General and Comparative Endocrinology **101**, 242–255 (1996)
Article No. 0027

Interrelationship of Day Length and Temperature on the Control of Gonadal Development, Body Mass, and Fat Score in White-Crowned Sparrows, *Zonotrichia leucophrys gambelii*

John C. Wingfield, Thomas P. Hahn,[1] Masaru Wada,* Lee B. Astheimer,† and Stephan Schoech

*Department of Zoology, NJ-15, University of Washington, Seattle, Washington 98195; *Department of General Education, Tokyo Medical and Dental University, 2-8-30 Kohnodai, Ichikawa, Chiba, 272 Japan; and †Department of Biomedical Science, Wollongong University, New South Wales, Australia*

Accepted August 25, 1995

We tested the effects of naturally relevant environmental temperatures on long day-induced reproductive development in male and female white-crowned sparrows, *Zonotrichia leucophrys gambelii.* Transfer from short days (8L 16D) to long days (20L 4D) resulted in rapid testicular development and partial ovarian development as has been reported many times previously. Exposure of experimental groups to low (5°), moderate (20°), and high (30°) temperature during photostimulation had only subtle effects on plasma levels of follicle-stimulating hormone and luteinizing hormone over time and no effects on the size of testes, cloacal protuberance, ovaries, or brood patch at Day 30 of treatment. Long days resulted in the well known increase in body mass and fat score, indicative of preparations for migration. In females, treatment with low temperature resulted in a reduction in the premigratory increase in fat and body mass when transferred to long days. This was accompanied by an increase in plasma levels of corticosterone during the early stages of photostimulation at low temperature. Temperature regimes had no effects on fattening or body mass in males, despite an early increase in plasma corticosterone at low temperature. Circulating levels of thyroxine (T4) and triiodothyronine (T3) increased to varying degrees following photostimulation. Temperature treatment had no effect on plasma levels of thyroid hormones in males, but low temperature did inhibit thyroid hormone secretion (particularly T4) in females. Although reproductive development appears to be resistant to naturally relevant temperature extremes in both sexes, low environmental temperature impaired preparations for migration in females but not males. This effect may be mediated through glucocorticosteroids and not thyroid hormones. Reasons for the sexual dimorphism in this response are unknown, but may be related to sexual selection for males to arrive on the breeding grounds ahead of females regardless of local weather conditions.

The environmental control of seasonal breeding has received almost continual experimental investigation for 7 decades. However, in birds most of these studies have focused on effects of the annual change in day length and less on other environmental factors (e.g., Farner and Follett, 1979; Follett, 1984; Wingfield and Kenagy, 1991). Although there is no question that pho-

[1] Current address: Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544-1003.

0016-6480/96 $18.00

toperiodic regulation of gonadal development and regression is of major importance in at least north temperate species, it must be borne in mind that other environmental cues are also critical for the regulation of gonadal development (e.g., Marshall, 1970; Immelmann, 1971, 1973; Wingfield, 1983; Wingfield and Kenagy, 1991). More recently, Wingfield *et al.,* (1992, 1993) have suggested that the degree to which populations integrate environmental signals to time gonadotropin secretion leading to gonadal development depends upon the predictability of their breeding seasons.

Cohen (1967) suggested that if a future event such as onset of breeding was highly predictable, then only one, or few, reliable environmental cues should be required to time breeding. Many other environmental signals could be ignored. On the other hand, if the breeding season is less predictable, i.e., varies due to cold or warm spring seasons etc., then an individual should integrate many environmental cues to ensure that reproduction is timed optimally. The idea that long term predictive cues (i.e., initial predictive information of Wingfield, 1983) would be important if the breeding season itself is predictable, has been applied to demographic data on breeding in several species of birds (Wingfield *et al.,* 1993). Log-linear analysis of egg-laying dates organized in matrices by month for as many years as there were data available, revealed that the white-crowned sparrow, *Zonotrichia leucophrys gambelii,* has a highly predictable breeding season and that initial predictive information such as photoperiod should provide the major cue to time gonadal development (Wingfield *et al.,* 1993).

Colwell (1974) pointed out that predictability has two components, constancy (i.e., the environment is predictable because it is always the same) and contingency (i.e., there are major, but predictable, fluctuations in environmental conditions). Wingfield *et al.* (1992) applied Colwell's formulae to matrices of egg-laying dates in birds and suggested that the ratio of contingency to constancy (called the environmental information factor, I_e) indicated the degree to which an individual within a population should integrate several environmental cues, i.e., both long term (initial predictive) and short term (supplementary cues of Wingfield, 1983). The data for *Z.l. gambelii* revealed that predictability was high, favoring a strong effect of photoperiod, and that the I_e factor was about 1.13. Comparisons with other taxa of *Zonotrichia* showed that populations of *Z.l. pugetensis* and *Z.l. nuttalli* (that breed at lower latitudes and have more flexible breeding seasons) had higher I_e factors (4.27 and 6.26, respectively, Wingfield *et al.,* 1992, 1993). Log-linear analysis indicated that all these taxa should, nevertheless, be highly responsive to photostimulation (see below). On the other hand, the I_e factors suggest that supplementary cues such as local temperature, food availability, etc. would be of rising importance as I_e increases. This does not mean that *Z.l. gambelii* with the smallest I_e would not also be responsive to temperature, food, and other cues, but rather that it should be less responsive than *Z.l. pugetensis* and *Z.l. nuttallii.* Wingfield *et al.,* (1993) point out that the I_e factor is relative and caution should be exercised when attempting to infer how many cues a population of interest should be responsive to based on specific I_e ratios.

There is much evidence that at least four taxa of white-crowned sparrow respond to increasing day length with gonadal development (e.g., Farner and Lewis, 1973). This is consistent with the predictions of log-linear analysis (Wingfield *et al.,* 1993). However, the degree to which other environmental signals, such as temperature, influence photoperiodically induced gonadal development has been less well studied. The hypothesis derived from the calculations of Wingfield *et al.,* (1992, 1993) is that *Z.l gambelii,* with a highly predictable breeding season in the subarctic and arctic regions of North America, should respond primarily to increased day length and be less responsive to high or low temperature. Previous studies have suggested that male and female Gambel's white-crowned sparrows were indeed resistant to the effects of low temperature on gonadal development induced by exposure to long days (Farner and Mewaldt, 1952; Lewis and Farner, 1973). It nonetheless remains unclear whether temperature may affect photoperiodically induced changes in hormones related to reproductive function.

In this paper we have tested the hypothesis derived from Wingfield *et al.* (1992, 1993) by exposing photostimulated male and female *Z.l. gambelii* to long days at low, moderate, and high environmental temperatures within the realistic range experienced by free-living birds. We investigate possible effects on both reproductive physiology (i.e., gonadal development,

secondary sexual characters) and premigratory physiology (i.e., body mass and fat deposition) further by measuring circulating gonadotropin levels as well as concentrations of corticosterone (to assess potential stress of treatment) and thyroid hormones (to assess whether any effects could be attributed to a mechanism of action via the thyroid gland). We also wish to point out that other related taxa, with higher I_e factors, have also been investigated and reports on their responses will follow.

MATERIALS AND METHODS

Capture and housing of birds. Male and female *Z.l. gambelii* were captured in Japanese mist nets during their autumn migration (September and October) at Sunnyside Game Refuge, Yakima County, Washington (47°N). Birds were transported to Seattle and held in roof aviaries at the Department of Zoology for at least 3 weeks. Birds were then housed in cages (55 × 25 × 25 cm), one per cage, in environmental chambers held at 20° and a daily photoperiod of 8 hr light 16 dark (8L 16D). Each chamber contained both males and females and both sexes could see and hear one another throughout the experiment, although contact was prevented by housing one bird per cage. Birds were assigned to treatment groups at random. Groups of both sexes contained adults and first year birds (identified by crown color). Previous studies have shown that the photoperiodic responses of first year birds and adults are identical (Farner and Lewis, 1973; J. C. Wingfield, unpublished observations). Sample sizes were 11 for males and 12 for females eventually exposed to 5°; 7 and 9, respectively, at 20°; and 9 and 7, respectively, at 30°. All birds had seeds, Mazuri small bird chow (PMI Feeds Inc., St. Louis, Missouri) and water *ad libitum.*

Sampling techniques. Birds were sexed by unilateral laparotomy at least 1 month before the experiment began. Further laparotomy was performed at Day 30 of photostimulation and temperature treatment. Just prior to the surgical procedure, a local anesthetic was applied to the exposed left flank. This procedure has been in routine use for over 30 years and has been approved for these investigations by the University of Washington Institutional Animal Care and Use Committee. Gonads can be easily observed through a single incision between the last pair of ribs on the left side. Testis length and width were measured to the nearest mm, as was diameter of the largest ovarian follicle. Volume of the testis was calculated from the formula for volume of an ovoid sphere (see Boswell, 1991):

$$V = 4/3\ \pi a^2 b$$

where V is volume, a is the radius of the testis at its widest point, and b is half the long axis.

