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The Annual Cycle of Plasma irLH and Steroid Hormones in Feral Populations of the White-crowned Sparrow, Zonotrichia leucophrys gambelii

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ABSTRACT

Plasma levels of immunoreactive luteinizing hormone (irLH) and 5 steroid hormones have been measured through the course of the winter breeding season and vernal and autumnal migration in both sexes of the White-crowned Sparrow, *Zonotrichia leucophrys gambelii*. Birds were captured in mist nets or traps on wintering grounds in Washington and California and on the breeding area in the vicinity of Fairbanks, Alaska. Blood samples were collected from a wing vein as soon after capture as possible. Birds were then individually marked with a unique combination of 1 or 2 colored leg bands for identification in field observations and laparotomy performed to assess the reproductive state. After sampling, all birds were released for subsequent observation and recapture.

During autumn and winter, the levels of irLH and sex hormones in the plasma are low in both sexes. In spring (April and May), they begin to increase becoming maximal coincidentally with maximal gonadal weights, establishment of territory, mating and courtship. During incubation and feeding of young, plasma irLH and sex steroid levels decline to basal levels, but there is only a gradual decrease in weight of the testes. By the time parent birds are feeding fledglings, there is a rapid involution of the gonads followed closely by the onset of postnuptial molt.

Plasma levels of corticosterone are high in both sexes in January, but much lower in the early spring. In males during vernal migration, there is a dramatic increase to a high level that persists throughout the breeding season. In females, plasma hormone levels remain low during vernal migration, but then increase during the time of ovulation and oviposition. Basal levels in plasma corticosterone occur in both sexes during postnuptial molt. In contrast to vernal migration, a slight increase in plasma corticosterone occurs during autumnal migration in females, but not in males.

INTRODUCTION

We (Wingfield and Farner, 1976) have recently described techniques for procurement of serial blood samples from individually marked live birds in the field. These methods have the distinct advantage of permitting a close correlation between the plasma levels of hormones with the phases of the reproductive cycle. Changes in hormone levels in the plasma during the reproductive cycle of *Zonotrichia leucophrys pugetensis* have already been described (Wingfield and Farner, 1977, 1978). We present here information on the changes in plasma levels of luteinizing and sex hormones in *Z. l. gambelii*. Birds of this race migrate as much as 5,000 km between wintering grounds in the southwestern United States and northern Mexico and breeding areas in Alaska (Cortopassi and Mewaldt, 1964). Breeding at such high latitudes, because of the brief summer, is restricted to a single brood per season in comparison with the midlatitude *Z. l. pugetensis* which raises 2 and sometimes 3 broods in the longer summer season of northwest Washington. The natural history of *Z. l. gambelii* has been described in detail by Blanchard and Erickson (1949) and King et al. (1966) and many aspects of the environmental control of reproductive cycles have been examined experimentally (e.g., Farner and Lewis, 1971, 1973). Therefore, a detailed study of changes in plasma levels of hormones in the course of the natural reproductive cycle is important as a test of the results of laboratory experiments and to provide greater insight into the control of the cycle.

MATERIALS AND METHODS

Birds were captured with Japanese mist nets or Potter traps at the Sunnyside Game Refuge, 5 km southeast of Mabton, Yakima County and at Camano Island, Island County, Washington (48°N); at San

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Jose, California (37°N) and in the vicinity of Fairbanks, Alaska (64°N). Methods for the procurement and processing of blood samples in the field have been described by Wingfield and Farner (1976). Briefly, blood is obtained from a wing vein by capillary uptake in heparinized tubes. In Washington, samples were centrifuged in the field and the plasma samples were transported frozen on dry ice to the laboratory where they were stored at −20°C. In California and Alaska, birds were trapped in the vicinity of a power source and freezing facilities. These samples were transported frozen by air to Seattle.

After capture, each bird was banded with a numbered aluminum band (U. S. Fish and Wildlife Service) along with a unique combination of plastic color bands for individual identification in the field. Laparotomy was performed to assess sex and gonadal size; estimated testicular weights are based on comparisons with preserved testes of known weight. Birds were weighed to the nearest 0.1 g and examined for fat reserves recorded on an arbitrary scale, inspected for molt and development of brood patch, cloacal protuberance length (CPL) measured to the nearest mm and then released for subsequent observation and recapture. All of these techniques have been described in detail by Wingfield and Farner (1976).

Plasma immunoreactive luteinizing hormone (irLH) was measured by the double antibody radioimmunoassay of Follett et al (1972) as modified for use on the White-crowned Sparrow (Follett et al, 1974). Levels are expressed as ng/ml and the coefficient of variation of consecutive determinations with respect to a pool of Mallard plasma assayed at 3 dilutions (Table 1) as a measure of interassay variation.

