

## ANNUAL GONADAL CYCLES AND PITUITARY GONADOTROPINS IN *ZONOTRICHIA LEUCOPHRYS GAMBELII*

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The essential role of the anterior pituitary gland in the development and the function of the avian gonad has been recognized for more than three decades. However, little is known concerning annual variation of gonadotropic function in species with marked gonadal cycles. The only extensive information available appears to be that of Greeley and Meyer (1953) who investigated the gonadotropic activity of the anterior pituitary in relationship to the annual testicular cycle in Ring-necked Pheasants (*Phasianus colchicus*) held in captivity under semi-natural conditions.

In order to extend our understanding of the regulation of the hypothalamo-hypophysio-gonadal axis in birds under natural conditions, we have investigated the annual cycle of pituitary gonadotropic potency in relation to gonadal cycles in free populations of the White-crowned Sparrow, *Zonotrichia leucophrys gambelii* Nuttall. Such data are also indispensable in the interpretation of the results of experiments conducted under controlled conditions. The annual testicular cycle of White-crowned Sparrows has been investigated by Blanchard and Erickson (1949), by Oakeson (1954), and by Oakeson and Lilley (1960). Our data extend these investigations, and describe also the annual ovarian cycle.

### MATERIALS AND METHODS

Migrating and wintering White-crowned Sparrows were either shot or caught with mist nets near Pullman, Washington (46.5° N latitude). Breeding and post-breeding birds were collected by these methods near Fairbanks, Alaska (64.8° N). Net-caught birds were killed by decapitation; the pituitary glands were dissected free within one minute after death and placed individually in separate vials containing about 3 ml of dry acetone. The acetone was subsequently replaced twice with fresh acetone, and the glands were stored at 0° C until assayed.

The gonads and, in breeding females, the oviducts were dissected free and fixed in alcohol-formalin-acetic acid mixture for 48 hours, after which they were transferred to 70 per cent ethanol. Eggs or ova contained in the oviduct were removed at this time. After at least five days in ethanol, the specimens were carefully debrided of extraneous tissue (leaving the oviducal ligaments intact), blotted, and weighed within 15 seconds on torsion balances accurate to  $\pm 0.02$  mg for specimens weighing 25 mg or less or to  $\pm 0.1$  mg for heavier specimens. Because of the impossibility of uniform blotting of large ovaries and oviducts, the wet weights of these organs are accurate to only about  $\pm 5$  per cent. At the time of weighing of the ovaries, the maximum diameter of the three largest follicles was measured with an accuracy of  $\pm 0.05$  mm.

Pituitary glands were collected from wintering and migrating birds near Pullman during 1964 and 1965, and from breeding birds near Fairbanks in the spring of 1965. Specimens of sex organs were collected over a longer span of time, beginning in 1952. Since we cannot detect any significant differences among the testicular cycles of different years, the data have been combined except as otherwise noted. This is true also for ovarian weights except during the breeding season; we have therefore combined the data obtained during several years from migrating and winter-resident females.

For assay of gonadotropin, a modification of the method of Breneman, Zeller, and Creek (1962) was used. Details of this modification are given by Follett and Farner (1966). The assay is based on the gonadotropin-induced increase in the uptake of radioactive phosphorus by the testes of chicks of the domestic fowl. A preparation of luteinizing hormone (NIH-LH-S7) was used as a standard, and the results of the assays are expressed as  $\mu\text{g}$  LH equivalents per gland. The data were subjected to a full analysis of variance; mean values are given together with the 95 per cent confidence limits and the index of precision,  $\lambda$ . The value of  $\lambda$  is the ratio of the standard deviation to the slope of the dose-response line (Bliss, 1952). The values obtained show that the procedure compares favorably with other methods of gonadotropin bioassay. In most cases, pituitary glands from several sparrows were pooled for assay. The glands from Alaskan birds, however, was assayed individually. In these cases, the values given are the means and 95 per cent confidence limits from the several individual assays.

In addition to samples of the free population, we examined the gonadal status and pituitary gonadotropic potency of White-crowned Sparrows retained through the summer at Pullman. These birds were exposed to local weather and photoperiod in large outdoor aviaries under conditions previously described in detail (Farner and Wilson, 1957).