Volume of the largest ovarian follicle was calculated from the formula for a sphere (Boswell, 1991):

$$V = 4/3\ \pi r^3$$

where V is volume, and r is the radius of the largest ovarian follicle. The volumes of both the testes and largest ovarian follicles were expressed as cubic millimeters.

Blood samples were collected in heparinized microcapillary tubes from a wing vein after puncture with a 26-g needle. Approximately 200–250 μl of whole blood were collected from each bird. Plasma was separated by centrifugation, harvested with a Hamilton micro syringe, and stored at −20° until assay.

Body mass was measured to the nearest 0.1 g on a Pesola spring scale for weighing wild birds. Fat score was assessed in the furcular and abdominal regions using an arbitrary scale (see Wingfield and Farner, 1978) from 0 to 5, in which 0 = no visible fat, and 5 = gross bulging fat bodies that far exceed the normal limits for wild birds. Stage 5 is usually only seen during the height of migration or under artificial conditions in the laboratory.

Where appropriate, secondary sex characters were also assessed. In males, the length of the cloacal protuberance (an androgen-dependent copulatory organ) was measured to the nearest millimeter. In females, presence of a brood patch was assessed by examining the ventral skin of the breast and abdomen. Absence of a patch was scored as 0% development. Percentage defeathering can be estimated (i.e., 10, 50, 80, or 100%) because the patch has a very clear edge (see Wingfield and Farner, 1976).

We checked for prebasic moult in all birds by examining the number of primary flight feathers absent or growing on both wings. True moult is symmetric, i.e., both wings show the same number of primaries (and

other feathers) shed and growing in. Asymmetric moult often occurs as a result of accidental loss of feathers and subsequent replacement, and was thus ignored in these experiments. At no time during the experiments described below was any pre-basic moult observed.

Experimental protocol. All birds were maintained on 8L 16D at 20° for at least 10 weeks prior to onset of experimental treatments. Exposure to short days for this period is known to result in complete recovery of photosensitivity (i.e., all birds would have been photorefractory when captured in autumn) in this species (e.g., Farner and Follett, 1979). Five days prior to transfer to long days, all birds were bled, weighed, and fat score, cloacal protuberance length (CPL), and brood patch assessed. At Day 0, all three constant environment chambers were transferred to 20L 4D, and one chamber set to 5°, a second maintained at 20°, and a third adjusted to 30°. These temperatures were selected as representative of extremes likely to be experienced by free-living *Z.l. gambelii* during gonadal development and vernal (April to early May) migration. Examination of weather records at selected locations along the migratory route from southwestern U.S.A. to Alaska (see Wingfield *et al.,* 1992) confirms these selections as appropriate.

Day 1 after transfer to experimental treatments, all birds were bled, weighed, and fat score assessed. The same sampling procedure was performed on all birds at Day 10 of treatment also. At Day 30, when the rapid phase of gonadal development was nearing completion (e.g., Farner and Follett, 1979; Wingfield and Farner, 1980), all birds were bled, weighed, and fat score, CPL, and brood patch assessed. Unilateral laparotomy was also performed on all birds to determine developmental state of the gonads.

It should be noted that because plasma levels of corticosterone rise as a result of capture and handling (e.g., Wingfield *et al.,* 1982), we recruited several laboratory personnel to assist the sample collection process. Each chamber was entered independently and blood samples were collected from all birds within the range 0.5–10 min (most within 5 min). It is likely that some increase in corticosterone had occurred by 5–10 mins of entering the chambers, but this would still be well below the maximum attained during capture, handling, and restraint (see Wingfield *et al.,* 1982). Furthermore, all chambers were entered and birds sampled in an identical manner and thus we feel that any effects of stress on corticosterone were minimal.

Hormone assays. Plasma levels of luteinizing hormone (LH) were measured by a double antibody, postprecipitation radioimmunoassay for chicken LH as developed by Follett *et al.* (1972) and updated by Sharp *et al.* (1987). We have used this assay extensively for determination of LH levels in many passeriform species (see Wingfield *et al.,* 1991 for details). The assay uses purified chicken LH for standards and for iodination by the chloramine T method. Plasma volumes for assay were between 10 and 20 μl. All samples were measured in duplicate within a single assay to avoid interassay variation. Intra-assay variation was assessed by duplicate determinations of three dilutions of high and low LH plasma pools from white-crowned sparrows. Intra-assay variation was within limits (15% coefficient of variation) seen in previous assays (Wingfield *et al.,* 1991).

Circulating follicle-stimulating hormone (FSH) was also measured by a double antibody postprecipitation radioimmunoassay (Sakai and Ishii, 1985) using chicken FSH for standards and iodination (again by the chloramine T method), and an anti-chicken FSH serum that shows very low cross-reaction with chicken LH (Sakai and Ishii, 1985). All samples were assayed within a single assay, in 20-μl duplicates and intra-assay variation determined on the breeding and nonbreeding (i.e., high and low FSH) plasma pools from white-crowned sparrows. As for LH, intra-assay variation was less than 15% (coefficient of variation).

Corticosterone in plasma was measured by radioimmunoassay after extraction in freshly redistilled dichloromethane. Approximately 10-μl aliquots of plasma were equilibrated with 2000 cpm of tritiated corticosterone (for determination of percentage recovery following extraction in each sample) and diluted to 200 μl in distilled water. After equilibration, samples were extracted in 5 ml dichloromethane and the organic phase was aspirated and taken to dryness under a stream of nitrogen in a water bath at 45°. Dried extracts were then reconstituted in 550 μl phosphate-buffered saline with gelatin and sodium azide, and 200-μl aliquots assayed in duplicate. A small (100 μl) aliquot was taken to a scintillation vial for determination of percentage recovery following extraction. The assay followed that of Wingfield *et al.* (1991). Bound and free fractions were separated by addition

of dextran-coated charcoal. Assay reliability criteria were well within the limits (intra-assay variation <9.6%; inter-assay variation <12.6%) described by Wingfield and Farner (1975) and Wingfield *et al.* (1991).

Plasma levels of thyroxine (T4) and tri-iodothyronine (T3) were assayed by separate radioimmunoassay with polyethylene glycol precipitation for separation of bound and free counts (after Tasaki *et al.*, 1986; Wada, 1993). The antibodies were purchased from Endocrine Sciences (Tarzana, CA) and used in appropriate dilutions (×1000 for T4 and ×3000 for T3) determined by preliminary binding tests. Stock solutions for standards were prepared by diluting T4 (free salt) and T3 (sodium salt), both purchased from Calbiochem (La Jolla, CA), into methanol and distilled water (1:1) containing 1% ammonium hydroxide solution. These were then further diluted with methanol to make a final solution of 1 μg/ml. Working standard solutions (32 ng/ml) were made up fresh from these stocks using 0.11 *M* barbital buffer (pH 8.6) containing 1% gelatin. Standard curves were generated by serial dilution from 32 ng/ml in barbital buffer containing 8-anilino-1-naphthalenesulfonic acid (0.55 mg/ml) and bovine gamma globulin (15 mg/ml). All the samples were assayed within a single assay in 5-μl duplicates. Intra-assay variation (<15%) was determined on breeding and nonbreeding plasma pools from white-crowned sparrows.

