

OVARIAN GROWTH IN TREE SPARROWS (*SPIZELLA ARBOREA*)¹

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THE gonadal responses of several species of birds exposed to stimulatory daily photoperiods of constant duration conform in that an initial rectilinear relationship exists between the logarithm of testicular weight and time on long days. They conform further in that the rate of photo-induced testicular growth, as determined from the rectilinear relationship, is a function of daylength. These and other observations support the thesis that increased daylength plays an indispensable role in initiating vernal testicular growth and determining its rate in many species. The current consensus is, however, that long days are gonadostimulatory not because they exceed a critical length, but because they illuminate a photoinducible phase of a circadian oscillation in photosensitivity. (For reviews see Farner, 1959, 1961, 1964a, 1964b, 1970; Hamner, 1966; Lofts et al., 1970.)

Quantification of photoperiodic gonadal responses has been limited essentially to males. To our knowledge, only four definitive studies—one on White-crowned Sparrows (Farner et al., 1966) and three on Japanese Quail (Follett and Farner, 1966; Sayler and Wolfson, 1967; Follett and Sharp, 1969)—describe the temporal course and/or rate of ovarian growth induced by constant daylengths, and only one suggests that rate of ovarian growth varies with daylength (Follett and Sharp, 1969). Moreover, although a circadian oscillation in photosensitivity apparently is involved in the control of ovarian growth in Japanese Quail (Follett and Sharp, 1969), there is no evidence for or against involvement of a similar oscillation in the control of photoperiodic ovarian growth in species that breed seasonally. In this report, we describe the temporal course and rate of ovarian growth in Tree Sparrows (*Spizella arborea*) exposed to daylengths of 8, 11, 15, or 20 hours and examine rate of photoinduced ovarian growth as a function of daylength. In addition, we report results of a preliminary experiment that suggest a circadian oscillation operates in the photoperiodic ovarian response of the Tree Sparrow. The pineal body is examined as the possible seat of the circadian oscillator.

MATERIALS AND METHODS

In the first of three experiments, 53 female Tree Sparrows captured between

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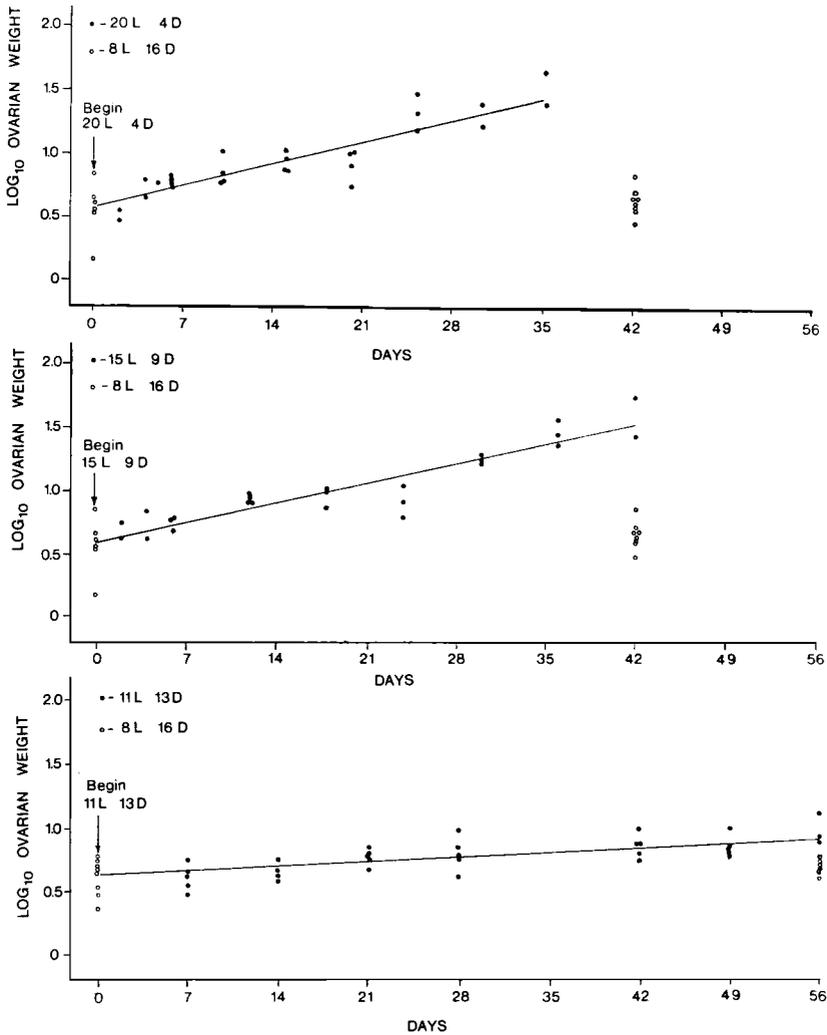