Steroid hormones measured by radioimmunoassay were 17β-hydroxy-5α-androstan-3-one (DHT). testosterone, estrone and estradiol-17β. Corticosterone was measured by a competitive protein binding assay. These assays have been described in detail by Wingfield and Farner (1975). For recovery determinations, each sample was equilibrated with 2,000 cpm of [H^3]-labelled hormones for each of those to be assayed. Each sample was then extracted with 5 ml of dichloromethane and the dried extracts transferred to the top of Celite:propylene glycol:ethylene glycol (6:1.5:1.5, w:v:v) with a total of 1.5 ml of 10% ethyl acetate in isooctane. Steroid fractions were then eluted in order of polarity with increasing concentrations of ethyl acetate in isooctane. Dried eluates were assayed in duplicate with an aliquot removed for recovery determinations. Standard curves extended over the range of 0–1,000 pg (except for corticosterone which ranges from 0–10,000 pg) and separation of bound and free counts was effected by addition of dextran coated charcoal. Two 1 ml samples of distilled water were included with each assay as solvent blanks. In addition, 0.5 ml of a pool of plasma obtained from roosters was also included with each assay as a measure of interassay variation. Solvent blanks and the coefficients of variation for respective steroid hormones measured in the pool of rooster plasma are presented in Table 1.

In plasma samples from females, both testosterone and estrone were measured. Since these are not separable in the chromatographic system used, 20–40 μl aliquots of each plasma sample from the same stage of the cycle were pooled in sets of 2 or 3 for the separate determination of testosterone. Thus, the number of determinations of testosterone levels in females is correspondingly smaller.

In all figures, results are expressed as means ± SEM. Levels of significance were determined by the Student's t test.

RESULTS

Since the stages of the breeding season are not synchronous within the population, the data on gonadal weights and plasma levels of hormones (Figs. 1–4) have been organized by stages in the reproductive cycles rather than by calendar time. The stages are described with ranges of dates in Tables 2 and 3.

Plasma levels of irLH (Figs. 2, 3) in both sexes increased from early spring and through migration to maxima coincident with courtship and copulation on the breeding territories ($P<0.001$ in both cases). In males (Fig. 2), the increase in testosterone levels was parallel with irLH ($P<0.001$). Plasma DHT levels increased significantly from October to February ($P<$

TABLE 1. Solvent blanks and interassay variation of hormone radioimmunoassays.

Hormone assay system	Interassay variation (%)[a]	Solvent blanks (pg)[b]
LH	11.04	...
DHT	9.06	4
Testosterone	12.64	4
Estrone	12.40	4
Estradiol-17β	13.50	4
Corticosterone	15.58	10

[a]Expressed as coefficient of variation on a Mallard plasma pool (irLH) or a rooster plasma pool (steroid assays).

[b]Amount of steroid apparent from % bound in the solvent blank.

TABLE 2. Stages in the annual cycle of male *Zonotrichia leucophrys gambelii* (Figs. 1, 2, 5 and 7).

Stage	Description	Dates[a]	Sample size
1	On wintering grounds, Mabton, Washington	October	10
2	On wintering grounds, Mabton, Washington	January	10
3	On wintering grounds, San Jose, California	February	8
4	On wintering grounds, prenuptial molt, Mabton, Washington	April	9
5	Spring migration, Camano Island, Washington. Early migration	4–6 May	5 (4 for testis wt. and CPL)
6	Spring migration, Fairbanks, Alaska. Late migration	14–17 May	6
7	Territorial males, unmated	13–23 May	16
8	Territorial males, mated, courtship and copulation	13 May–6 June	25
9	Early incubation	29 May–11 June	15
10	Late incubation	4–17 June	14
11	Feeding nestlings	12–29 June	12
12	Feeding fledglings	29 June–9 July	8
13	Early postnuptial molt	1–17 July	7
14	Mid postnuptial molt	5–19 July	6
15	Late postnuptial molt	28 July–12 Aug	7
16	Autumnal migration, Fairbanks, Alaska. Early migration	5–17 Aug	7
17	Autumnal migration, Nr. Mabton, Washington Late migration	September	8

[a]Dates represent the period in which birds in this stage of the cycle were captured. These dates do not necessarily represent the period for each stage in the population as a whole.

0.001), but no further change occurred until the courtship phase in later May and early June. In contrast, the level of DHT in females (Fig. 4) was very high in February and early May and decreased considerably by the time of arrival on the breeding grounds ($P<0.05$). Plasma testosterone levels in females began to increase in April reaching a maximum on arrival in Alaska and during the courtship and nest building phases ($P<0.01$).