Statistics. Changes in hormone levels, body mass, and fat deposition over time were assessed by ANOVA for repeated measures with one grouping factor (temperature treatment). When necessary, hormone data were natural logarithm transformed to reduce heteroscedasticity. Univariate *F*-tests were used for post hoc comparisons both between different sampling dates within temperature treatments and between the different temperature treatments at each sampling date. In general, significance was set at $P = 0.05$, and post hoc comparisons were only considered significant if they met the Bonferroni criterion of P divided by the total number of comparisons made. When sampling date was the only significant ANOVA effect, then we made nine pairwise comparisons of the adjacent sampling dates within each treatment, and we considered the differences robust if $P = 0.006$ or less. When temperature treatment and/or the interaction of sampling date and temperature treatment also were significant in the ANOVA, then we compared each temperature treatment at each sampling date as well (12 additional comparisons). In this case we considered differences to be robust if $P = 0.002$ or less. In one case (T3 levels in females) we made further within treatment comparisons of some nonadjacent sampling dates in an attempt to understand the complex variation that occurred; here we considered the difference truly robust only if $P = 0.001$ or less. This approach to the statistics is conservative; some of the "marginally" significant differences that failed to survive adjustment for multiple comparisons (see Tables 1 and 2) may reflect biologically real phenomena.

Data on gonadal condition and length of the cloacal protuberance (CPL) in relation to temperature were analyzed by one factor ANOVA since repeated measures were not taken (i.e., only at day 30 of photostimulation).

RESULTS

Effects of Temperature on Photoperiodically Induced Testicular and Ovarian Development

Temperature did not affect photoinduced development of testes and cloacal protuberance in males (Fig. 1). By Day 30 after transfer to 20L 4D, testes showed similar enlargement in all three groups (ANOVA, $F = 0.568$, $P = 0.5741$, $df = 2,24$, Fig. 1A). Likewise, cloacal protuberance length was similar in the three temperature treatments after 30 days of photostimulation ($F = 0.36$, $P = 0.7016$, $df = 2,25$, Fig. 1B). As in males, the effects of temperature on photoperiodically induced ovarian follicle development in females were not significant at 30 days of treatment ($F = 1.199$, $P = 0.3182$, $df = 2,25$, Fig. 2). There was no development of the brood patch (i.e., 0% defeathering) by Day 30 in any group.

Effects of Temperature on Photoperiodically Induced Changes in Plasma Gonadotropins

Following photostimulation, plasma levels of both LH and FSH increased in males (Fig. 3; Table 1; note that all further statistics for males appear in Table 1).

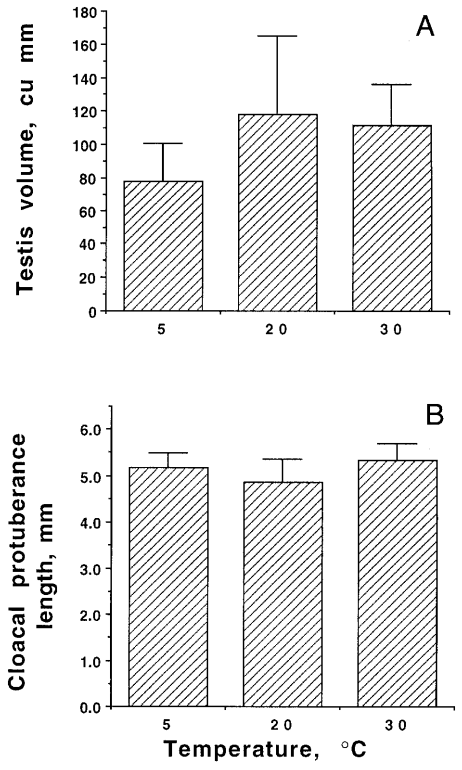

FIG. 1. Effects of temperature on testicular development (A) and length of the cloacal protuberance (B) in male white-crowned sparrows exposed to 20L 4D and three different experimental temperatures for 30 days. Bars are means ± standard errors.

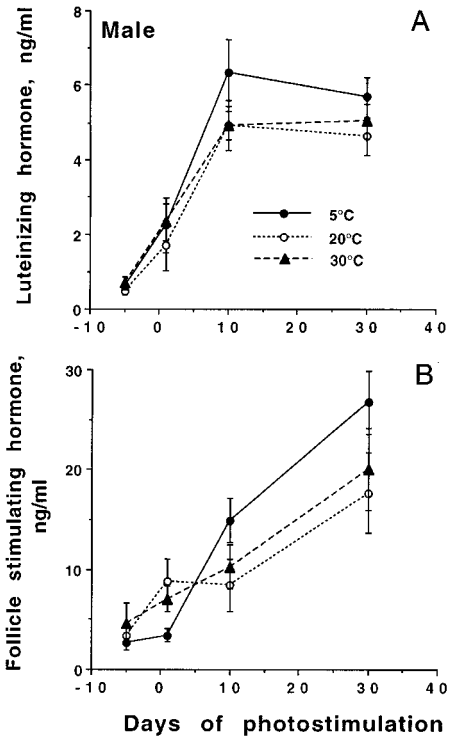

FIG. 3. Effects of temperature on plasma levels of luteinizing hormone (A) and follicle-stimulating hormone (B) in male white-crowned sparrows transferred to 20L 4D from 8L 16D at Day 0. Each point is the mean and vertical lines are the standard errors.

LH rose significantly by Day 1 and then increased further by Day 10 in all three treatments except in the 5° group, where the change between Day −5 and Day 1 was not significant. Note, however, that there was neither a main effect of temperature on LH nor an interaction of temperature with sampling date in males (Table 1). LH levels on Day 30 were similar to those on day 10 in all treatments. Although there was no main effect of temperature on FSH levels, the temporal profiles of FSH in the three treatments were different (Fig. 3B; Table 1). The increase in FSH following photostimulation appeared to be delayed in the 5° birds. However, the increase by Day 1 within the 20° and 30° birds, as well as the differences between the 5° and the 20° and 30° birds on Day 1, only approached significance (Table 1). The only robust change in FSH levels between adjacent sampling dates within any temperature treatments occurred in the 5° group between Day 1 and Day 10 of photostimulation (Table 1).

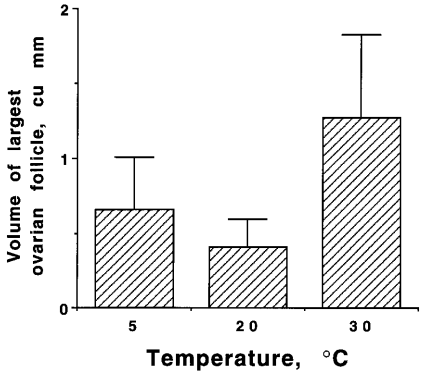

FIG. 2. Effects of temperature on ovarian follicle development in female white-crowned sparrows exposed to 20L 4D and three different experimental temperatures for 30 days. Bars are means ± standard errors.

Likewise in females, plasma levels of LH and FSH increased following transfer to long days (Fig. 4; Table 2; note that all further statistics for females, except where noted in the text, appear in Table 2). There was no main effect of temperature on plasma FSH in females, and although there was a trend toward more rapid elevation of FSH on Day 1 in the 30° group (Fig. 4B), the interaction of date and temperature was not significant; none of the groups showed significant el-