## RESULTS AND DISCUSSION

### THE TESTICULAR CYCLE

The testes are in a fully regressed or undeveloped condition when White-crowned Sparrows reach southeastern Washington in September (fig. 1; table 1). There were no statistically significant changes in testicular weight during the winter, although there appear to be trends in the means toward midwinter minima in both adults (November) and first-year birds (December-January). In general, first-year birds have smaller testes in winter than do adults, although the differences are significant ( $P < 0.05$ ) in our samples only in September, December, and February. In *Agelaius phoeniceus* (Wright and Wright, 1944) and *Quiscalus mexicanus* (Selander and Hauser, 1965) the weight of the testes of first-year birds also averages less than that of adults in winter.

The mean testicular weight of adult White-crowned Sparrows in Alaska in late August is the same as that of adults at Pullman in September. However, in first-year birds the mean testicular weight of Alaskan birds is significantly less ( $P < 0.001$ ) than that of migrants in September near Pullman. Although this difference may in part reflect a continued postjuvenile growth of the testes, it is also possible that it results from the sampling of different geographic populations. We do not know the migratory itinerary or the location of the wintering area of the population that breeds near Fairbanks. It seems improbable that birds arriving in southeastern Washington in mid-September are derived wholly from the population leaving central Alaska in late August and early September. Therefore, we suspect that our samples of migrants in September come predominantly from populations in which the postjuvenile development of the testes is more rapid or reaches a greater resting level than in the populations at Fairbanks.

Prenuptial increase in testicular weight first was detected in adult White-crowned Sparrows in southeastern Washington in mid-February. A statistically significant increase was noted by the period of 1 to 10 March. Testicular development began

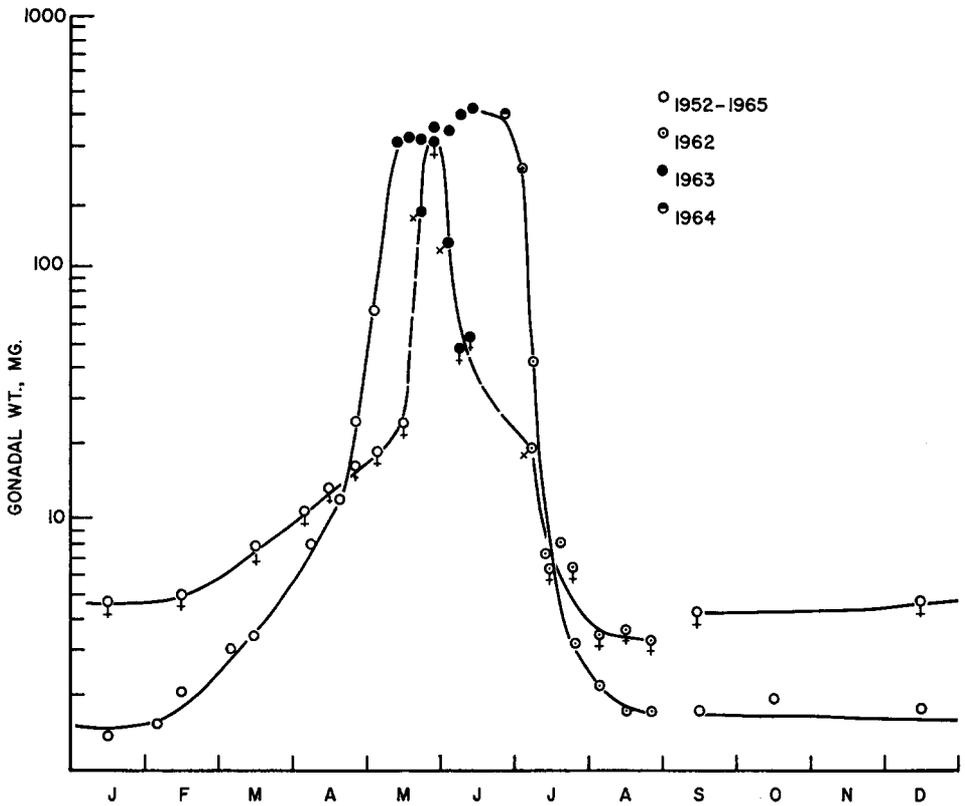


Figure 1. Interrelationship of testicular cycle and ovarian cycle in adult *Zonotrichia leucophrys gambelii* (see table 1, table 4). All symbols not indicating ovaries refer to testes.