The vernal increases in testosterone were closely parallel with increases in testis weight and CPL (Fig. 1), reaching a maximum soon after arrival on the breeding grounds. Females (Figs. 3, 4) arrived on the breeding grounds with ovarian follicles 1–2 mm in diameter. It is at this time that estrone and estradiol-17β levels (Fig. 3) were first detected in the plasma. In mated females, yolk deposition and the rapid final maturation of the follicles occurred at this time culminating in oviposition in late May and early June. Plasma levels of irLH, androgen and estrogen remained high throughout this period.

During incubation, there was a precipitous decline in testosterone levels in males (Fig. 2, $P<0.001$) and only a gradual decline in plasma irLH levels ($P<0.001$) and plasma DHT ($P<0.02$). This is also apparent in Fig. 5 in which individual hormone levels from serially sampled birds are given with corresponding reproductive stages (Table 2). The decline in plasma irLH levels continued through the period of feeding young to low levels at the onset of postnuptial molt in July ($P<0.01$). Testosterone and DHT levels were also basal at this time. A similar trend occurred in the females (Figs. 3, 4) not only for plasma irLH and androgens, but also the estrogens. Figures 5 and 6 show individual hormone levels in the plasma of serially sampled birds. Basal levels were attained by the time postnuptial molt began in July. In both males and females, this decline in gonadal hormones was also accompanied by a gonadal involution to the completely regressed state. Gonadal hormone levels remained low throughout postnuptial molt and autumnal migration.

In males, there was a 4-fold increase in plasma corticosterone (Fig. 7, $P<0.01$) during vernal migration. These levels remained high throughout the nesting period, but had declined precipitously as postnuptial molt approached

TABLE 3. Stages in the annual cycle of female *Zonotrichia leucophrys gambelii* (Figs. 3, 4, 6 and 8).

Stage	Description	Dates[a]	Sample size
1	On wintering grounds, Mabton, Washington	October	8
2	On wintering grounds, Mabton, Washington	January	8
3	On wintering grounds in San Jose, California	February	7
4	On wintering grounds, prenuptial molt, Mabton, Washington	April	0
5	Spring migration, Camano Island, Washington. Early migration	4–6 May	5
6	Spring migration, Fairbanks, Alaska. Late migration	18–21 May	6 (3)[b]
7	Newly arrived on breeding territory, mated	18–30 May	9 (4)
8	Yolk deposition, courtship, nest building and copulation	18 May–22 June	11 (5)
9	Large ovarian follicles, about to ovulate	21 May–22 June	10 (5)
10	Egg in oviduct, i.e., postovulation	28 May–21 June	8 (4)
11	Early incubation	19 May–8 June	6 (3)
12	Late incubation	2–17 June	9 (4)
13	Feeding nestlings	2 June–6 July	14 (6)
14	Feeding fledglings	20 June–9 July	17 (8)
15	Early postnuptial molt	7–19 July	5 (3)
16	Mid postnuptial molt	15 July–1 Aug	6 (3)
17	Late postnuptial molt	29 July–13 Aug	7 (3)
18	Autumnal migration, Fairbanks, Alaska. Early migration	9–17 Aug.	10 (5)
19	Autumnal migration, Nr. Mabton, Washington. Late migration	September	6 (3)

[a]Dates represent the period in which birds in this stage of the cycle were captured. These dates do not necessarily represent the period for each stage in the population as a whole.

[b]Figures in parentheses represent sample sizes for testosterone plasma levels only.

($P<0.01$). In contrast, there was no increase in plasma corticosterone in females during vernal migration (Fig. 8, $P>0.3$), but a maximum occurred coincidently with ovulation and oviposition ($P<0.001$). Corticosterone levels remained somewhat elevated above spring levels ($P<0.05$) during incubation and feeding of young, but declined precipitously at the onset of postnuptial molt ($P<0.001$). There was an increase in plasma corticosterone ($P<0.001$) in females at the onset of fall migration in mid August, but no change was detected in the males until they were on the wintering grounds in October ($P<0.05$).

In both males and females, there was a

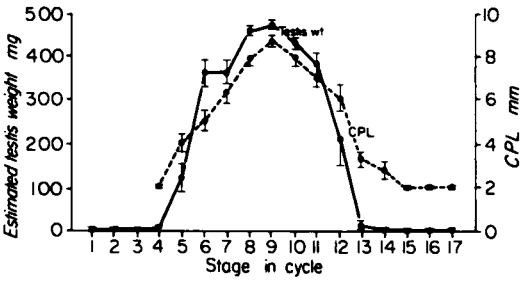

FIG. 1. Estimated testis weights and cloacal protuberance length in male *Z. l. gambelii*. See Table 2 for key to figure and sample sizes.