Figure 1. Relationships between ovarian weight and time in Tree Sparrows after daily photoperiods were changed from 8 to 20 (upper), 8 to 15 (middle), and 8 to 11 (lower) hours. Regression lines were obtained by the method of least squares.

14 January and 10 February 1967 were held on 8-hour daily photoperiods (08:30–16:30 CST) until 21 June 1967 when they were divided into groups designated PSL-20 and PSL-15 and caged in adjacent rooms on 20- (08:30–04:30 CST) and 15-hour (08:30–23:30 CST) daily photoperiods, respectively. Six birds sacrificed on 21 June permitted us to estimate ovarian weight at the beginning of the long-day treatments, and eight birds (group PSS-42) continued on 8-hour daily photoperiods (8L) for 42 days served as terminal short-day controls. Thirty-three

other females (group PSL-11) captured between 19 December 1969 and 21 February 1970 were transferred from 8- (08:30-16:30 CST) to 11-hour (08:30-19:30 CST) daily photoperiods on 30 April 1970. Eight birds killed on that day served as initial controls, and five birds (group PSS-56) killed after 56 additional days on 8L, as terminal controls. Birds of groups PSL-20, -15, and -11 were sacrificed at selected intervals during 5, 6, and 8 weeks of photostimulation, respectively. Minimum intensity was 400 lux.

In the second experiment, Tree Sparrows captured between 17 February and 11 March 1967 were held on 20-hour daily photoperiods (08:30-04:30 CST) until 5 July 1967 when the photoperiod was reduced 1 hour per day to 8 hours (08:30-16:30 CST). Beginning 17 July, 24 females were sacrificed over a 12-month period during which the photoperiod remained at 8 hours.

In the third experiment, female Tree Sparrows captured between 11 November 1967 and 31 January 1968 were held on 8L (08:30-16:30 CST) until mid-July 1968 when they were subjected to pinealectomy or to sham pinealectomy (for procedures see Donham, 1968) or sacrificed as initial controls ($N = 8$). Pinealectomized birds were divided into groups designated PC ($N = 9$) and PD ($N = 12$) and continued on 8L (08:30-16:30 CST and 08:30-14:30, then 01:30-03:30 CST, respectively). Sham-pinealectomized birds were divided into similar groups designated SPC ($N = 11$) and SPD ($N = 14$). All birds were sacrificed during the next 100 days.

Tree Sparrows used in these experiments were captured with mist nets from wintering populations near Manhattan, Kansas, and held, four or five per cage, in small cages ($51 \times 27 \times 27$ cm or $23 \times 25 \times 41$ cm). Illumination was by overhead fluorescent lamps, sometimes in combination with incandescent lamps; extraneous light was excluded. Food (a vitamin- and mineral-enriched chick-starter crumble supplemented with commercially prepared parakeet foods) and water were freely available. Temperature varied within a few degrees of 21°C . Sex was determined by laparotomy under Nembutal anesthesia (Donovan, 1958) prior to each experiment. Immediately after sacrifice by decapitation, ovaries were removed and placed in an aqueous solution of acetic acid, formalin, and ethanol (AFA); 5 days thereafter they were transferred to 70 percent ethanol. Ten days after sacrifice, ovaries were freed of extraneous tissue and weighed to the nearest 0.01 mg on a torsion balance. Logarithmic ovarian growth-rate constants and their 95 percent confidence intervals were estimated using formulae described by Simpson et al. (1960).