slightly later in first-year birds with the first significant ( $P < 0.001$ ) increase between the first two 10-day periods of March. This later initiation of testicular growth in first-year birds resembles the lag reported in *Quiscalus mexicanus* (Selander and Hauser, 1965). It infers a minor difference in the characteristics of the photoperiodic testicular response in these age groups. This may be reflected also in the slightly higher rate of testicular growth (1.2 times the rate for adults) in photostimulated first-year *Z. l. gambelii* (Farner and Wilson, 1957). Hence, under natural conditions, the delayed inception of testicular development in first-year birds appears to be compensated by a greater rate of growth, bringing both groups into reproductive condition at approximately the same time.

In adult *Z. l. gambelii* at Davis, California ( $38.5^{\circ}$  N latitude), testicular enlargement began in mid-February in 1943; at Santa Barbara, California ( $34.4^{\circ}$  N), enlargement began in late February or early March during this year (Blanchard and Erickson, 1949). Taken together with our data for several years at  $46.5^{\circ}$  N, these observations suggest that the inception of testicular growth begins on about the same date through about 12 degrees of latitude. Although this apparent similarity requires additional examination and confirmation, it implies that different geographic populations of *Z. l. gambelii* possess slightly different responses to the annual photo-

TABLE 1  
 TESTICULAR WEIGHTS IN FREE POPULATIONS OF *Zonotrichia leucophrys gambelii*

Period	Adults		Immatures	
	N	Mean $\pm$ SE	N	Mean $\pm$ SE
September	13	1.8 $\pm$ 0.22 mg	57	0.9 $\pm$ 0.06 mg
October	8	2.0 $\pm$ 0.46	21	1.0 $\pm$ 0.16
November	7	0.9 $\pm$ 0.35	17	0.8 $\pm$ 0.10
December	15	1.7 $\pm$ 0.15	21	0.7 $\pm$ 0.08
January	15	1.3 $\pm$ 0.47	23	0.7 $\pm$ 0.07
1-10 February	5	1.5 $\pm$ 0.23	—	—
11-20	11	2.0 $\pm$ 0.19	12	1.0 $\pm$ 0.14
21-28	2	1.4	—	—
1-10 March	9	3.1 $\pm$ 0.43	5	1.0 $\pm$ 0.24
11-20	17	3.4 $\pm$ 0.08	14	2.9 $\pm$ 0.15
21-31	1	2.4	8	5.0 $\pm$ 0.69
1-15 April	9	8.8 $\pm$ 0.58	—	—
16-25	16	11.8 $\pm$ 0.11	—	—
26-30	12	26.4 $\pm$ 2.87	—	—
1- 5 May	6	64.4 $\pm$ 2.89	—	—
11-15 <sup>a</sup>	24	315 $\pm$ 15	—	—
16-20 <sup>a</sup>	32	339 $\pm$ 16	—	—
21-25 <sup>a</sup>	11	316 $\pm$ 25	—	—
26-31 <sup>a</sup>	13	357 $\pm$ 18	—	—
1- 5 June <sup>a</sup>	10	353 $\pm$ 23	—	—
6-10 <sup>a</sup>	1	428	—	—
11-15 <sup>a</sup>	11	442 $\pm$ 31	—	—
1- 5 July <sup>b</sup>	5	254 $\pm$ 12	—	—
6-10 <sup>b</sup>	6	42 $\pm$ 20	3	0.6
11-15 <sup>b</sup>	5	6.9 $\pm$ 1.8	—	—
16-20 <sup>b</sup>	4	8.1 $\pm$ 1.5	10	0.5 $\pm$ 0.07
21-25 <sup>b</sup>	7	3.2 $\pm$ 0.9	6	0.4 $\pm$ 0.04
1-10 August <sup>b</sup>	13	2.2 $\pm$ 0.2	8	0.3 $\pm$ 0.04
11-20 <sup>b</sup>	15	1.7 $\pm$ 0.6	19	0.4 $\pm$ 0.06
21-31 <sup>b</sup>	10	1.7 $\pm$ 0.2	12	0.5 $\pm$ 0.05

<sup>a</sup> 1963 only.

