, 1995), this seasonal decrease in plasma LH is associated with gonadal regression. If Ring Doves are subsequently maintained on short days, the gonads

recrudescence and plasma LH concentrations increase (Balthazart *et al.*, 1981; Lea *et al.*, 1986). A similar, nonphotoperiodic increase in plasma LH and gonadal size has been observed in captive Red Crossbills exposed to natural lighting (Tordoff and Dawson, 1965; Hahn, 1995). In the Ring Dove, the nonphotoperiodic increase in plasma LH during the winter and early spring is moderately augmented in the spring when daylength exceeds 12.5 h (Lea *et al.*, 1986). This seasonal photoperiodically induced increase in LH secretion is not sufficient to induce full breeding condition, which requires an adequate food supply and the presence of a mate and nest site. In the Ring Dove, breeding can occur in all months of the year but is rare in the fall and early winter when the birds are molting (Davies, 1974).

The interaction between annual changes in day length and nonphotoperiodic factors in the control of breeding in the Ring Dove may be similar in the White-winged Crossbill. The increase in plasma LH in late fall probably reflects an increase in the activity of GnRH-I neurons, marking the dissipation of photorefractoriness, which decreases the threshold response to nonphotoperiodic factors, such as food supply, required to stimulate full breeding activity. A further decrease in this threshold might be anticipated in spring, when day lengths become photostimulatory. In principle, crossbills would be expected to be able to breed several times a year, as do Ring Doves, provided that they can locate a suitable food source, mate, and nest site. Evidence supporting the hypothesis that individual White-winged Crossbills can breed more than once during a single year is not available. However, we note that 79% of the adult females sampled in May 1994, shortly after they moved into the study area, had an incubation patch and low LH levels. It is likely that these females had bred or had attempted to do so earlier that year outside the study area and then bred again locally.

Experimental evidence demonstrating the importance of the food supply as the proximal factor initiating breeding in crossbills comes from observations on captive Red Crossbills. When held on long days on a restricted diet, the moderately enlarged testes grow rapidly in response to *ad libitum* feeding (Hahn, 1995). Our results are consistent with the idea that food availability plays an important role in timing reproduction in White-winged Crossbills. Numerous birds

wintered in the study area in 1998 and bred locally in spring 1999, but did not continue to do so in summer 1999, when local production of new conifer cones was poor. Adults and hatching-year birds caught at this time tended to be in poorer body condition, as indicated by low body mass and fat scores (hatching-year crossbills actually lost weight between May–July and August 1999), than in 1994. This may explain why few birds were found in the study area by the end of summer 1999. However, birds moved into the region in late spring–early summer of 1994, when abundant new cones were ripening. These birds then started to breed and were locally abundant at the end of that year. The annual Christmas bird count of the National Audubon Society indicates that an exceptionally high number ($n = 830$) of White-winged Crossbills were present in the Fairbanks area in December 1994 compared to previous or following years (average for 1993–1999 excluding 1994: $n = 62$). The most parsimonious interpretation of these data is that spring (1999) and summer (1994) breeding took place in response to local food resources—or to an environmental factor correlated with these resources—rather than to an increase in photoperiod. The transitory increase in plasma LH followed by a more prolonged increase in plasma testosterone in male crossbills in fall 1998–March 1999 is likely to have been a direct or indirect response to an increase in food availability that occurred at this time. A similar transitory increase in plasma LH increase was not detected in females, possibly because of large individual variation. The increase in plasma LH in males in summer 1994, but not in 1999, is also consistent with a response to increased food triggering the late breeding episode in 1994.

A characteristic feature of seasonally breeding birds at mid-high latitudes is a photoinduced peak in prolactin secretion in late summer. This peak occurs in species that terminate breeding by the development of both absolute (Dawson, 1997; Silverin and Goldsmith, 1997; Dawson and Sharp, 1998; Sharp *et al.*, 1998; Deviche *et al.*, 2000) and relative (Boswell *et al.*, 1995) reproductive photorefractoriness. A similar elevation in plasma prolactin was observed in free-living White-winged Crossbills. The functional significance of this seasonal peak in prolactin has been investigated in the starling, a species that terminates breeding by the development of absolute photorefractoriness. Immunization of starlings against vasoactive intestinal

polypeptide (prolactin releasing factor) blocks the seasonal increase in plasma prolactin, delays the development of photorefractoriness, and abolishes the postnuptial molt (Dawson and Sharp, 1998). This observation suggests that the late summer/early fall peak in plasma prolactin observed in the White-winged Crossbill may also be causally related to the annual molt and, possibly, the development of photorefractoriness. The summer increase in plasma prolactin appears to be influenced by photoperiod since it occurred in 1999 and 1994, irrespective of whether birds bred early or late in the year. Factors other than photoperiod also contribute to regulation of prolactin secretion, notably the expression of parental behaviors (Goldsmith, 1983). Increased prolactin secretion in males feeding their incubating partners may account for the higher concentrations of this hormone in males between May and July 1994 than during the same period in 1999.

The annual molt induced, in part, by the seasonal increase in plasma prolactin secretion is likely to impose substantial metabolic constraints which contribute to a reduced likelihood of autumnal breeding (Murphy and Taruscio, 1995; Schieltz and Murphy, 1997). The energetic demands of molt are further augmented in the fall–early winter by low ambient temperature which also increases energy expenditure (Pohl and West, 1973; Dawson *et al.*, 1983). However, low ambient temperature alone is unlikely to account for the absence of fall–early winter breeding. Indeed, in 1999 at least some females were reproductively competent and laid eggs at the end of February or earlier, when temperature was similar to, and food equally or more likely less abundant than, that during the previous fall. Consistent with this hypothesis, initial signs of breeding activity (males singing, elevated plasma LH and testosterone levels) in 1998 were noticed in November, when birds were nearing molt completion, and breeding proceeded during the following months despite lower temperatures than those in September and October.

In males, an age-related difference occurred in concentration of plasma LH, with an increase occurring in adults but not in juveniles in November/December 1998. This difference may account for the fact that testosterone started to increase in December in adults, but not until January/February in juvenile males. Previous research on other male passerines has also

found seasonal reproductive development to be attenuated or delayed in young compared to adults (Dark-eyed Junco, *Junco hyemalis*: Ketterson and Nolan, 1992; Deviche *et al.*, 2000; Mountain White-crowned Sparrow, *Zonotrichia leucophrys oriantha*: Morton *et al.*, 1990; Great Tit, *Parus major*: Silverin *et al.*, 1997).

In conclusion, compared to other seasonally breeding passerines, White-winged Crossbills have developed an attenuated dependency on photoperiod and a relatively increased reliance on a nonphotic cue (food availability) to stimulate breeding. As a result, crossbills can breed on a flexible schedule. They nest at the end of winter and early spring if seeds produced during the previous summer are available and at the beginning of the summer if ripening and newly matured seeds are present in sufficient quantities. Breeding does not normally continue in fall and early winter, even in the presence of abundant food. Seasonal termination of breeding may result from prolonged exposure to long day length that stimulates increased prolactin secretion. Since increased prolactin secretion is likely to facilitate molt, it is suggested that increased energy expenditure required to meet the energetic demands of both the molt and the rapidly decreasing fall and early winter ambient temperatures may be a major factor preventing reproduction at this time. Additionally, it cannot be discounted that, in fall and early winter, the birds may become photorefractory, further decreasing the likelihood of breeding.

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