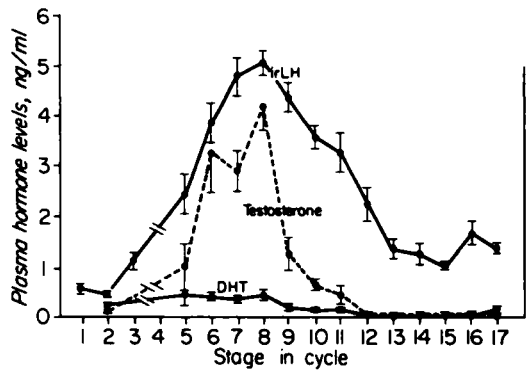

FIG. 2. Plasma irLH, testosterone and DHT levels in male *Z. l. gambelii*. See Table 2 for key to figure and sample sizes.

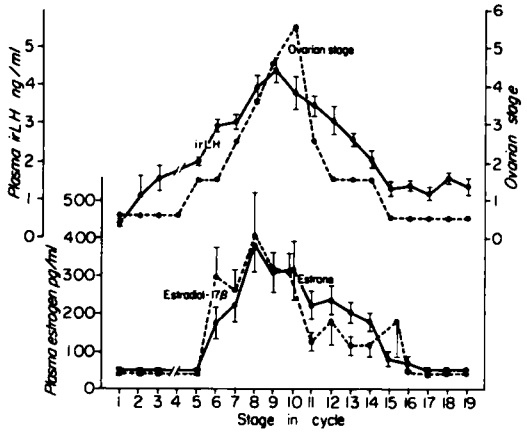

FIG. 3. Ovarian stage, plasma irLH estrone and estradiol-17β levels in female *Z. l. gambelii*. See Table 3 for key to figure and sample sizes. Ovarian stages are: 1) Ovary in winter condition, follicles less than 0.5 mm in diameter; 2) Follicles 0.5–1.0 mm in diameter; 3) Follicles 1–2 mm in diameter; 4) Follicles 3–5 mm in diameter, the yolk deposition phase; 5) Follicles greater than 5 mm in diameter about to ovulate; 6) Egg in oviduct, recently ovulated follicle apparent.

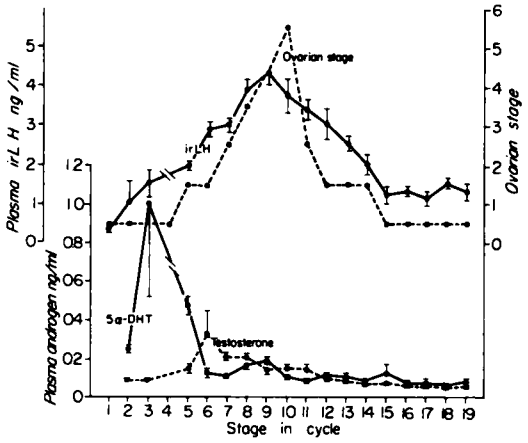

FIG 4. Ovarian stage, plasma irLH, DHT and testosterone in female *Z. l. gambelii*. See Table 3 for key figure and sample sizes. See legend to Fig. 3 for key to ovarian stage.

significant decrease in body weight and depot fat during vernal migration ($P<0.001$ in all cases). Body weights and depot fat remained low throughout the nesting period in males, but showed a gradual increase as the postnuptial molt progressed ($P<0.01$ and $P<0.02$, respectively). No further change was observed during autumnal migration. During the final maturation phase of the ovarian follicles, body weights of the females increased ($P<0.001$) to a maximum at ovulation and oviposition. Depot fat increased gradually throughout this period ($P<0.05$). While incubating and feeding young, both body weight and depot fat declined in females ($P<0.001$) to a minimum at the onset of postnuptial molt. However, both increased gradually throughout the postnuptial molt and autumnal migratory periods ($P<0.01$ and $P<0.001$, respectively).

DISCUSSION

The White-crowned Sparrow is one of the most extensively studied feral avian species in relation to environmental control of the reproductive cycle. As in many other avian species, the increasing day length of late winter and spring serves as basic information for the initiation of gonadal growth, premigratory fattening and spring migration (Farner and Follett, 1966; Lofts and Murton, 1968; Farner, 1970, 1975; Farner and Lewis, 1971; Dolnik, 1975, 1976). Gonadal growth, as induced by artificial long days in the laboratory, is generally consistent with development under natural conditions with the exception of the final maturation (yolk deposition) of the ovarian follicles which is induced by other environmental information (King et al., 1966; Farner and Lewis, 1971), including the presence and activities of a territorial male. Because the environmental and endocrine aspects of the reproductive cycle under natural conditions have already been described for the midlatitude breeding *Z. l. pugetensis* (Wingfield and Farner, 1977, 1978), the discussion in this communication is restricted largely to the major differences between long and short distance migration and single and multiple broodedness.