<sup>b</sup> 1962 only.

cycle. In this connection it is noteworthy that Dolnik (1963) found a lower photoperiodic threshold in a southern population (55° N) of *Fringilla coelebs* than in a northern population (60° N). In addition, the rate of testicular growth as a function of photoperiod above the threshold value was less in the southern population. It should be recalled that until the vernal equinox the daily photoperiods in winter are longer at southern stations, and that these differences in *F. coelebs* are in the direction that could be expected to bring different geographic populations into sexual activity on a uniform schedule throughout the area. A similar relationship may prevail in *Z. l. gambelii*.

TABLE 2  
 TESTICULAR WEIGHTS OF *Zonotrichia leucophrys gambelii* FROM HART'S PASS,  
 OKANOGAN COUNTY, WASHINGTON

Dates	N	Mean testicular wt. ( $\pm$ SE), mg	Testicular wt., Alaska, mg <sup>a</sup>
29 June 1957	3	460	420
6 July 1955	5	428 $\pm$ 40.6	200
14 July 1956	5	425 $\pm$ 30.1	8
18 July 1961	2	436	6

<sup>a</sup> Approximate testicular weight in population at Fairbanks, interpolated from table 1.

Approximately comparative data are available for two other forms of *Zonotrichia*. In nonmigratory *Z. l. nuttalli*, gonadal enlargement begins in January at Berkeley, California (37.6° N) and at Santa Barbara (Blanchard, 1941; Oakeson and Lilley, 1960). On the basis of histological examination and analysis of variation in testicular volume, Blanchard (1942) deduced evidence of considerable annual variation in the date of inception and the rate of testicular growth in this form. In *Z. l. pugetensis*, a short-range migrant, testicular growth begins in the span from late February to mid-March and, according to Blanchard (1941), shows somewhat less annual variation than *Z. l. nuttalli*. Miller (1960) has noted that *Z. l. nuttalli* and *Z. l. pugetensis* appear to be less dependent on the annual photoperiod as a timer of the reproductive cycle than do migratory forms of *Zonotrichia* that breed at high latitudes. Such an emphasis upon endogenous "circennial" rhythms or upon sources of environmental information that are annually less precise than the photoperiod could be expected to produce a less regular annual schedule of reproduction than that found in *Z. l. gambelii*.

The phase of most rapid testicular growth in *Z. l. gambelii* occurs principally during the migratory period (fig. 1, table 1). Testicular weight probably continued to increase slightly after the arrival of the birds in the breeding area. Although we lack samples from the earliest arrivals in 1963 (about 7 May), an analysis of testicular weights from 13 May to 15 June reveals a highly significant increase in weight ( $r = 0.404$ ;  $P < 0.001$ ) at about 4 mg per day ( $b \pm S_b = 4.2 \pm 0.92$ ).

Full functional status of the testes, as indicated by the plateau of testicular weight and by the presence of the cloacal papilla, is maintained for 40 to 50 days at Fairbanks. Testicular regression occurs very rapidly during the period in which broods are being fledged, beginning in late June and early July. Data from a small series of samples collected at Hart's Pass, Okanogan County, Washington (49° N), indicate that the schedule of regression is not uniform throughout the breeding area (table 2). At Hart's Pass during two different years testicular size was maintained at maximum for at least two weeks beyond the onset of regression at Fairbanks.

Captives retained through the summer in outdoor aviaries at Pullman attained full testicular size (table 3), but regression began in mid-June, preceding by about two weeks the beginning of regression in Alaska, and by at least a month the beginning of regression at Hart's Pass, only 2.5 degrees of latitude north of Pullman. This disparity emphasizes the role of factors other than the daily photoperiod in the maintenance of sexual activity under natural conditions.

Postnuptial molt, first detected in males on 7 July in 1962, at Fairbanks, was correlated with the period of testicular regression. In general, molt did not begin

TABLE 3  
TESTICULAR WEIGHTS OF CAPTIVE *Zonotrichia leucophrys gambelii* AT PULLMAN, WASHINGTON

Dates	N	Mean testicular wt. ( $\pm$ SE), mg	Testicular wt., Alaska, mg <sup>a</sup>
14-20 May	5	293 $\pm$ 45.0	340
24-31 May	16	364 $\pm$ 28.0	360
3 June	5	353 $\pm$ 16.4	360
28 June	2	80.1	400
6 July	5	8.4 $\pm$ 1.89	250
15 July	9	4.2 $\pm$ 0.71	8
30 July	5	2.5 $\pm$ 0.21	3

<sup>a</sup> Approximate testicular weight in population at Fairbanks, interpolated from table 1.

until testicular weight had decreased to less than 25 mg, although one male of the 31 examined during July was molting the first three primaries of each wing while testicular weight was 128 mg.