TABLE 1
Statistical Analysis for Males

		LH		FSH		MASS		FAT		B		T4		T3	
EFFECT		F	P	F	P	F	P	F	P	F	P	F	P	F	P
TEMPERATURE		1.301	0.293	0.184	0.833	2.195	0.134	0.878	0.429	0.099	0.906	1.214	0.318	0.591	0.562
DATE		122.337	*0.001* **	36.130	*0.001* **	6.367	*0.001* **	13.387	*0.001* **	6.806	*0.001* **	25.573	*0.001* **	5.933	*0.001* **
DATE*TEMPERATURE		1.119	0.362	3.358	*0.008* **	0.225	0.967	0.502	0.805	2.425	*0.034* **	1.339	0.254	0.513	0.796
Comparisons within treatments:															
DAY -5 vs 1	5°	2.130	0.159	1.089	0.307	0.003	0.954	3.099	0.091	15.304	*0.001* **	1.177	0.291	0.144	0.708
	20°	10.062	*0.005* **	6.118	0.021 *	2.190	0.152	0.067	0.799	0.105	0.748	1.261	0.275	1.251	0.275
	30°	18.979	*0.001* **	5.086	0.034 *	0.230	0.636	0.000	1.000	1.221	0.280	6.120	0.022 *	0.075	0.787
DAY 1 vs 10	5°	29.625	*0.001* **	23.581	*0.001* **	2.183	0.153	15.478	*0.001* **	3.844	0.061	13.158	*0.002* **	2.504	0.127
	20°	23.621	*0.001* **	0.069	0.796	1.378	0.252	8.992	*0.008* **	4.933	0.036 *	16.249	*0.001* **	3.572	0.071
	30°	12.253	*0.002* **	0.704	0.410	0.711	0.408	2.607	0.119	2.265	0.145	2.927	0.103	2.183	0.153
DAY 10 vs 30	5°	0.743	0.399	4.586	0.043 *	0.319	0.577	0.409	0.529	2.770	0.109	3.505	0.076	0.182	0.674
	20°	0.914	0.350	5.438	0.028 *	0.833	0.371	0.000	1.000	0.014	0.908	2.342	0.142	0.182	0.673
	30°	0.521	0.478	3.038	0.094	0.552	0.465	0.562	0.461	2.322	0.140	0.674	0.421	0.671	0.421
Comparisons between treatments:															
DAY -5	5° vs 20°	-	-	0.854	0.365	-	-	-	-	0.005	0.945	-	-	-	-
	5° vs 30°	-	-	0.878	0.358	-	-	-	-	0.709	0.408	-	-	-	-
	20° vs 30°	-	-	0.000	0.991	-	-	-	-	0.508	0.483	-	-	-	-
DAY 1	5° vs 20°	-	-	6.405	0.018 *	-	-	-	-	5.577	0.026 *	-	-	-	-
	5° vs 30°	-	-	5.414	0.029 *	-	-	-	-	7.657	0.010 *	-	-	-	-
	20° vs 30°	-	-	0.036	0.851	-	-	-	-	0.091	0.766	-	-	-	-
DAY 10	5° vs 20°	-	-	6.076	0.021 *	-	-	-	-	1.208	0.282	-	-	-	-
	5° vs 30°	-	-	2.064	0.164	-	-	-	-	0.066	0.799	-	-	-	-
	20° vs 30°	-	-	0.913	0.349	-	-	-	-	0.661	0.424	-	-	-	-
DAY 30	5° vs 20°	-	-	3.763	0.064	-	-	-	-	0.044	0.835	-	-	-	-
	5° vs 30°	-	-	2.432	0.132	-	-	-	-	0.209	0.652	-	-	-	-
	20° vs 30°	-	-	0.125	0.727	-	-	-	-	0.049	0.826	-	-	-	-

Note. Summary of statistical analyses for male Gambel's white-crowned sparrows. First three rows show main repeated measures ANOVA results for main temperature treatment effect, sampling date effect (whether the variable changed over time during the experiment), and the date by temperature interaction (whether the different temperature treatments varied in their temporal profiles of the variable). Remaining rows summarize post hoc comparisons for adjacent sampling dates within treatments, and for the same sampling date between treatments. Robust differences (i.e., differences that remained significant after adjustment for multiple pairwise comparisons, see Methods section under "Statistics") are in italics and marked with double stars. Less robust differences (i.e., post hoc comparisons with $P \leq 0.05$, but greater than P adjusted for multiple comparisons) are marked with single stars. Due to variation in amount of available plasma for different birds and occasional loss of individual blood samples for a variety of reasons, sample sizes are not identical for all analyses. For temperature effect, degrees of freedom (df) vary from 2,21 to 2,25. For date effect, df vary from 3,63 to 3,75. For date*temperature effect, df vary from 6,63 to 6,75. For post hoc comparisons, df vary from 1,21 to 1,25.

evation of FSH by Day 1 compared with Day -5 (Table 2). In all three treatments, FSH first rose significantly between Day 1 and Day 10 and then declined significantly by Day 30. In contrast to FSH, temperature significantly affected LH levels, producing both a main effect and an interaction with sampling date (Fig. 4A; Table 2). LH in the 30° group increased by Day 1, but did not do so until day 10 in the other two groups. These profile differences resulted in significantly greater LH levels after 1 long day in the 30° group than in both of the other groups. By Day 10 LH levels were indistinguishable in the three groups. The apparent declines in LH between Days 10 and 30 were only marginally significant (Table 2).

Effects of Temperature and Photoperiod on Body Mass and Fat Score

Photostimulation resulted in an increase of body mass in males (Fig. 5A; Table 1); temperature had no effect. Despite the main effect of sampling date, none of the adjacent dates within any treatment differed significantly, reflecting the gradual nature of the changes. Fat score also varied significantly over time (Fig. 5B; Table 1), but again temperature treatment was without effect. The significant date effect appears to be primarily due to sharp increases between Days 1 and 10 in the 5° and 20° groups (Table 1). Curiously, body mass did not change significantly concomitant with these changes in fat deposition (Table 1).

Similarly in females, body mass increased following photostimulation (Fig. 6A; Table 2); however, unlike in males there was a significant main effect of temperature, with the 5° birds showing little gain of body mass and the 30° group showing the greatest mass. The only robust difference in mean body mass between treatments occurred between the 5° and 30° birds at Day 10, when the 30° birds had reached a peak and the 5° birds had not changed; several other comparisons approached significance (Table 2). The tem-

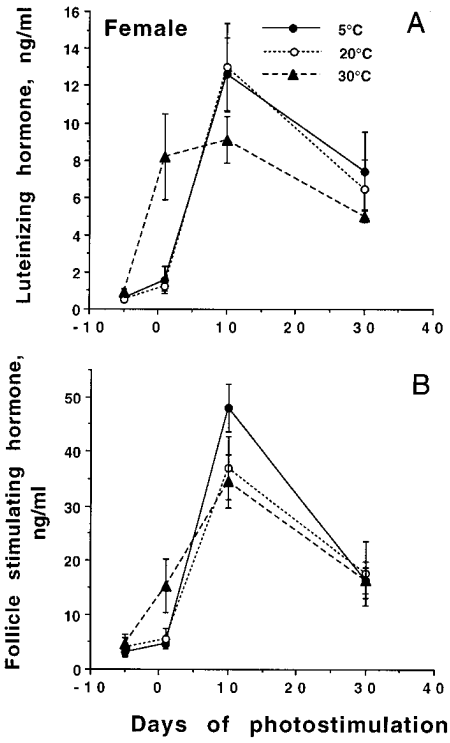

FIG. 4. Effects of temperature on plasma levels of luteinizing hormone (A) and follicle-stimulating hormone (B) in female white-crowned sparrows transferred to 20L 4D from 8L 16D at Day 0. Each point is the mean and vertical lines are the standard errors.

poral pattern of change in fat deposition in females paralleled that of body mass (Fig. 6B; Table 2).

Effects of Temperature and Photoperiod on Circulating Levels of Corticosterone

There was a general trend for increased plasma levels of corticosterone following transfer to long days in both sexes (Fig. 7; Tables 1 and 2). Experimental temperature treatments had no main effect on plasma corticosterone concentrations in males, but there was a significant date by temperature interaction. This appears to be due to the fact the 5° males showed a significant increase in B levels after exposure to 1 long day, while neither of the other temperature treatments changed during this interval (Table 1). None of the post hoc comparisons between treatments at each date were significant after adjustment for multiple comparisons, though it is notable that the comparisons between the 5° group and the 20° and 30° groups approached significance on Day 1.

Temperature also affected B levels in females, in this case emerging as a main effect (Fig. 7B; Table 2). The 5° group appeared to show high and sustained levels of corticosterone compared with the other two treatments. None of the post hoc comparisons between treatments were significant after adjustment for multiple comparisons, but as for males the difference between the 5° and 30° groups after 1 long day approached significance. The temporal changes in female corticosterone levels were very gradual, and there were no differences between adjacent dates within any of the treatments.