RESULTS AND DISCUSSION

A rectilinear relationship exists between the logarithm of ovarian weight and time (in days) when photosensitive Tree Sparrows, held on 8L since capture, are exposed to constant daily photoperiods of 20, 15, or 11 hours (20L, 15L, or 11L) for at least 35, 42, or 56 days, respectively (Figure 1). That relationship, which depicts the approximate temporal course of photoinduced ovarian growth and defines its rate, may be expressed algebraically as

$$\log W_t = kt + \log W_0 \quad (1)$$

where W_t is ovarian weight in mg after t days on 20L, 15L, or 11L; W_0 is ovarian weight at day 0; and k is the logarithmic ovarian growth-rate

TABLE 1
RATE OF OVARIAN GROWTH (k) AS A FUNCTION OF DAYLENGTH

Group	Daylength, hours	k^1 , days ⁻¹	log W_0^2
PSL-20	20	0.025 ± 0.004 (34)	0.567 ± 0.048 (34)
PSL-15	15	0.022 ± 0.004 (31)	0.597 ± 0.053 (31)
PSL-11	11	0.005 ± 0.002 (41)	0.615 ± 0.035 (41)
PSS-42	8	0.002 ± 0.005 (14)	0.560 ± 0.098 (14)
PSS-56	8	0.001 ± 0.003 (13)	0.617 ± 0.076 (13)

¹ Logarithmic ovarian growth-rate constant ± 95 percent confidence interval. For groups PSL-20, -15, and -11, k is estimated for days 0-35, 0-42, and 0-56, respectively. For groups PSS-42 and -56, k is estimated for days 0-42 and 0-56, respectively. Number of birds in each sample is indicated in parentheses.

² Ordinate-intercept (equation 1) ± 95 percent confidence interval. Number of birds in each sample is indicated in parentheses.

constant (= coefficient of regression; see Figure 1) in days⁻¹. On the other hand, when photosensitive Tree Sparrows are continued on 8L in short-term experiments, ovarian weight is ostensibly independent of time (Table 1; Figure 1).

Rate of photoinduced ovarian growth (k) in Tree Sparrows is examined as a function of daylength (p) in Figure 2. Although it is apparent that k depends on p , at least over part of the range, the real relationship between k and p remains somewhat uncertain for several reasons: (1) Relatively small changes in ovarian weight with respect to natural variability make estimation of k difficult in short-term experiments when p is short; (2) an approximately fourfold increase in k over the interval $p = 11-15$ hours, coupled with a lack of data for intermediate values of p , makes charting the rate of change in k difficult; and (3) k may be slightly underestimated when $p = 11$ hours if, as in some male passerines (Farner, 1962), photosensitivity increases with retention on 8L. (Because of inadequate numbers of Tree Sparrows in one year and scheduling problems in another, it was necessary to determine k for PSL-11 birds some 6-7 weeks earlier on a calendar basis than for PSL-15 and -20 birds.) For these reasons, the curve in Figure 2, fitted to the points by eye, should be regarded as approximate, especially over the intervals $p = 8-11$ hours and $p = 11-15$ hours. In birds of equal photosensitivity, the rate of ovarian growth induced by 15L is not significantly different from that induced by 20L.

For males of most photoperiodic species, there is a complete daily photoperiod below which testicular growth is not induced as well as one that induces testicular growth maximally. Between those limits,