It is widely recognized that reproductive activity and molt are usually mutually exclusive. In the few temperate-zone species that have been adequately studied in this respect (e.g., *Agelaius phoeniceus*; Wright and Wright, 1944; Dunson, 1965), a close correlation has been found between gonadal regression and the onset and progress of postnuptial molt. Assenmacher (1958) and Wagner and Müller (1963) attribute this to the dissipation of an inhibition of molt by gonadal steroids.

#### THE OVARIAN CYCLE

Because of the paucity of females in the northern wintering populations of *Z. l. gambelii* (King *et al.*, 1965), our data on the ovarian cycle are fewer than for the testicular cycle. Nevertheless, they are sufficient to show (fig. 1, table 4) that the ovary of first-year birds weighs approximately half that of adults during the postnuptial period and early winter. The ovary of first-year birds continued to grow during the winter, however, and was essentially equivalent in weight to that of adults by January (see table 4, and Farner *et al.*, 1966). The vernal phase of ovarian growth in adults began in February (fig. 1), apparently concurrently with the onset of testicular recrudescence. From experimental investigations we know that the initial phase of ovarian growth, to a weight of about 50 mg, is photoperiodically induced. However, the attainment of full functional status requires additional stimuli that are associated with migration and the breeding area or the removal of some inhibitory component of the cage environment (Farner, 1964; Farner and Follett, 1966; Farner *et al.*, 1966).

Ovarian and oviducal development apparently continues during migration and the early days of residence in the breeding area. In 1963, female *Z. l. gambelii* began to arrive at Fairbanks on 18 May; pairs were formed immediately. Ovarian and oviducal growth continued in the population until late May. The relationships of ovarian development and the beginning of the incubation period are shown in figure 2. The peak of gonadal development preceded the peak of incubation-starts by about five days, or the span required in egg-laying. The mean clutch-size during this year was 4.67 eggs.

In late May the sex organs began a rapid regression that continued through the termination of our field work on 14 June. The terminal phase of ovarian regression is shown in data for 1962 (fig. 1), indicating decline to the resting level by late July.

TABLE 4  
 OVARIAN WEIGHTS IN FREE POPULATIONS OF *Zonotrichia leucophrys gambelii*

Period	Adults		Immatures	
	N	Mean $\pm$ SE	N	Mean $\pm$ SE
September	16	4.3 $\pm$ 0.28 mg	35	2.2 $\pm$ 0.17 mg
October	—	—	24	2.4 $\pm$ 0.21
November	—	—	—	—
December	9	4.8 $\pm$ 0.67	45	3.8 $\pm$ 0.22
January	6	4.7 $\pm$ 0.51	2	6.2
February	3	5.0	2	4.4
March	2	7.8	—	—
1-10 April	2	10.8	—	—
11-20	2	13.2	—	—
21-30	17	16.2 $\pm$ 0.94	—	—
1-10 May	24	18.2 $\pm$ 1.03	—	—
11-20	5	24.1 $\pm$ 8.63	—	—
20-25 <sup>a</sup>	12	166 $\pm$ 61	—	—
26-31 <sup>a</sup>	13	326 $\pm$ 86	—	—
1- 5 June <sup>a</sup>	13	124 $\pm$ 33	—	—
6-10 <sup>a</sup>	2	47.7	—	—
11-15 <sup>a</sup>	9	52.7 $\pm$ 3.87	—	—
1-10 July <sup>b</sup>	7	19.2 $\pm$ 4.02	—	—
11-20 <sup>b</sup>	11	6.5 $\pm$ 0.57	—	—
21-31 <sup>b</sup>	5	6.5 $\pm$ 0.65	8	1.0 $\pm$ 0.13
1-10 August <sup>b</sup>	4	3.4 $\pm$ 0.80	4	1.6 $\pm$ 0.16
11-20 <sup>b</sup>	9	3.6 $\pm$ 0.42	2	0.7
21-31	6	3.3 $\pm$ 0.59	8	1.8 $\pm$ 0.14

<sup>a</sup> 1963 only.