Effects of Temperature and Photoperiod on Thyroxine (T4) and Tri-Iodothyronine (T3) Levels

T4 levels in males showed parallel increases following photostimulation in all three temperature treatments (Fig. 8A; Table 1). The most striking increase occurred between Days 1 and 10 in the 5° and 20° birds; the mean T4 level of the 30° birds on Day 10 was similar to the other two groups, but there was substantially greater variability and the change from Day 1 levels was not significant in this group (Table 1). The changes in T3 over time in males were much less dramatic (Fig. 8B), but still significant (Table 1). As for T4 levels there was no effect of temperature treatment. Changes in T3 levels of males were gradual, and none of the post-hoc comparisons of adjacent dates within temperature treatments were significant (Table 1).

Patterns of T4 and T3 secretion in females differed from those of males (Fig. 9; Table 2). As for males, female T4 levels changed over time, but there also was a significant interaction of date and temperature, indicating that the female T4 profiles differed among the temperature treatments (Table 2). This appears to be due to increases between Days 1 and 10 of photostimulation in the 20° and 30° birds but not in the 5° birds. These increases in the 20° and 30° birds are not quite significant after adjustment for multiple comparisons, but they are very nearly so (Table 2). T3 levels of females also varied significantly over time (Fig. 9B), but the variation was rather complex. There was no main temperature effect, but there was a significant interaction of date and temperature treatment. This interaction appears to be due to the fact that levels did not change in the 20° birds (within treatment comparisons between all dates, including nonadjacent

TABLE 2
Statistical Analysis for Females

		LH			FSH			MASS			FAT			B			T4			T3		
EFFECT		F	P		F	P		F	P		F	P		F	P		F	P		F	P	
TEMPERATURE		3.627	*0.043*	**	1.142	0.336		7.819	*0.002*	**	8.240	*0.002*	**	3.407	*0.050*	**	0.819	0.456		1.611	0.225	
DATE		89.294	*0.001*	**	58.717	*0.001*	**	7.419	*0.001*	**	10.604	*0.001*	**	6.673	*0.001*	**	17.945	*0.001*	**	5.919	*0.001*	**
DATE*TEMPERATURE		5.832	*0.001*	**	0.854	0.533		1.419	0.219		1.030	0.413		0.909	0.493		3.188	*0.009*	**	3.464	*0.005*	**
Comparisons within treatments:																						
DAY -5 vs 1	5°	2.580	0.122		0.769	0.389		2.759	0.109		0.000	1.000		2.316	0.141		0.070	0.794		0.855	0.366	
	20°	2.374	0.137		0.785	0.385		3.004	0.095		0.000	1.000		0.781	0.386		4.757	0.042	*	2.149	0.158	
	30°	30.781	*0.001*	**	2.966	0.098		1.345	0.257		1.250	0.274		0.647	0.429		1.602	0.221		1.475	0.239	
DAY 1 vs 10	5°	49.906	*0.001*	**	58.588	*0.001*	**	0.439	0.514		1.853	0.186		0.110	0.743		1.755	0.201		1.145	0.297	
	20°	53.344	*0.001*	**	34.491	*0.001*	**	0.552	0.465		4.672	0.040	*	0.599	0.446		9.069	0.007	*	0.221	0.644	
	30°	0.948	0.340		11.442	*0.002*	**	9.254	0.005	*	6.007	0.022	*	0.847	0.366		11.144	0.003	*	7.454	0.013	*
DAY 10 vs 30	5°	4.985	0.036	*	33.505	*0.001*	**	5.761	0.024	*	6.563	0.017	*	0.164	0.689		1.852	0.190		3.827	0.065	
	20°	9.419	0.005	*	19.809	*0.001*	**	0.294	0.593		0.034	0.855		0.144	0.707		10.618	0.004	*	0.824	0.375	
	30°	4.337	0.049	*	10.382	*0.004*	**	1.260	0.272		0.703	0.410		5.286	0.031	*	1.128	0.301		2.302	0.145	
Comparisons between treatments:																						
DAY -5	5° vs 20°	0.190	0.667		-	-		1.361	0.254		1.594	0.218		1.258	0.273		0.244	0.627		1.598	0.221	
	5° vs 30°	3.645	0.069		-	-		6.103	0.021	*	0.246	0.130		2.410	0.134		1.325	0.264		5.056	0.036	*
	20° vs 30°	5.127	0.033	*	-	-		1.718	0.202		0.140	0.711		0.239	0.629		0.497	0.489		1.229	0.281	
DAY 1	5° vs 20°	0.016	0.901		-	-		1.708	0.203		1.481	0.235		3.625	0.069		1.163	0.294		0.136	0.717	
	5° vs 30°	19.559	*0.001*	**	-	-		5.868	0.023	*	5.845	0.023	*	5.638	0.026	*	0.022	0.882		0.342	0.565	
	20° vs 30°	19.708	*0.001*	**	-	-		1.306	0.264		1.481	0.235		0.336	0.567		0.597	0.449		0.753	0.396	
DAY 10	5° vs 20°	0.266	0.611		-	-		5.448	0.028	*	7.235	0.013	*	1.461	0.239		5.109	0.036	*	0.031	0.861	
	5° vs 30°	0.338	0.567		-	-		26.900	*0.001*	**	23.529	*0.001*	**	2.559	0.123		4.854	0.040	*	1.532	0.230	
	20° vs 30°	1.078	0.310		-	-		8.136	0.009	*	4.947	0.035	*	0.209	0.652		0.052	0.821		1.786	0.196	
DAY 30	5° vs 20°	0.147	0.705		-	-		1.513	0.230		0.154	0.698		1.854	0.186		3.586	0.074		19.853	*0.001*	**
	5° vs 30°	0.654	0.427		-	-		8.697	0.007	*	2.192	0.151		0.085	0.773		0.323	0.577		6.797	0.017	*
	20° vs 30°	0.195	0.663		-	-		2.914	0.100		1.111	0.302		2.232	0.148		4.708	0.043	*	1.446	0.243	

Note. Summary of statistical analyses for female Gambel's white-crowned sparrows. See Table 1 caption for detailed explanation. As for males, due to variation in amount of available plasma for different birds and occasional loss of individual blood samples for a variety of reasons, sample sizes are not identical for all analyses. For temperature effect, degrees of freedom (df) vary from 2,19 to 2,25. For date effect, df vary from 3,57 to 3,75. For date*temperature effect, df vary from 6,57 to 6,75. For post hoc comparisons, df vary from 1,19 to 1,25.

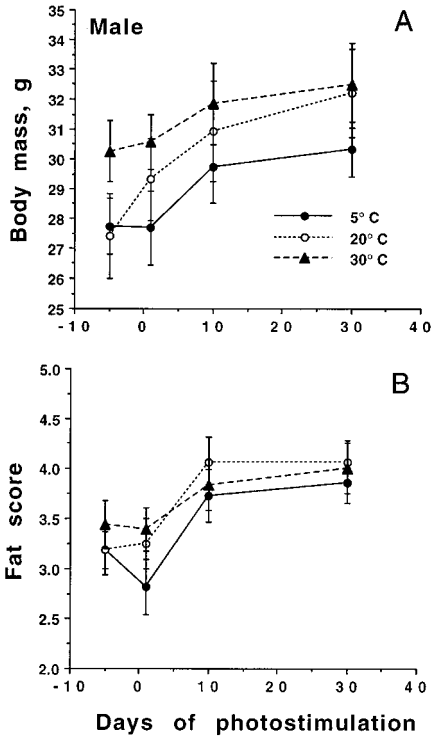

FIG. 5. Effects of temperature on body mass (A) and fat score (B) in male white-crowned sparrows transferred to 20L 4D from 8L 16D at Day 0. Each point is the mean and vertical lines are the standard errors.

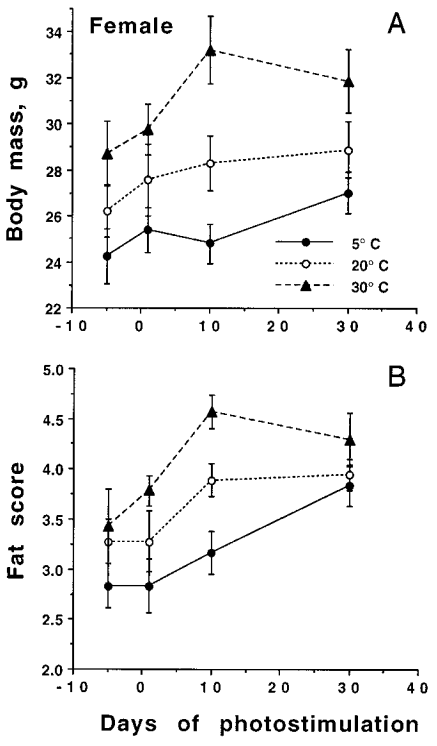

FIG. 6. Effects of temperature on body mass (A) and fat score (B) in female white-crowned sparrows transferred to 20L 4D from 8L 16D at Day 0. Each point is the mean and vertical lines are the standard errors.