It has now been established that in the North American species of *Zonotrichia*, the gonads under the influence of long days are essential for vernal premigratory hyperphagia, fattening and migratory restlessness (*Zugunruhe*), at least in males (see Weise, 1967; Berthold, 1973; Mattocks, 1976). In this investigation, both plasma irLH and testosterone levels were found to increase during migration in both males and females and are also elevated over winter levels just prior to the migratory phase (Figs. 2, 4). The significant levels of testosterone (and, early in the year, of DHT) in the plasma of females suggests that this hormone may be involved in regulating vernal migration which

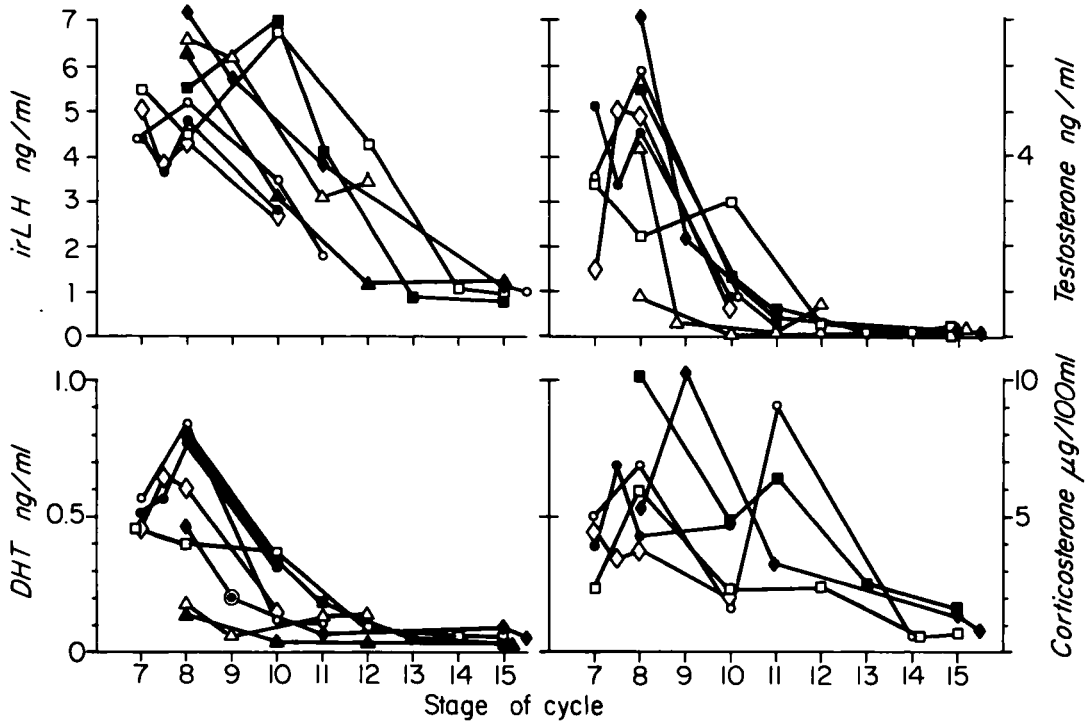

FIG. 5. Plasma levels of irLH, DHT, testosterone and corticosterone in individual male *Z. l. gambelii* sampled 3 or more times on their breeding grounds in Alaska. See Table 2 for key to figure.

in many respects is identical to migration in the males. Before the advent of microradioimmunoassay techniques for measurement of plasma levels of steroid hormones, it had been assumed that estrogens had identical effects to androgens (Farner, 1950; Berthold, 1973) in species with migratory behavior. Since testosterone has been identified in the plasma of laying hens (O'Malley et al., 1968) and measured by radioimmunoassay in the plasma of females of several species (Peterson et al., 1973; Common, 1973; Wingfield and Farner, 1975), it is entirely possible that

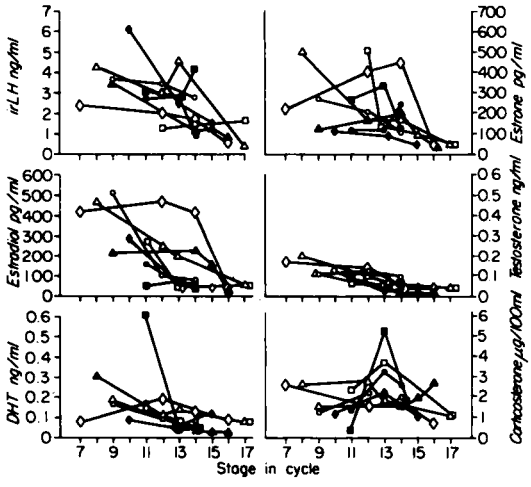

FIG. 6. Plasma levels of irLH, DHT, estrone, estradiol-17β and corticosterone in individual female *Z. l. gambelii* caught 3 or more times on their breeding grounds in Alaska. See Table 3 for key to figure.