<sup>b</sup> 1962 only.

Although the breeding seasons were phenologically similar in 1962 and 1963, it appears that ovarian regression may have occurred somewhat later in 1962 than in 1963 (see fig. 1).

The temporal relationship between the testicular and ovarian cycles is shown in figure 1. The rapid increase in ovarian weight above about 50 mg can be attributed to the growth of ovarian follicles during the phase of nest-building and oviposition. It is noteworthy that testicular size was maintained essentially at a maximum from the time that males arrived in the breeding area, 10 to 15 days in advance of the females, until the broods were fledged. This long maintenance of spermatogenesis relative to ovarian maturation is presumably an adaptation that allows a considerable latitude of accommodation to a female gonadal cycle that is affected more strongly than that of the male by local ecological conditions. Its significance as a pre-adaptation in case of first-nest failure is also apparent.

In *Z. l. gambelii* maintained in outdoor aviaries through the spring and summer at Pullman, ovarian development only very rarely progresses to a fully functional condition. We have found single eggs in the aviaries only twice in 15 years of observation, and with equal rarity under conditions of artificial photostimulation

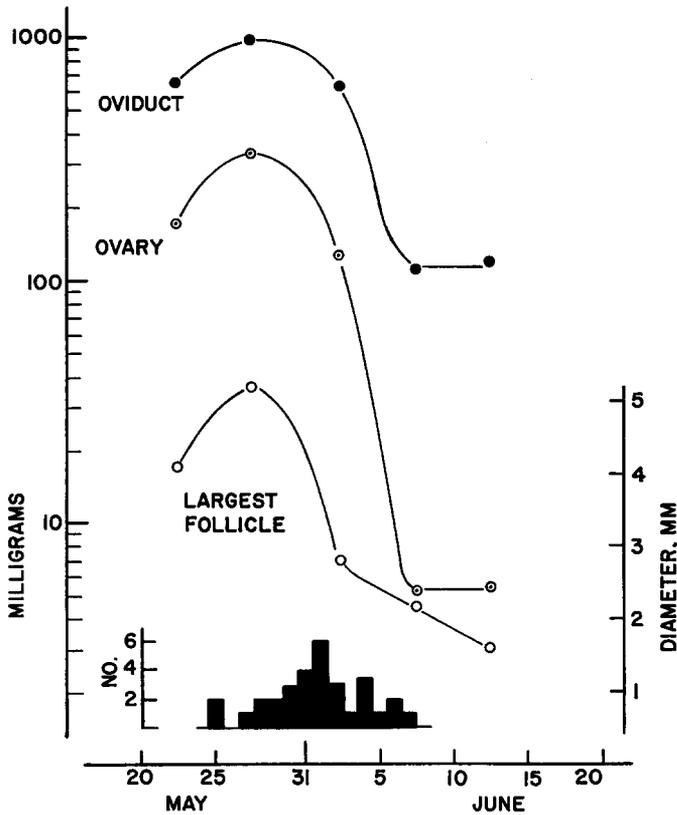


Figure 2. Interrelationship of mean ovarian and oviducal weight (scale at left), mean diameter of largest ovarian follicle (scale at right), and frequency of incubation starts (histogram with scale at left) in *Zonotrichia leucophrys gambelii* at Fairbanks, Alaska (1963).

(e.g., Merkel, 1963). The mean ovarian weight in five birds in the outdoor aviary on 3 June 1965 was  $30.2 \pm 2.8$  mg. In a group of seven females collected as field controls at Fairbanks during 26 May–1 June 1965, mean ovarian weight was  $327 \pm 129.1$  mg (table 5). It is obvious that the phase of follicular enlargement was not initiated in the captives. In a later sample taken from the aviaries on 6 July 1965 mean ovarian weight was  $6.8 \pm 0.55$  mg, representing a regression from the condition of a month earlier. We do not have concurrent field controls for this group, but the mean ovarian weight of a comparable sample from Fairbanks in 1962 was 19.2 mg (table 1). The role of fear (Phillips, 1964) and of sensory deprivation (Poli-karpova, 1940; Vaugien, 1948; Brockway, 1964) in the blockade of gonadotropic function in female birds requires additional investigation.