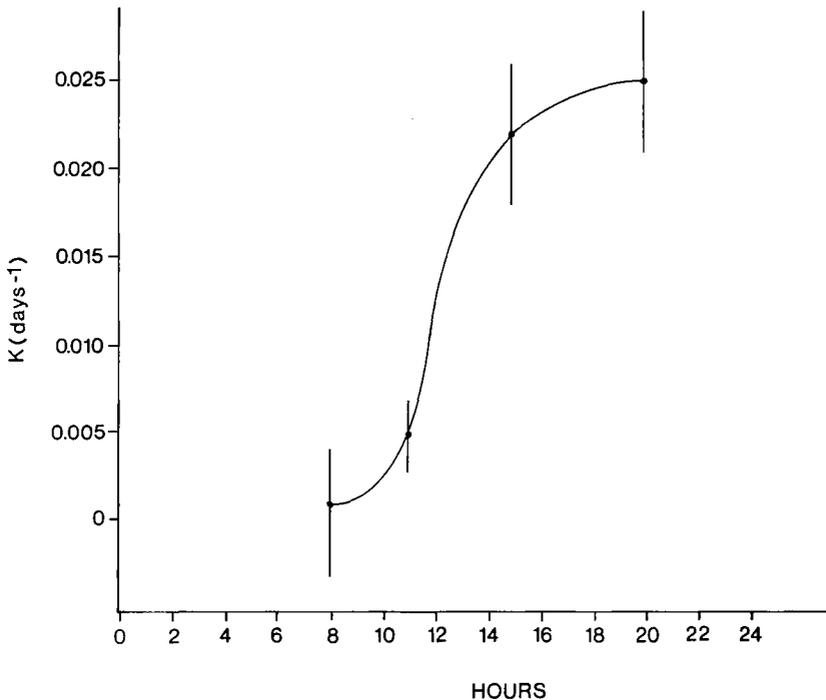


Figure 2. Rate of ovarian growth (K) as a function of daylength (hours). Vertical lines represent 95 percent confidence intervals. The estimate at 8 hours is based on group PSS-56 (Table 1).

rate of testicular growth (k) varies with daylength (p), and k is sometimes directly proportional to p . These principles have emerged from quantitative examinations of photoperiodic testicular responses of several avian species (see Lofts et al., 1970); our data on female Tree Sparrows raise the possibility that similar principles may apply to photoperiodic ovarian responses and, further, that differences between photoperiodic testicular and ovarian responses may be primarily quantitative, at least initially. The latter possibility seemingly has been confirmed, for when photosensitive male and female Tree Sparrows (Table 1; cf. Wilson and Hands, 1968), White-crowned Sparrows (Farner et al., 1966), or Japanese Quail (Follett and Farner, 1966; Follett and Sharp, 1969) are exposed to 20L at approximately the same time of year, gonadal growth initially follows a log-linear function of time, but k for testicular growth is two to four times greater than k for ovarian growth, which for Tree (Table 1) and White-crowned (Farner et al., 1966) Sparrows is 0.025 and 0.027 days⁻¹, respectively, when $p = 20$ hours. The rate of ovarian growth in

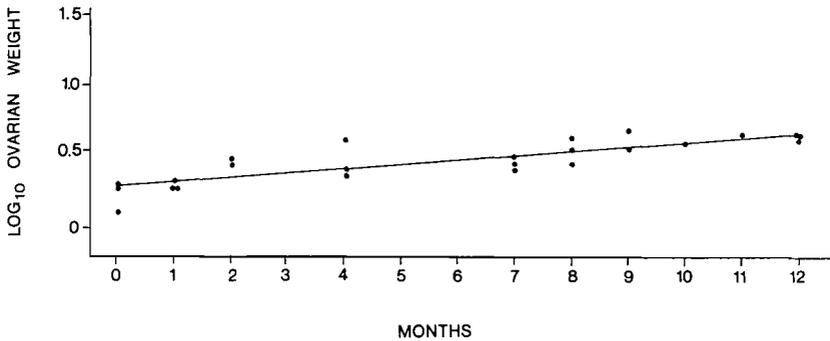


Figure 3. Relationship between ovarian weight and time in initially photorefractory Tree Sparrows held 0-12 months on 8-hour daily photoperiods. The regression line was obtained by the method of least squares.

Japanese Quail exposed to similar photoperiodic conditions (Follett and Farner, 1966; Follett and Sharp, 1969) is two to three times more rapid. Logarithmic growth continues in White-crowned Sparrows (Farner et al., 1966) and Japanese Quail (Follett and Sharp, 1969) until ovarian weights of about 50 and 100 mg, respectively, are achieved. Although equation (1) is valid for Tree Sparrows until ovarian weight reaches approximately 45 mg (Figure 1), its validity beyond 45 mg has not been established.

As noted, ovarian weight did not increase significantly when photosensitive Tree Sparrows were continued on 8L in short-term (42- or 56-day) experiments (Table 1; Figure 1). However, when initially photorefractory Tree Sparrows were transferred from 20L to 8L and sacrificed over the course of a year, it became apparent that ovarian growth did occur during retention on 8L (Figure 3). Such growth showed good conformance with equation (1), but its rate ($k = 0.00095 \pm 0.00026$ days⁻¹) was very slow; an increase from 4 to 45 mg, as might occur within 5 weeks after transferring photosensitive birds to 20L, would require approximately 3 years. It should be emphasized that ovarian growth in birds on 8L was similar, except for rate, to that in birds on 11L, 15L, or 20L (cf. Figures 1 and 3), even though birds on 8L (unlike those on 11L, 15L, or 20L) were initially photorefractory (see Materials and Methods). As photosensitivity doubtlessly gradually increased from zero (photorefractoriness) during the year (see Farner, 1964a), ovarian growth occurred apparently independently of physiological state. Non-photoperiodic ovarian growth—i.e. that which is independent of physiological (photorefractory vs. photosensitive) state or daylength—also has been noted in White-crowned Sparrows (Farner et al., 1966). In first-