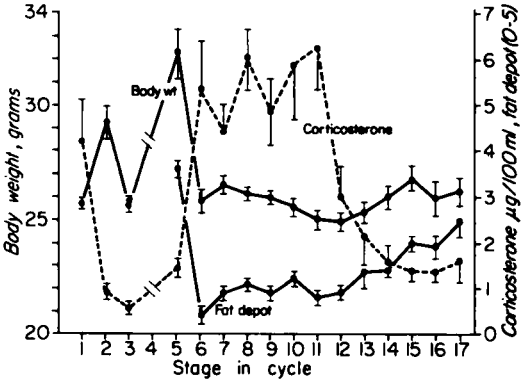

FIG. 7. Body weights, depot fat and plasma corticosterone levels in male *Z. l. gambelii*. See Table 2 for key to figure and sample sizes.

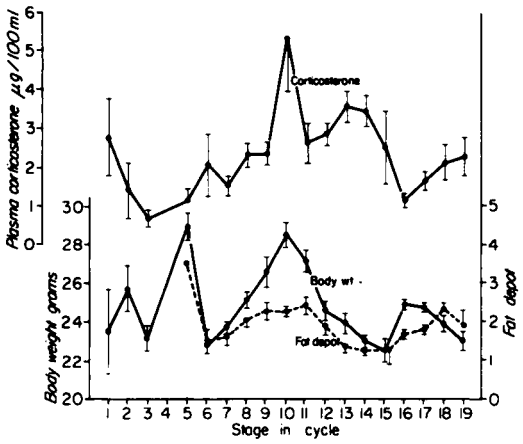

FIG. 8. Body weights, depot fat and plasma corticosterone levels in female *Z. l. gambelii*. See Table 3 for key to figure and sample sizes.

testosterone is involved in the development of vernal migratory behavior in both sexes. However, a possible role for estrogens in females cannot yet be precluded.

The levels of plasma corticosterone in breeding males are higher than those in females and also higher than captive male *Z. l. gambelii* during a photoperiodically induced gonadal cycle (Wingfield, Mattocks and Farner, unpublished). High levels of corticosterone occur in male *Z. l. pugetensis* only during the time of the second brood and in females of both races during egg laying (Wingfield and Farner, 1977, 1978 and Fig. 8). The high levels of corticosterone in male *Z. l. gambelii* contrast with the results of Lorenzen and Farner (1964) who showed by histological techniques that the adrenocortical cells of the interrenal gland regressed during the late spring and summer. Only during postnuptial molt are corticosterone levels basal, correlating with a minimal relative fractional cortical volume at this time (Lorenzen and Farner, 1964). Especially intriguing is the very significant increase in plasma corticosterone in vernal migration in male *Z. l. gambelii* (Fig. 7). This increase is greater than that of *Z. l. pugetensis* (Wingfield and Farner, 1977, 1978) and may be related to the stress of long distance migration and the generally more compressed breeding season at higher latitudes. However, such a hypothesis is tenuous since such an increase does not occur in females (Fig. 8) which perform a similar vernal migration and in which high corticosterone levels are instead associated with the egg laying period. The reason for this striking difference is at present unclear. Recently Péczely (1976), in a study of several European species, showed that corticosteroid secretion by the interrenals (*in vitro*) was lowest in the spring and highest during postnuptial molt in nonmigrants, while maximal levels were coincident with the vernal and autumnal migratory periods in migratory species. No change in plasma corticosterone was observed during autumnal migration in the males of this study (Fig. 7), but a slight increase in corticosterone was recorded at this time in the plasma of females (Fig. 8, $P<0.001$). Much further work is necessary to elucidate the role(s) of corticosterone and the mechanism of the control of its plasma level during migration and reproduction in birds.