The onset and progress of postnuptial molt in females was less regular than in males, and the correlation between gonadal regression and the molt is accordingly less clear-cut. Although molt in the females was first detected slightly earlier (3 July 1962) than in the males, it did not reach maximum intensity until late July, or about two weeks later than in the males. In 26 females examined in July, molt in no case had begun with ovarian weight greater than 25 mg.

TABLE 5  
GONADAL WEIGHT AND PITUITARY GONADOTROPIC ACTIVITY IN *Zonotrichia leucophrys gambelii*

Class	Male				Female			
	Mean testicular wt. ( $\pm$ SE), mg	N	Gonadotropin* $\mu$ g/gland	N $\lambda$	Mean ovarian wt. ( $\pm$ SE), mg	N	Gonadotropin* $\mu$ g/gland	N $\lambda$
Autumn migrants (22 September)								
Adult	2.0 $\pm$ 0.07	16	0.7 (0.4-1.4)	18 0.097	4.4 $\pm$ 0.35	15	1.1 (0.5-1.9)	11 0.097
Immature	1.0 $\pm$ 0.07	45	0.7 (0.4-1.0)	23 0.128	2.6 $\pm$ 0.21	20	0.5 (0.3-0.7)	20 0.097
Winter-resident adults (14 January)								
	1.1 $\pm$ 0.09	21	3.0 (2.6-3.6)	17 0.098	4.9 $\pm$ 0.53	5	3.1 (2.3-4.1)	5 0.118
Spring migrants (4 May)								
	47.7 $\pm$ 4.00	13	11.1 (9.4-14.4)	4 0.116	18.6 $\pm$ 1.50	14	11.1 (8.4-14.7)	5 0.156
Breeding, Alaska (26 May- 1 June)								
	408 $\pm$ 14	23	14.8 (13.5-16.1)	12 —	327 $\pm$ 129	7	9.8 (7.5-12.1)	7 —
Captives, Pullman (3 June)								
	353 $\pm$ 16	5	16.9 (12.6-22.8)	2 0.127	30.2 $\pm$ 2.80	5	2.7 (1.6-4.4)	4 0.181
Captives, Pullman (6 July)								
	8.4 $\pm$ 1.89	5	6.3 (4.9-7.6)	4 0.094	6.8 $\pm$ 0.55	7	4.3 (3.5-5.3)	4 0.100

\* Gonadotropic potency estimated by single (2 + 2) assay from pooled sample of glands. Potency for Alaskan groups calculated as mean of individual estimates for each gland. The 95 per cent confidence limits are given in parentheses.

#### PITUITARY GONADOTROPIC POTENCY

Variation in the quantity of gonadotropin per gland obviously can result from variation in the rate of synthesis, the rate of release, or both. As an approximation, the rate of gonadotropin release can be regarded as proportional to the rate of gonadal growth. This makes it possible to reach some provisional conclusions about the relative rates of release and of synthesis during various phases of the annual cycle.

Pituitary gonadotropic potency was at an annual minimum in migrant White-crowned Sparrows in September (table 5). There were no significant differences between males and females or between adult and first-year birds at this time. By midwinter, the mean pituitary gonadotropin levels had approximately tripled ( $P < 0.05$ ) in both sexes, but without significant increases in gonadal weight. Presumably, this signifies a continued low level of synthesis with little or no release, and a consequent increase in storage concentration. Greeley and Meyer (1953) describe a post-nuptial increase of pituitary gonadotropins in male Ring-necked Pheasants, although the resurgence occurred in August and September in this species.

In samples of White-crowned Sparrows taken in early May there was an additional three-fold to four-fold increase in gonadotropic potency above the winter level.

This accompanied the period of accelerating gonadal growth (see fig. 1) and must have involved an augmentation of synthesis to exceed the increased rate of gonadotropin release. In birds taken during the period of egg-laying in Alaska there were no significant increases of gonadotropin levels above those of May, although gonadal weight and activity were maximal at this time. We can assume that pituitary gonadotropins were at a steady-state level, with maximum rates of synthesis and release of hormone(s). There was no correlation between gonadal weight and pituitary gonadotropic potency in individual birds.

