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Effects of Day Length and Temperature on Gonadal Development, Body Mass, and Fat Depots in White-Crowned Sparrows, *Zonotrichia leucophrys pugetensis*

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We tested the effects of ambient temperature (5°, 20°, and 30°) on photoperiodically induced reproductive functions in male and female white-crowned sparrows, *Zonotrichia leucophrys pugetensis.* Transfer from short days (9L 15D) to long days (16L 8D) resulted in rapid testicular growth and partial ovarian development in all three temperature treatments. There were no differences in sizes of testes and cloacal protuberance following 30 or 70 days of exposure to long days at the different temperatures. However, brood patch and follicular development were enhanced in females at 30° compared with the 5° and 20° groups. Many of these females exposed to 30° had large yolky follicles by Day 70. This enhancement was evident only when females were housed in the same room with males, however. Despite the effects of high temperature on ovarian development, there were no differences among groups in plasma levels of follicle-stimulating hormone or luteinizing hormone, suggesting that differential ovarian development may have been mediated by gonadal sensitivity to gonadotropins rather than by differential secretion of these hormones. We examined circulating levels of corticosterone (B) and both tri-iodothyronine (T_3) and thyroxine (T_4) as possible regulators of this differential ovarian sensitivity to gonadotropins. Plasma B levels showed transitory increases in males at 5° and 20°, but were suppressed in males at 30°. Titers of B were not influenced by temperature treatments in females. Circulating T_4 increased following photostimulation in both sexes, but this increase was reduced at 5°. T_3 concentrations in plasma were highly variable and not influenced by either photoperiod or temperature in males, but were significantly lower in females exposed to 30° by Day 70. Thus, B and T_4 levels do not appear to help explain differential ovarian development, but circulating T_3 levels cannot yet be excluded as a regulator of ovarian sensitivity to gonadotropins. Long days resulted in no change, or a gradual decrease, in body mass and fat deposit in males and females, and temperature regimes had no further effects on fattening or body mass. Thus, reproductive development under long days appears to be resistant to naturally relevant temperature extremes in male *Z.l. pugetensis,* whereas follicular development (i.e., yolk deposition in follicles leading to ovulation and onset of nesting) can be enhanced by high temperature. Reasons for the dimorphism in this response are unknown, but may be explained by the role of females in determining onset of final ovarian maturation and nesting in relation to favorable environmental conditions. In a second experiment, in which the sexes were isolated from one another, we determined the effects of the same treat-

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ments on *Z.l. pugetensis.* Again there was no effect of temperature on photoperiodically induced testicular growth, and the enhancement of follicular development in females at 30° was greatly reduced in the absence of males. We also continued this experiment up to 116 days of treatment to investigate effects on onset of photorefractoriness (spontaneous gonadal regression) and onset of prebasic moult. In both sexes it was clear that low temperature (5°) retarded gonadal regression and high temperature (30°) advanced it. Similarly, the prebasic moult score was greater at 30° and less at 5° in both sexes. There were no effects of temperature on plasma levels of LH at Day 116 of treatment, but plasma levels of T_4 were higher in the 5° group of both males and females sampled at Day 116. Clearly, the effects of temperature can have different effects on gonadal recrudescence, onset of breeding (yolk deposition), *and* termination of breeding. Whether these influences of temperature on reproductive function at different stages in the breeding cycle have different mechanisms remains to be determined.

The role of the annual change in day length in regulation of vertebrate reproductive cycles has received detailed attention over the past few decades (e.g., Farner and Follett, 1979; Follett, 1984; Wingfield and Kenagy, 1991). Although it is well established that influences of photoperiod are of major importance, it must be made clear that other environmental cues are also critical for regulation of gonadal development (e.g., Marshall, 1970; Immelmann, 1971, 1973; Wingfield 1983; Wingfield and Kenagy, 1991).

Wingfield (1983) suggested that there are two distinct types of environmental signals that regulate gonadal development and regression. One type provides long-term predictive signals (initial predictive information) and initiates gonadal maturation several weeks in advance of the breeding season. The second type includes many cues from the immediate environment (e.g., temperature, food, rainfall, etc.) that provide short-term predictive signals (supplementary information). The latter can speed up or slow down gonadal development regulated by initial predictive cues such as photoperiod (see also Marshall, 1959; Wingfield and Kenagy, 1991; Wingfield *et al.,* 1992a). It should also be noted that the reproductive maturation process involves regulation of gonadal growth to a "prebreeding" stage, and then final onset of breeding is triggered when local conditions are favorable (Marshall, 1959, 1970). The latter process is most easily identified in females and involves synthesis and deposition of yolk leading to ovulation (e.g., King *et al.,* 1966; Wingfield and Farner, 1980; Wingfield and Kenagy, 1991). Supplementary factors may act on both processes, although the mechanisms remain essentially unknown.