Maximum levels of testosterone in both males and females are coincident with maximal gonadal weights, defense of territory, courtship and nesting activity (Figs. 2, 4) as is the case in *Z. l. pugetensis* before and during the first brood (Wingfield and Farner, 1977, 1978). Males generally arrive on the breeding grounds first and acquire a breeding territory before the appearance of the females. When the females arrive, pairing, territorial encounters and courtship chases occur. The singing frequency of the males (determined by counting the number of songs per minute at frequent intervals during the day) may be as high as 400/h at this time. It should also be noted that females may assist in defense of territory. Maximal levels of androgens and maximal gonadal weights are also correlated with high plasma irLH in both sexes. Pituitary gonadotropin levels are maximal at this time in birds sampled from the same population (King et al., 1966) suggesting that rates of synthesis and release are also maximal. It seems likely that the high levels of testosterone at this time induce sexual and territorial behavior. Certainly testosterone has been implicated in the expression of sexual behavior in male birds (Hutchinson, 1970) and high "androgen" levels correlate well with the intensive phase of courtship activity in the male Eider duck, *Somateria mollissima* (Gorman, 1977). Other androgens not measured in this study may possibly be also effective since androstenedione as well as dehydroepiandrosterone have been measured in higher concentrations than testosterone in the plasma of male pigeons (Rivarola et al., 1968). Androsterone is also apparently the most potent androgen in restoring courtship behavior to

castrate pigeons (Pietras and Wenzel, 1974), although in this experiment, all androgen injections were well above the physiological range.

There is a wealth of information on the environmental control, particularly photoperiodic, of the gonadal cycle in birds (for reviews see, Dolnik, 1975, 1976; Farner and Lewis, 1971; Farner, 1975; Lofts and Murton, 1968). It has also been shown that if photosensitive male *Z. l. gambelii* held on daily photocycle of 8hL:16hD are transferred to 20hL:4hD photocycles, there is an increase in plasma irLH to a maximum within 5 days. These levels remain elevated for a further 50 days after which they decline and the testes regress as the birds become photorefractory (Follett et al., 1975). Plasma testosterone levels begin to increase about Day 10 and are maximal by Day 35 of 20hL:4hD. Thereafter, there is a precipitous decrease despite a continued high plasma level of irLH (Lam and Farner, 1976). Since LH undoubtedly has a steroidogenic function in birds (Brown et al., 1975; Ishii and Furuya, 1975; Maung and Follett, 1977), such a disparity between LH and testosterone levels in the plasma is puzzling. A similar disparity has been demonstrated in *Z. l. pugetensis* in which a second maximum plasma level of irLH occurs at the time of nesting for the second brood without a corresponding increase in plasma testosterone (Wingfield and Farner, 1977, 1978). The precipitous decline in testosterone levels in males early in the incubation period without a significant change in irLH demonstrated in this investigation is a reflection of the same phenomenon. This is particularly apparent in measurements in plasma from serially sampled males (Fig. 5). One possible explanation for this is an increase in the metabolic clearance rate of testosterone at this time as has been shown in the Pekin duck (Jallageas et al., 1974). Other explanations include a decreased sensitivity of the Leydig cells to LH (see Wingfield and Farner, 1978). This intriguing problem is now under further investigation.

Estrone and estradiol-17β are undetectable in the plasma of males (<40 pg/ml), but in females, maximum levels are correlated with yolk depostion and egg laying (Fig. 3). Again, this is a pattern similar to that in *Z. l. pugetensis* (Wingfield and Farner, 1977, 1978) and we refer the reader to those papers for a more extensive discussion.

During incubation, there is a decline in plasma irLH and sex steroids in both sexes similar to that in *Z. l. pugetensis.* Although the males do not incubate, singing is greatly reduced (to less than 5 songs/h) and very few territorial encounters occur. Males do feed nestlings and fledglings and by this time, plasma irLH, sex steroids and gonadal weights have declined even further in both sexes. Interestingly, the plasma testosterone levels in male *Z. l. gambelii* decline to basal levels by the time young are being fed, whereas in *Z. l. pugetensis* plasma testosterone remains at about 1 ng/ml throughout the period of the first brood, but declines very rapidly during the time of the second brood (Wingfield and Farner, 1977, 1978). Since *Z. l. pugetensis* remains on the same territory and with the same mate for the second brood, it is possible that the higher plasma testosterone levels are related to maintaining a territory through a longer period than in the single brooded *Z. l. gambelii.* This hypothesis is supported by some circumstantial observations of territoriality in these two forms. Both sexes respond to tape recorded songs of the White-crowned Sparrow by singing and with intense wing fluttering and chattering during the courtship and nesting phase of the cycle. Songs played back to territorial pairs during incubation and feeding of young, elicit only a mild or often no territorial response in *Z. l. gambelii,* whereas in *Z. l. pugetensis* both sexes responded intensively during the period of incubation and during the time of feeding of young of the first brood. Often, no response is elicited during the time of feeding young of the second brood, a phase in the cycle that is comparable to that of the *Z. l. gambelii* after a single brood, i.e., just prior to rapid gonadal involution and the onset of postnuptial molt. Catchpole (1977) has shown that the aggressive responses of male Sedge Warblers (*Acrocephalus schoenbaenus)* to songs played from tape decreased markedly after pairing with a female. Further, the responses of 3 species of North American sparrows to playbacks of distress calls is strongest early in the season, i.e., before egg laying and less so during incubation and feeding of young (Stefanski and Falls, 1972). Thus, the decrease in plasma testosterone during incubation may be adaptive in reducing the singing frequency and territorial defense in males with an established territory in sufficient time to begin feeding young that hatch within 12–14 days thereafter. In addition, testosterone-induced courtship at a time when the female is incubating would