Mean gonadal weight and gonadotropic potency in captive males at Pullman in early June did not differ significantly from levels in the feral population. However, in captive females the gonadotropic potency was only about one-fourth ( $P < 0.01$ ) that of breeding females in Alaska, and ovarian growth was arrested. The significant decline of gonadotropic potency from the level observed in spring migrants suggests that reproductive failure in captivity may involve blockade of both synthesis and release of gonadotropins.

In captive males sampled during the period of gonadal regression in July, gonadotropic potency had significantly ( $P < 0.01$ ) decreased. The rapid decline of testicular weight implies a cessation of gonadotropin release, and the concurrent reduction of pituitary gonadotropin indicates that synthesis of the hormone(s) was also drastically reduced or stopped. In contrast, cytological evidence in the Pekin duck suggests that gonadotropin release ceases suddenly at the onset of testicular regression, but that synthesis may continue for several weeks into the refractory period, producing a high level of hormone storage (Tixier-Vidal *et al.*, 1962).

#### SUMMARY AND CONCLUSIONS

The annual cycles of gonadal weight and pituitary gonadotropic potency were investigated in free populations of *Zonotrichia leucophrys gambelii* in central Alaska (breeding) and in southeastern Washington (migrating or wintering). Some observations were made also on captives retained during the breeding season in outdoor aviaries in Washington.

Pre-nuptial increase of testicular weight begins in mid-February in adults. In first-year birds testicular development begins significantly later. Ovarian enlargement also begins during February. Testicular maturation progresses essentially to completion during the period of migration, and testicular weight increases only slightly after arrival of the birds in the breeding area. Females have fully developed ovaries and oviducts at the time of their arrival in the breeding area about 15 days later than the first males. There is a minor increase in ovarian and oviducal weight to a maximum coinciding with the peak of oviposition in late May. This is followed by rapid regression of both ovary and oviduct. Testicular weight is maintained at a maximum until the broods were fledged in early July, and then decreases rapidly to minimal levels reached in early August. The onset of post-nuptial molt in both sexes is correlated with gonadal regression.

In captives retained outdoors in southeastern Washington, the testicular cycle is indistinguishable from that of the free-living birds in late winter and spring, but regression begins about two weeks earlier than in the natural population in Alaska. In captive females, ovarian development is arrested at a weight of about 40 mg, before the onset of significant follicular enlargement.

Pituitary gonadotropin level is at an annual minimum in free-living birds during autumn migration. During winter, preceding detectable gonadal growth, the gonado-

tropin level approximately triples, suggesting a low level of synthesis with little or no release, and consequently a storage during this period. During the phase of accelerating gonadal growth in May, gonadotropin levels again approximately triple, presumably as a consequence of increase in both synthesis and release of the hormone(s). There is no additional increase in gonadotropic potency in birds in Alaska during the breeding season. In captive males, gonadotropic potency is the same as that of free-living males in June; during testicular regression in captives in July the gonadotropin level is significantly decreased, indicating that the withdrawal of gonadotropic stimulus includes decrease of synthesis as well as cessation of hormone release. In captive females, pituitary gonadotropic potency is only about one-fourth that of reproductively active females in Alaska, suggesting that the reduced gonadotropic function in captivity involves a failure or partial failure of gonadotropic synthesis as well as release.

Combined with the results of experimental investigations, our data show that testicular development to a fully functional status can be achieved by photostimulation, but that the period of testicular function is prolonged by stimuli associated with the breeding area. In females, in contrast, only the initial stages of ovarian development can be photoperiodically induced in cages. The full development of the ovary and oviduct does not occur with significant frequency in captivity. This gonadotropic blockade may result from fear or from deprivation of stimuli encountered during migration and in the breeding area.

#### ACKNOWLEDGMENTS

This investigation was supported by funds provided for Medical and Biological Research by State of Washington Initiative Measure No. 171, by a Research Career Development Award from the National Institute of Arthritis and Metabolic Diseases (5-K3-AM-18 370), and by grants from the National Institutes of Health (NB 01353, NB 04587) and the National Science Foundation (GB 1380). We are indebted to the Endocrinology Study Section, National Institutes of Health, for supplies of NIH-LH and NIH-FSH, and to Brina Kessel and Laurence Irving for making available the facilities of their laboratories at the University of Alaska.

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