The degree to which individuals integrate these two types of environmental signals that time gonadal development and thus regulate secretion of gonadotropins depends upon the predictability of their breeding seasons (see Wingfield *et al.,* 1992a). Cohen (1967) suggested that if a future event such as onset of breeding is highly predictable, then only one or a few reliable environmental cues should be required to time breeding, and many other environmental signals can be ignored. On the other hand, if the breeding season is less predictable, i.e., variation due to cold or warm spring seasons etc., then an individual should integrate many environmental cues to ensure that reproduction is timed optimally. Construction of matrices of egg-laying dates by month for as many years as there are data available and subjected to log linear analysis revealed that the Puget Sound white-crowned sparrow, *Zonotrichia leucophrys pugetensis,* has a highly predictable breeding season and that initial predictive information such as photoperiod would provide the major cue to time gonadal development (Wingfield *et al.,* 1993). However, 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 fluctuations in environmental conditions). Wingfield *et al.* (1992a) applied Colwell's formulae to the matrices of egg-laying dates in birds and suggested that the ratio of contingency to constancy (called the environmental information factor, I_e) indicates 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. pugetensis* revealed that predictability was high, suggesting a strong effect of photoperiod, and that the I_e factor was higher (about 4.0) than that of *Z.l. gambelii* (about 1.0), a taxon breeding at high latitude in which the spring season is

very short. This higher I_e factor in *Z.l. pugetensis* suggests that supplementary cues such as local temperature, food availability, etc. would be important in timing gonadal development and onset of breeding (Wingfield *et al.*, 1992a, 1993).

Hypotheses derived from the calculations of Wingfield *et al.* (1992a, 1993) suggest that *Z.l. pugetensis*, with a predictable breeding season in the Pacific Northwest region of the United States, should respond to both increased day length and environmental temperature. Here we exposed photostimulated male and female *Z.l. pugetensis* to long days at low (5°C), moderate (20°C), and high (30°C) environmental temperatures. Circulating gonadotropin levels as well as concentrations of corticosterone (to assess potential stress of treatment) and thyroid hormones (to assess whether potential effects could be attributed to a mechanism of action via the thyroid gland) were also measured.

Wingfield *et al.* (1993) point out that these mathematical approaches may also be applied to seasonal phenomena other than gonadal development, such as preparations for migration, termination of breeding (gonadal regression), and moult. To test this we have determined the effects of temperature treatments on photoperiodically induced premigratory functions such as changes in body mass and fat depot and later on gonadal regression and prebasic moult. Since in the first experiment males and females were in visual and auditory contact, we conducted an additional experiment with sexes isolated to determine whether social cues might also influence responses to the physical environment.

MATERIALS AND METHODS

Capture and Housing of Birds

Male and female *Z.l. pugetensis* were captured in Japanese mist nets during the postbreeding season. Capture sites were at breeding grounds in Pierce and San Juan Counties in western Washington State (48°N). Birds were transported to Seattle and held in outdoor aviaries at the Department of Zoology for at least 3 weeks. They were then housed in cages (55 × 25 × 25 cm) one per cage, in environmental chambers held at 20°C, and a daily photoperiod of 9 hr light 15 dark (9L 15D). In experiment 1 (see below), each chamber contained males and females and both sexes could see and hear one another throughout the experiment. In experiment 2, each chamber contained only a single sex. In both experiments contact was prevented by housing one bird per cage. All birds were fed mixed seeds, Mazuri small bird maintenance diet, and water *ad libitum*.

Sampling Techniques

Birds were sexed by unilateral laparotomy at least 1 month before the experiment began. Further laparotomies were performed at Days 30 and 70 (experiment 1) or Days 31, 75, and 116 (experiment 2) of photostimulation and temperature treatment. 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 millimeter, as was the 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 simple 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. All laparotomies were performed with birds under light general anesthesia using Metofane (Pittman-Moore, Inc.).