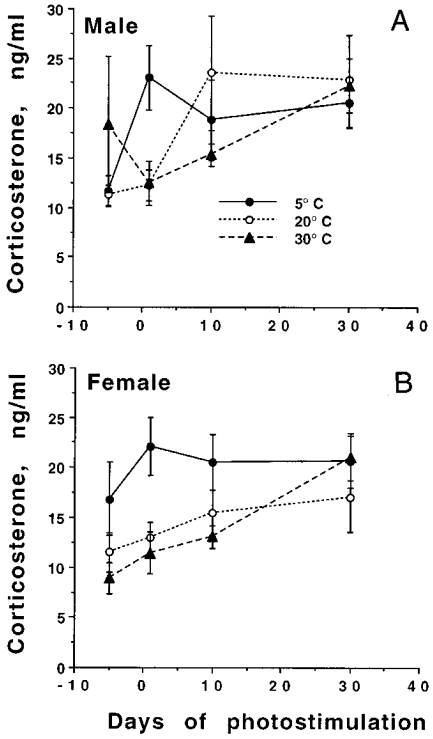

FIG. 7. Effects of temperature on circulating levels of corticosterone in male (A) and female (B) white-crowned sparrows transferred from 8L 16D to 20L 4D at Day 0. Each point is the mean, and vertical lines are the standard errors.

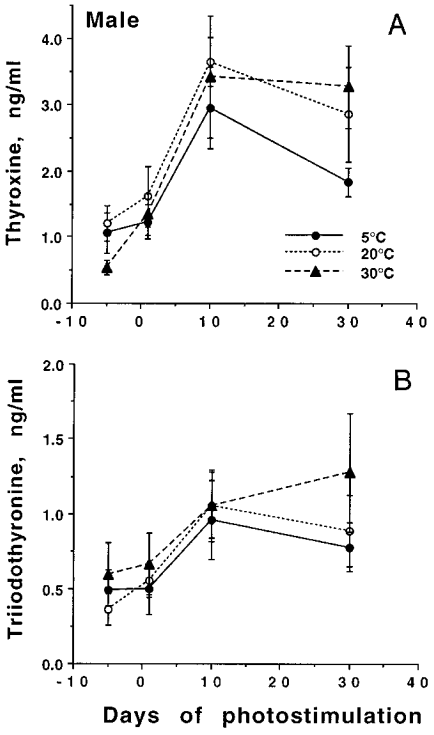

FIG. 8. Effects of temperature on plasma levels of thyroxine (A) and tri-iodothyronine (B) in male white-crowned sparrows transferred to 20L 4D from 8L 16D at Day 0. Each point is the mean and vertical lines are the standard errors.

ones, all $F < 3.827$, all $P > 0.064$, all $df = 1,20$), but did fluctuate in the other two treatments. In particular, T3 levels appeared elevated in the 30° birds by Day 10 as compared with Day −5, though this difference only approached significance when corrected for multiple comparisons ($F = 9.290$, $P = 0.006$, $df = 1,20$). The increase by Day 30 in the 5° birds was the most robust change of all (Day 1 versus Day 30, $F = 24.060$, $P < 0.001$, $df = 1,20$), and it was on Day 30 that T3 levels of 5° birds exceeded those in the 20° birds (Table 2).

DISCUSSION

The failure of low environmental temperature to inhibit photoperiodically induced testicular development or for high environmental temperature to enhance it in *Z.l. gambelii* is consistent with the observations of Farner and Mewaldt (1952) and Lewis and Farner (1973). Female *Z.l. gambelii* were also unaffected by environmental temperature, at least during the period of ovarian development. Furthermore, there was no main effect of temperature on plasma levels of LH and FSH in males consistent with the observations of Wingfield *et al.* (1982) for brief episodes of low (5°) and high (30°) temperature. The somewhat sluggish increase in FSH in males at 5° suggests that FSH secretion in males can be modulated by temperature. However, by Day 10 this effect was no longer evident. Furthermore, this slight delay in FSH increase did not appear to influence testicular development. A similar argument can be made for the high level of LH in females of the 30° group at Day 1 of treatment. In this case LH levels were higher than in both the 5° and 20° groups. By Day 10, however, LH titers in blood had increased in the other two groups to the level of the 30° group, and there was no effect on ovarian follicle development at Day 30. It is possible that gonadal development was enhanced or inhibited during early maturation before we performed laparotomies at Day 30. However, we feel that Day 30 is the

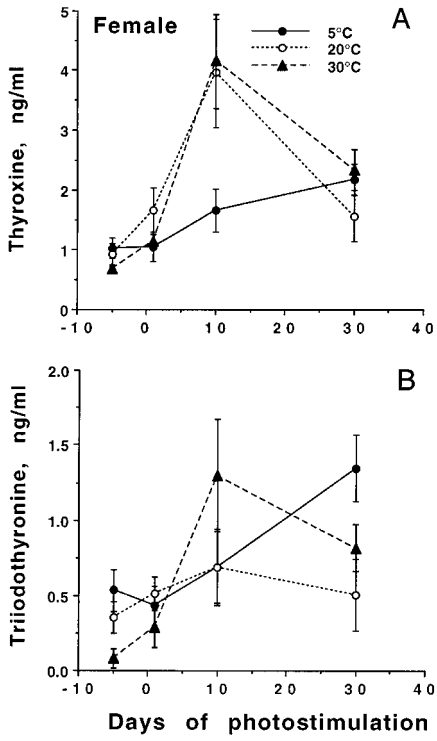

FIG. 9. Effects of temperature on plasma levels of thyroxine (A) and tri-iodothyronine (B) in female white-crowned sparrows transferred to 20L 4D from 8L 16D at Day 0. Each point is the mean and vertical lines are the standard errors.

time when an effect will most likely be seen because although the logarithmic phase of growth is ending, full maturation (at a slower rate) is not attained for another 10–20 days (e.g., Farner and Mewaldt, 1952). In other species studied, temperature effects on gonadal development were certainly clear by this stage (see Wingfield *et al.*, 1992 for review).

In other species it has been shown that low temperatures may inhibit testicular development, possibly related to migratory status (see Wingfield *et al.*, 1992 for review). Lewis and Farner (1973) did detect a slight effect of low temperature on oviduct development in Gambel's white-crowned sparrows, suggesting that although ovarian growth was not affected, its endocrine function may have been. Our data on gonadotropin levels suggest that if there is an effect of temperature on ovarian endocrine secretions, it is not mediated through gonadotropin release.

In willow tits, *Parus montanus,* photostimulation at 20° resulted in advanced maturation up to 2 weeks earlier than in willow tits photostimulated at 10° or 4° (Silverin and Viebke, 1994). However, maximum testis size attained was rather small. No such effect was found following similar treatment of great tits, *P. major* (Silverin and Viebke, 1994). It is also interesting that the patterns of LH secretion in willow tits were identical despite differences in temperature-modulated photostimulation of testis growth. In contrast, LH levels in great tits exposed to 20° were markedly higher than in groups held at 10° and 4°, despite no effect on gonadal development. Silverin and Viebke (1994) suggest that the increase of LH in great tits at 20° may be related to androgen release and activation of singing/aggressive behavior that occur early in the breeding season of this species. These results resemble ours in the sense that temperature-induced differences in photoinduced gonadotropin secretion do not necessarily translate into differences in gonadal development.