be maladaptive in a monogamous species such as *Z. leucophrys*. The former explanation has also been put forward for the polygamous Pied Flycatcher (*Ficedula hypoleuca*) which undergoes a rapid testicular involution during incubation (Silverin, 1975, 1977). *Z. l. gambelii* forage in territories other than their own, sometimes in loose flocks of up to 6 birds. For example, in our study area, 1 levee about 200 m in length, was particularly favored by foraging birds. However, this levee also had 5 occupied territories along its length with the regular singing perches of the territorial males on top of the levee. Apparently, these birds tolerated foraging individuals from adjacent or nearby territories, as on each of 3 days in late June, over 15 birds were mist netted or identified by color bands as they foraged along the levee. Such tolerance correlates well with the low testosterone levels in males at this time as compared to earlier in the season when the territories were first established. Similar foraging flocks were seen during both broods in *Z. l. pugetensis* by Lewis (1971) and Wingfield and Farner (1978). In both races, foraging birds remain inconspicuous when they are away from their own territory and almost never sing in this situation. Experiments in the field involving implants of steroid hormones, which are now feasible, could be particularly revealing with respect to the hormonal control of sexual and territorial behavior.

The cycles in testicular weight and size of ovarian follicles (Figs. 1, 3) are very similar to those reported by King et al. (1966) and Blanchard and Erickson (1949) in the same population. While feeding fledglings (late June and early July), a rapid involution of the gonads occurs so that by the time the young are independent of their parents, postnuptial molt is beginning or is imminent. It is at this time that the birds are photorefractory, that is, unresponsive to long days insofar as gonadotropin secretion and gonadal growth are concerned (King et al., 1966, Farner and Lewis, 1971, Farner, 1975). The basal levels of plasma irLH and sex hormones during postnuptial molt are in agreement with current opinion that molt and reproductive capability are mutually exclusive in this species (King et al., 1965) and are normally separated in time by the control system.

Mattocks et al. (1976) have shown that the cycle in plasma levels of irLH in male *Z. l. gambelii* held in aviaries under the natural light regimen in Seattle (48°N) is similar to that of feral males and decline in levels precedes the testicular regression in late June and early July. Plasma irLH also declines in castrate *Z. l. gambelii* at this time suggesting that gonadal steroid hormones are not involved in the onset of photorefractoriness. The cycles in plasma testosterone and DHT are, as one would expect, more or less synchronous with that of irLH (Lam and Farner, unpublished).

The plasma levels of irLH in females increase in response to lengthening days both in the laboratory, 20hL:4hD (Yokoyama and Farner, 1976), and in birds held in roof aviaries under natural day lengths (Mattocks and Farner, unpublished). However, the levels in females are considerably lower than those in males under similar conditions and may be related to the failure of the ovary of captives of *Z. l. gambelii* and of many other species of birds to develop beyond the preyolk deposition phase. The final maturation of the ovarian follicles apparently requires the presence of a mate with suitable breeding territory (King et al., 1966) and doubtless other supplemental environmental information. Thus, it is not surprising that the levels in feral females during yolk deposition and ovulation (Figs. 3, 4) are higher than those of captive females (1–2 ng/ml). It should also be mentioned here that Yokoyama and Farner (1976) have shown that visual information may suppress irLH levels in females since levels of 8.9 and 6.0 ng/ml were measured in bilaterally and unilaterally enucleated females, respectively, subjected to a photoregimen of 20hL:4hD. Whether or not the eyes may impart some inhibitory information in males in captivity is not known, but it is interesting to note that the maximum testosterone levels recorded in the laboratory (1 ng/ml) by Lam and Farner (1976) and Wingfield and Farner (unpublished) are considerably lower than those recorded in the field (4–10 ng/ml) in both *Z. l. pugetensis* and *Z. l. gambelii* (Wingfield and Farner, 1977, 1978 and Fig. 2).

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