Blood samples were collected in heparinized microcapillary tubes from a wing vein after puncture with a 26-gauge needle. Approximately 200–250 µl of whole blood was collected from each bird. Plasma was separated by centrifugation, harvested with a Hamilton microsyringe, 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 from 0 to 5, in which 0 is no visible fat and 5 is gross bulging fat bodies that far exceed the normal limits within the furcular and

abdominal fat pads of birds (see Wingfield and Farner, 1978). 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, the presence of a brood patch was assessed by examining the ventral skin of the breast and abdomen. If no patch was present this represented 0% development. The percentage of defeathering of the patch can be estimated (i.e., 10, 50, 80, or 100%) since the patch has a very clear edge that is unambiguous (see Wingfield and Farner, 1976). The percentage of defeathering was then converted to a brood patch score by dividing by 10 (i.e., a brood patch score of 0–10).

In experiment 2 we assessed all birds for the prebasic moult by inspecting primary wing feathers on each wing at Days 70 and 116 of experimental treatment. There are nine primaries and the number in moult on each wing (absent or growing) was counted and their positions in the moult sequence were noted. Moult data are presented as the mean position in the moult sequence to give a moult score (stage in moult). The higher the score, the more advanced the moult stage.

Experiment 1

All birds were maintained on 9L 15D 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 in this species (i.e., all birds would have been photorefractory when captured in late summer, e.g., Farner and Follett, 1979). Five days prior to transfer to long days, all birds were bled and weighed, and fat score, cloacal protuberance length (CPL), and brood patch assessed. At Day 0, all three constant environment chambers were transferred to 16L 8D, and one chamber was set to 5°, a second maintained at 20°, and a third set to 30°. These temperatures were selected as representative of the range of temperatures likely to be experienced by free-living *Z.l. pugetensis* during gonadal development and vernal migration (March to mid-April). These assumptions were confirmed by examination of weather records at selected locations along the migratory route from central California to western Washington (see also Wingfield *et al.*, 1992a).

Day 1 after transfer to experimental treatments, all birds were bled and weighed, and fat score was assessed. The same sampling was repeated on all birds at Days 10 and 20 of treatment. 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 and weighed, and fat score, CPL, and brood patch assessed. Unilateral laparotomy was performed on all birds to determine developmental state of the gonads. Similar data were also collected at Day 70 of treatment to determine if any final maturation of the reproductive system (especially ovary) had occurred. Note that although males and females within each treatment group were in visual and auditory contact, all birds were caged singly and thus tactile stimuli were precluded.

Experiment 2

The experiment described above was repeated but this time sexes were isolated in separate chambers. This was to check for possible social effects on temperature-modulated responses to long day lengths. Temperature and photoperiod treatments were as described above. Unilateral laparotomy was performed and blood samples were collected just prior to experimental treatment and then at Days 30, 75, and 116 following transfer to long days and temperature treatments. Thus we could follow gonadal development and hormone levels in relation to temperature regimes with isolated sexes. The sample at Day 116 allowed us to assess possible effects of temperature on the termination of breeding and onset of moult. Gonadal volume was assessed as described above.

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 passeriforme species (see Wingfield *et al.*, 1992b, 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.*, 1992b).

Circulating follicle-stimulating hormone (FSH) was also measured by a double-antibody postprecipitation radioimmunoassay as developed by Sakai and Ishii (1985) using chicken FSH for standards and iodination (by the lactoperoxidase method) and 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. Intra-assay variation was 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. Approximately 10-µl aliquots of plasma were equilibrated with 2000 cpm of tritiated corticosterone (for determination of percentage of recovery following extraction in each sample) and diluted to 200 µl in distilled water. After equilibration, samples were extracted in 5 ml freshly redistilled dichloromethane, organic phase aspirated, and taken to dryness under a stream of nitrogen in a water bath at 45°. Dried extracts were then reconstituted in phosphate-buffered saline with gelatin and sodium azide and assayed in duplicate. A small (100 µl) aliquot was taken to a scintillation vial for determination of percentage of recovery following extraction. The assay followed that of Wingfield *et al.* (1992c). Bound and free fractions were separated by addition of dextran-coated charcoal. Assay reliability criteria were well within the limits described by Wingfield and Farner (1975) and Wingfield *et al.* (1992c).