TABLE 2
 RATE OF OVARIAN GROWTH (k)
 IN PINEALECTOMIZED AND SHAM-PINEALECTOMIZED TREE SPARROWS
 EXPOSED TO 8 HOURS OF CONTINUOUS OR DISCONTINUOUS LIGHT PER DAY

Group ¹	Light (L) - dark (D) schedule, hours	k^2 , days ⁻¹	Days
PC	8L ³ -16D	0.002 ± 0.003 (11)	76
SPC	8L ³ -16D	0.000 ± 0.001 (13)	100
PD	6L ⁴ -11D-2L ⁵ -5D	0.012 ± 0.006 (8)	49 ⁶
SPD	6L ⁴ -11D-2L ⁵ -5D	0.008 ± 0.003 (11)	79 ⁶

¹ Prefixes P and SP refer to pinealectomy and sham pinealectomy, respectively.

² Logarithmic ovarian growth-rate constant ± 95 percent confidence interval. Number of birds in each sample is indicated in parentheses.

³ 08:30-16:30 CST.

⁴ 08:30-14:30 CST.

⁵ 01:30-03:30 CST.

⁶ Ovarian growth approximated a logarithmic function of time for number of days indicated (see Figures 4 and 5).

year birds, and to a lesser extent in adults, ovarian growth, which began in midsummer during photorefractoriness, continued at a slow rate through early winter and, then, after transfer to 8L. The contribution of non-photoperiodic ovarian growth to overall ovarian development in both Tree and White-crowned Sparrows is minimal, especially in highly photosensitive birds exposed to long daily photoperiods. However, its occurrence emphasizes the necessity of determining ovarian weight at the beginning of the interval over which k is to be estimated, for to assume that ovarian weight is maintained at some "resting" value during retention on short days is clearly invalid.

Differences in rate of ovarian growth when $p = 8, 11,$ or 15 (or 20) hours indicate that photosensitive females can measure, within limits, the duration of the daily photoperiod. Time measurement in avian photoperiodic gonadal responses is presumed to be effected through an endogenous circadian oscillation in photosensitivity. According to that idea, which derives support from the investigations of Hamner (1963, 1964, 1966) and others on male passerines and from the study of Follett and Sharp (1969) on male and female Japanese Quail, a long day is gonadostimulatory not because it exceeds a critical length, but because a portion of it illuminates a photoinducible phase of a circadian rhythm in photosensitivity. By the same token, a short day is nonstimulatory because light and photoinducible phase are not coincident. We found in a preliminary test that, although 8 hours of continuous light in a 24-hour cycle (6L-2L-16D) failed to induce ovarian growth in either

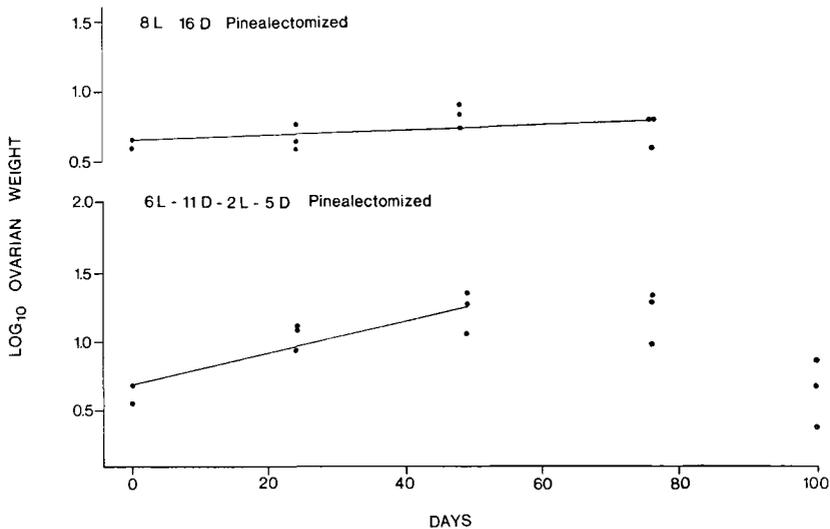


Figure 4. Relationship between ovarian weight and time in pinealectomized Tree Sparrows exposed to 8 hours of continuous or discontinuous light per day. Regression lines were obtained by the method of least squares.