In migratory species that have a highly predictable and brief breeding season, low ambient temperature had no effect on testicular recrudescence (Suomalainen, 1937; Engels and Jenner, 1952; Rowan 1925). Sedentary species with longer or more flexible breeding seasons showed reductions in rates of testicular recrudescence at low temperatures (e.g., Kendeigh, 1941; Jones 1986; Storey and Nicholls, 1982). Data for ovarian development are sparse but support the results for males (Ibid). The mechanisms by which temperature may modulate gonadal recrudescence remain unknown.

It is possible that greater extremes of temperature would have had an effect on gonadal development in *Z.l. gambelii,* especially since there were no effects of 5° on circulating levels of thyroid hormones in males. Interestingly, temperature did affect thyroid hormones in females, but these effects were only apparent later in the experiment, when gonadotropin levels were similar in all three treatments. Thus, differences in thyroid hormone secretion do not appear to help explain accelerated gonadotropin secretion in females at 30° on Day 1. Furthermore, T4 levels tended to be *reduced* in females at low temperature. Smith (1982) has shown that low ambient temperature can *increase* circulating T3 and T4, suggesting that in this experiment, the temperature limits were within a range that will not directly influence thyroid hormone secretion. However, we were careful to select low and high temperatures that were within the normal (i.e., predict-

able) range that *Z.l. gambelii* would experience during spring (see Wingfield and Kenagy, 1991). Exposure to temperatures outside this range may potentially elicit a stress response and gonadal development would be affected by a different mechanism. In other words, temperatures outside the normal range to be expected for the season may act as modifying factors — a different category of environmental signals with potentially very different transduction pathways within the central nervous system (see Wingfield, 1983, 1988).

In the domestic fowl, low environmental temperature has the potential to increase circulating levels of corticosterone that in turn inhibits the reproductive system (e.g., Siegel, 1980). Elevated adrenocortical secretions can have profound inhibitory effects on the reproductive systems of most vertebrates (e.g., Moore and Miller, 1984; Greenberg and Wingfield, 1987). In this study, corticosterone levels were indeed somewhat elevated in the 5° birds of both sexes on Day 1. This difference in corticosterone secretion on Day 1 could explain the slightly reduced FSH secretion in males at 5° on that day; apparently it had no effect on LH in males. In females, on the other hand, gonadotropin secretion was similarly sluggish in the 5° and 20° birds on Day 1, whereas corticosterone was (marginally) elevated only in the 5° birds on that day. After Day 1, there were no differences in corticosterone levels among treatments, and the highest corticosterone levels were still well below those induced by stress (Wingfield *et al.,* 1982), indicating that although the experimental temperatures were at the limits normally expected during gonadal development, they were not stressful. In any case, none of the differences observed in corticosterone or other hormones translated into differences in ovarian development. These data suggest that temperatures which may potentially have dramatic effects on gonadal recrudescence in passeriform species breeding at lower latitudes are ineffective in modulating photoperiodically induced gonadal development in *Z.l. gambelii* consistent with the hypothesis from Wingfield *et al.* (1992, 1993).

It is also possible that a combination of photostimulation at shorter day lengths with the same temperature treatments may have resulted in a significant effect on gonadal development. This aspect could be tested but would require more birds and environmental chambers than were feasible in the current investigation. The plan here was to test the possibility that temperature may modulate gonadal development in a bird with a highly predictable breeding season and a low I_e factor so that these results can be compared with other closely related taxa with higher I_e factors. To do this requires careful comparisons, and we felt that each taxon should be tested at the maximal day length normally experienced by that population to avoid possible artifacts of longer or shorter day lengths. We feel that additional experiments are certainly warranted, but this does not detract from the conclusions being made here. Note especially that other experiments investigating effects of low temperature on testis development in *Z.l. gambelii* used different day lengths. Farner and Mewaldt (1952) exposed males to 15L 9D; Lewis and Farner (1973) used 18L 6D, and we used 20L 4D. In all cases the effects of temperature on gonadal development were nonexistent or very slight, suggesting that the photoperiod we chose did not mask effects of temperature.

The classification of types of environmental factors regulating reproduction (Wingfield, 1983) has also been applied to the regulation of vernal and autumnal migration (Wingfield *et al.,* 1990). It was proposed that initial predictive information such as photoperiod provides long term predictive cues to begin preparations for migration. Then short term predictive factors such as temperature, wind, food availability, etc. (i.e., supplementary factors) are integrated to varying degrees depending upon the predictability of the migration season. There have been extensive investigations on the control of body mass and fat depots for migration by photoperiod in several avian species (e.g., Ramenofsky, 1990; Wingfield *et al.,* 1990), but few have studied integration of photoperiodic responses with temperature. In male *Z.l. gambelii,* photoperiodically induced increases in body mass and fat score were unaffected by temperature, but in females, the 5° group showed less accumulation of fat and lower body mass than the other groups (particularly than the 30° group), suggesting that low temperature may indeed inhibit preparations for migration in females.

These data are unlike the results of Lewis and Farner (1973) who found that premigratory fattening progressed normally at low temperatures but was inhibited at higher temperatures (e.g., 34.5°). Reasons for this disparity are not clear, but may be related to different temperature regimes used, length of the experimental long days, or the fact that their birds were

acclimated gradually to the experimental temperature regimes. Clearly, temperature has an effect on vernal premigratory functions in female *Z.l. gambelii,* but further experiments are needed to clarify the relationship.

In the investigations reported here, the low temperature groups of both sexes had marginally higher circulating levels of corticosterone, but only after the first day of photostimulation and temperature treatment. Whether or not this early rise in corticosterone may represent a possible mechanism in females for reduced fattening later in the photostimulation cycle remains to be determined; it appears not to have this effect in males. As noted above it is unlikely that the birds were stressed since the highest corticosterone levels were still well below those measured in chronically stressed individuals (Wingfield *et al.,* 1982), and furthermore, gonadal development was not affected. These data suggest that preparations for migration may be influenced more by temperature than is ovarian development, but the nature of the endocrine mechanisms require further work.

There is evidence from the field that bad weather, including low temperature, can delay onset, slow down, or even reverse vernal migration in several avian species (e.g., Elkins, 1983; Williams and Williams, 1990). Greater temperature sensitivity of the fattening response in females could reflect some propensity to modify the migratory schedule. However, the reasons underlying the sexual dimorphism of the fattening response in *Z.l. gambelii* are obscure. One could speculate that the resistance of males to an effect of temperature on migration physiology may be related to the selective pressure for them to arrive on the breeding grounds first. Females on the other hand arrive later when weather may be more benign.

Increased day length resulted in dramatic increases in T4 levels in both sexes. This is consistent with a role for T4 in the induction of photorefractoriness and postnuptial moult (e.g., Nicholls *et al.,* 1988). However, the 5° treatment appeared to suppress this cycle of T4 secretion in females which is contrary to expectations that low temperature should increase thyroid hormone release that is thought to regulate metabolism accordingly (e.g., Assenmacher, 1973; Smith, 1982). It does appear that temperature may have influenced the rate at which photoperiodically induced functions progress. This aspect clearly requires further study and may represent a hitherto unexpected effect of ambient temperature on photoperiodically induced vernal functions.

Clearly there is great flexibility of the control systems for reproductive and associated cycles and the mechanisms by which they integrate environmental signals. Further tests on other taxa that experience varying predictability and that have different I_e factors will indicate whether models for integration of environmental signals will prove to be useful in general.

ACKNOWLEDGMENTS

These investigations were supported by grant number DCB-9005081 from the National Science Foundation to J.C.W. Many thanks to Lynn Erckmann for her expert assistance with maintaining the birds and for performing the corticosterone assays. We are grateful to Marilyn Ramenofsky, Troy Smith, and Mark Stanback who helped us collect blood samples.

REFERENCES

Assenmacher, I. (1973). The peripheral endocrine glands. *In* "Avian Biology" (D. S. Farner and J. R. King, Eds.), Vol. 3, pp. 183–286. Academic Press, New York.

Boswell, T. (1991). "The Physiology of Migratory Fattening in the European Quail (*Coturnix coturnix*)." Ph.D. thesis, University of Bristol.

Cohen, D. (1967). Optimizing reproduction in a varying environment. *J. Theor. Biol.* **16,** 1–14.

Colwell, R. K. (1974). Predictability, constancy and contingency of periodic phenomena. *Ecology* **55,** 1148–1153.