pinealectomized or sham-pinealectomized Tree Sparrows (Figures 4 and 5), 8 hours of discontinuous light in a 24-hour cycle (6L-11D-2L-5D) stimulated ovarian growth at a slow rate for 49 days in pinealectomized birds and for 79 days in sham-pinealectomized birds (Figures 4 and 5; Table 2). These observations are consistent with the notion that an endogenous periodicity in photosensitivity is entrained to a 24-hour cycle with coincidence between light and photoinducible phase after termination of the main (6-hour) photoperiod (or after termination of the 2-hour photoperiod if "phase-jumping" occurred) and suggest that the circadian oscillator does not operate through the pineal body. In addition, the decline in ovarian weights of both pinealectomized and sham-pinealectomized birds at 100 days to or near initial values suggests that long-term exposure to 6L-11D-2L-5D, as to conventional long days, may cause ovarian regression.

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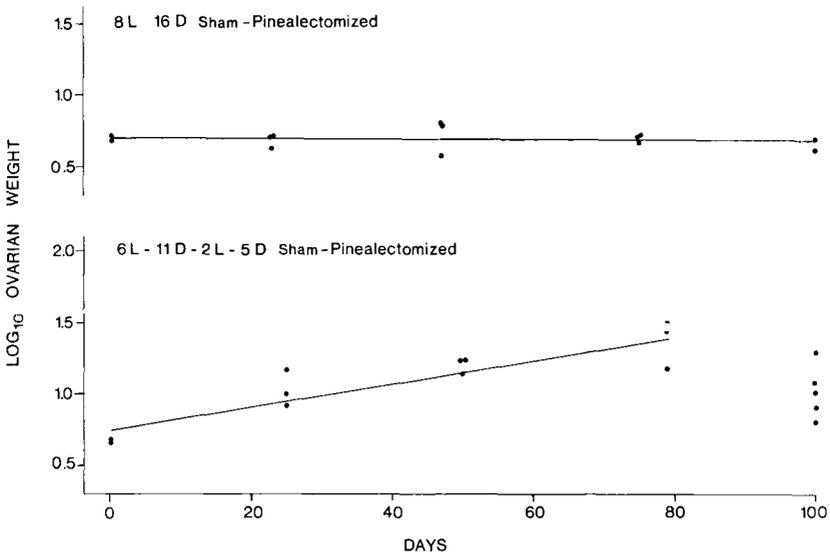


Figure 5. Relationship between ovarian weight and time in sham-pinealectomized Tree Sparrows exposed to 8 hours of continuous or discontinuous light per day. Regression lines were obtained by the method of least squares.

SUMMARY

Ovarian growth in photosensitive Tree Sparrows exposed to 20-, 15-, or 11-hour daily photoperiods, after being held on 8-hour daily photoperiods for several months, approximated a logarithmic function of time for at least 35, 42, or 56 days, respectively; photosensitive Tree Sparrows continued simultaneously on 8-hour daily photoperiods for 42 or 56 days failed to show detectable ovarian growth. Rate of photoinduced ovarian growth varied with daylength and was four to five times slower in birds exposed to 11-hour daily photoperiods than in birds exposed to 15- or 20-hour daily photoperiods. Though the rate at 15 hours was exceeded by that at 20, the difference was not statistically significant. An endogenous circadian oscillation in photosensitivity of the response mechanism was suggested by a slow rate of ovarian growth in both pinealectomized and sham-pinealectomized Tree Sparrows exposed to 6-hour daily photoperiods with an additional 2-hour light period beginning 11 hours after onset of darkness. Nonphotoperiodic ovarian growth, of minor importance quantitatively and of no apparent functional significance, was detected in initially photorefractory Tree Sparrows held up to 12 months on 8-hour daily photoperiods.

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