Elkins, (1983). "Weather and Bird Behaviour." Poyser Press, Calton, UK.

Engels, W. L., and Jenner, C. E. (1952). The effect of temperature on testicular recrudescence in juncos at different photoperiods. *Biol. Bull.* **110,** 129–137.

Farner, D. S., and Follett, B. K. (1979). Reproductive periodicity in birds. *In* "Hormones and Evolution" (E. J. W. Barrington, Ed.), pp. 829–872. Academic Press, New York.

Farner, D. S., and Lewis, R. A. (1973). Field and experimental studies of the annual cycles of white-crowned sparrows. *J. Reprod. Fertil.* **19**(suppl.), 35–50.

Farner, D. S., and Mewaldt, L. R. (1952). The relative roles of photoperiod and temperature in gonadal recrudescence in male *Zonotrichia leucophrys gambelii*. *Anat. Rec.* **113,** 106.

Follett, B. K. (1984). Birds. *In* "Marshall's Physiology of Reproduction, Vol. 1. Reproductive Cycles of Vertebrates" (G. E. Lamming, Ed.), pp. 283–350. Churchill Livingstone, Edinburgh.

Follett, B. K., Scanes, C. G., and Cunningham, F. (1972). A radioimmunoassay for avian luteinizing hormone. *J. Endocrinol.* **52,** 359–378.

Greenberg, N., and Wingfield, J. C. (1987). Stress and reproduction: Reciprocal relationships. *In* "Hormones and Reproduction in Fishes, Amphibians and Reptiles" (D. O. Norris and R. E. Jones, Eds.), pp. 461–503. Plenum, New York.

Immelmann, K. (1971). Ecological aspects of periodic reproduction. *In* "Avian Biology" (D. S. Farner and J. R.King, Eds.), Vol. 1, pp. 341–389. Academic Press, New York.

Immelmann, K. (1973). Role of the environment in reproduction as a source of predictive information. *In* "Breeding Biology of Birds" (D. S. Farner, Ed.), pp. 121–147. Natl. Acad. Sci. U.S.A., Washington, D.C.

Jones, L. R. (1986). The effect of photoperiod and temperature on testicular growth in captive black-billed magpies. *Condor* **88,** 91–93.

Kendeigh, S. C. (1941). Length of day and energy requirements for gonadal development and egg-laying in birds. *Ecology* **22,** 237–246.

Lewis, R. A., and Farner, D. S. (1973). Temperature modulation of photoperiodically induced vernal phenomena in white-crowned sparrows (*Zonotrichia leucophrys*). *Condor* **75,** 279–286.

Marshall, A. J. (1970). Environmental factors other light involved in the control of sexual cycles in birds and mammals. *In* "La Photorégulation de la Reproduction Chez les Oiseaux et les Mammifères" (J. Benoit and I. Assenmacher, Eds.), pp. 53–64. Cent. Natl. Rech. Sci.

Moore, F. L., and Miller, L. J. (1984). Stress-induced inhibition of sexual behavior: corticosterone inhibits courtship behaviors of a male amphibian (*Taricha granulosa*). *Horm. Behav.* **18,** 400–410.

Nicholls, T. J., Goldsmith, A. R., and Dawson, A. (1988). Photorefractoriness in birds and comparison with mammals. *Physiol. Rev.* **68,** 133–176.

Ramenofsky, M. (1990). Fat storage and fat metabolism in relation to migration. *In* "Bird Migration" (E. Gwinner, Ed.), pp. 214–231. Springer-Verlag, Berlin.

Rowan, W. (1925). Relation of light to bird migration and development changes. *Nature* **115,** 494–495.

Sakai, H., and Ishii, S. (1985). A homologous radioimmunoassay for avian follicle stimulating hormone. *In* "Current Trends in Comparative Endocrinology" (B. Lofts and W. N.Holmes, Eds.) pp. 195–197. Univ. Hong Kong Press, Hong Kong.

Sharp, P. J., Dunn, I. C., and Talbot, R. T. (1987). Sex differences in response to chicken LHRH-I and II in the domestic fowl. *J. Endocrinol.* **115,** 323–331.

Siegel, H. S. (1980). Physiological stress in birds. *BioScience* **30,** 529–534.

Silverin, B., and Viebke, P. A. (1994). Low temperatures affect the photoperiodically induced LH and testicular cycles differently in closely related species of tits (*Parus* sp). *Horm. Behav.* **28,** 199–206.

Smith, J. P. (1982). Annual cycle of thyroid hormones in the plasma of white-crowned sparrows and house sparrows. *Condor* **84,** 160–167.

Storey, C. R., and Nicholls, T. J. (1982). Low environmental temperature delays photoperiodic induction of avian testicular maturation and the onset of postnuptial photorefractoriness. *Ibis* **124,** 172–174.

Suomalainen, H. (1937). The effect of temperature on the sexual activity of non-migratory birds stimulated by artificial lighting. *Ornis Fenn.* **14,** 108–112.

Tasaki, Y., Inoue, M., and Ishii, S. (1986). Annual cycle of plasma thyroid hormone levels in the toad, *Bufo japonicus*. *Gen. Comp. Endocrinol.* **62,** 404–410.

Wada, M. (1993). Low environmental temperature and short days together induce thyroid activation and suppression of LH release in Japanese quail. *Gen. Comp. Endocrinol.* **90,** 355–363.

Williams, T. C., and Williams, J. M. (1990). The orientation of transoceanic migrants. *In* "Bird Migration" (E. Gwinner, Ed.), pp. 7–21. Springer-Verlag, Berlin.

Wingfield, (1983). Environmental and endocrine control of reproduction: an ecological approach. *In* "Avian Endocrinology: Environmental and Ecological Aspects" (S.-I. Mikami, and M. Wada, Eds.), pp. 149–166. Jpn. Sci. Soc. Press, Tokyo and Springer-Verlag, Berlin.

Wingfield, J. C. (1988). Changes in reproductive function of free-living birds in direct response to environmental perturbations. *In* "Processing of Environmental Information in Vertebrates" (M. H. Stetson, Ed.), pp. 121–148. Springer-Verlag, Berlin.

Wingfield, J. C., and Farner, D. S., (1975). The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. *Steroids* **26,** 311–327.

Wingfield, J. C., and Farner, D. S. (1976). Avian endocrinology—Field investigations and methods. *Condor* **78,** 570–573.

Wingfield, J. C., and Farner, D. S. (1978). The endocrinology of a naturally breeding population of the white-crowned sparrow (*Zonotrichia leucophrys pugetensis*). *Physiol. Zool.* **51,** 188–205.

Wingfield, J. C., and Farner, D. S. (1980). Environmental and endocrine control of seasonal reproduction in temperate zone birds. *Prog. Reprod. Biol.* **5,** 62–101.

Wingfield, J. C., and Kenagy, G. J. (1991). Natural control of reproduction. *In* "Vertebrate Endocrinology: Fundamentals and Biomedical Implications" (P. K. T. Pang and M. P. Schreibman, Eds.), Vol. 4B, pp. 181–242. Academic Press, New York.

Wingfield, J. C., Hahn, T. P., and Doak, D. (1993). Integration of environmental cues regulating transitions of physiological state, morphology and behavior. *In* "Avian Endocrinology" (P. J. Sharp, Ed.), pp. 111–122. *J. Endocrinol. Ltd.,* Bristol.

Wingfield, J. C., Hahn, T. P., Levin, R., and Honey, P. (1992). Environmental predictability and control of gonadal cycles in birds. *J. Exp. Zool.* **261,** 214–231.

Wingfield, J. C., Hegner, R. E., and Lewis, D. (1991). Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the coopertively breeding white-browed sparrow weaver, *Plocepasser mahali. J. Zool.* (*Lond.*) **225,** 43–58.

Wingfield, J. C., Schwabl, H., and Mattocks, P. W., Jr. (1990). Endocrine mechanisms of migration. *In* "Bird Migration" (E. Gwinner, Ed.), pp. 232–256. Springer-Verlag, Berlin.

Wingfield, J. C., Smith, J. P., and Farner, D. S. (1982). Endocrine responses of white-crowned sparrows to environmental stress. *Condor* **84,** 399–409.