Plasma levels of thyroxine (T_4) and tri-iodothyronine (T_3) were assayed by separate radioimmunoassay with polyethylene glycol precipitation for separation of bound and free counts (after Wada, 1993; and Tasaki *et al.*, 1986). The antibodies were purchased from Endocrine Sciences (Tarzana, California) and used in appropriate dilutions ($\times$1000 for T_4 and $\times$3000 for T_3) determined by preliminary binding tests. Stock solutions for standards were prepared by diluting T_4 (free salt) and T_3 (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 γ-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 gonadal condition, hormone levels, body mass, and fat deposition over time were assessed by ANOVA for repeated measures with one grouping factor (temperature treatment). When necessary, data were transformed to natural logarithms to reduce heteroscedasticity. Univariate *F* tests were used for post hoc comparisons both among different sampling dates within temperature treatments and between different temperature treatments at each sampling date. In general, significance was set at $P = 0.05$, and differences indicated by post hoc comparisons were only considered robust if the Bonferroni criterion of *P* divided by the total number of comparisons was met. In the tables summarizing statistics, these robust differences are indicated by double asterisks; less robust differences that may be real but not meeting this criterion (i.e., $P <= 0.05$ but > 0.05 when divided by the number of pairwise comparisons) are indicated by a single asterisk. When sampling date was the only significant ANOVA effect, we only made post hoc comparisons of adjacent sampling dates within temperature treatments to determine when hormone levels were changing during the study. When temperature treatment and/or the interaction of temperature and sampling date also were significant, then we made between-treatment comparisons at each sampling date as well.

In the case of changes in gonadal condition, precise measurements of testes and ovarian follicles were not

made when the birds were laparotomized prior to photostimulation; gonads at this time were simply noted to be uniformly regressed in both sexes. These data are included in the figures but statistical analyses were restricted to data collected at the laparotomies after photostimulation (Days 30 and 70 in experiment 1 and Days 31, 75, and 116 in experiment 2).

For data on secondary sex characters in experiment 1 we present analyses by one-factor ANOVA only at Day 30 (cloacal protuberance length) or at Day 75 (brood patch) of photostimulation.

RESULTS

Experiment 1: Sexes Together

Effects of temperature on photoperiodically induced testicular development and gonodotropin profiles in males. Photostimulation resulted in an increase in testicular development (Fig. 1a). There was a provocative trend for increasing temperature to enhance gonad growth but this was not significant; there were no significant effects in the ANOVA based on data collected at Days 30 and 70 (temperature effect: $F = 2.315$, $P = 0.121$, $df = 2,23$; date effect: $F = 0.067$, $P = 0.798$, $df = 1,23$; interaction of temperature and date: $F = 0.373$, $P = 0.693$, $df = 2,23$). Exposure to 16L 8D also resulted in the expected increases in plasma gonadotropins (Figs. 1b and 1c; Table 1; all further statistics for males in experiment 1 appear in Table 1 unless otherwise noted). Temperature had no effect on LH levels, which increased steadily in all groups from Day −5 through Day 10 and then leveled off (Fig. 1b; Table 1). In contrast, there was a significant date by temperature treatment interaction effect on FSH levels (Table 1), which apparently resulted from a slight acceleration of the FSH increase at 30° and delay in the 5° groups, but only on one day (Fig. 1c; Table 1).

Effects of temperature on photoperiodically induced ovarian development and gonadotropin profiles in females. Transfer to long days resulted in marked development of ovarian follicles (Fig. 2a). Repeated measures ANOVA on data from Days 30 and 70 revealed significant effects of temperature and sampling date but not the interaction of the two on follicle size (temperature: $F = 11.923$, $P = 0.001$, $df = 2,16$; date: $F = 6.595$, $P = 0.021$, $df = 1,16$; interaction of temperature and date: $F = 2.975$, $P = 0.080$, $df = 2,16$). There were no differences between treatments at Day 30 (all $F < 3.30$, all $P > 0.08$); however, follicular development of females at 30° increased significantly from Day 30 to Day 70 ($F = 12.846$, $P = 0.002$, $df = 1,16$), while follicle size did not change in the 5° and 20° females (both $F < 0.70$, both $P > 0.40$). By Day 70, several females at 30° had deposited yellow yolk in some of their follicles, indicative of having made the transition into rapid final ovarian maturation that immediately precedes egg-laying in the wild. At this time, the 30° females had significantly larger follicles than both the

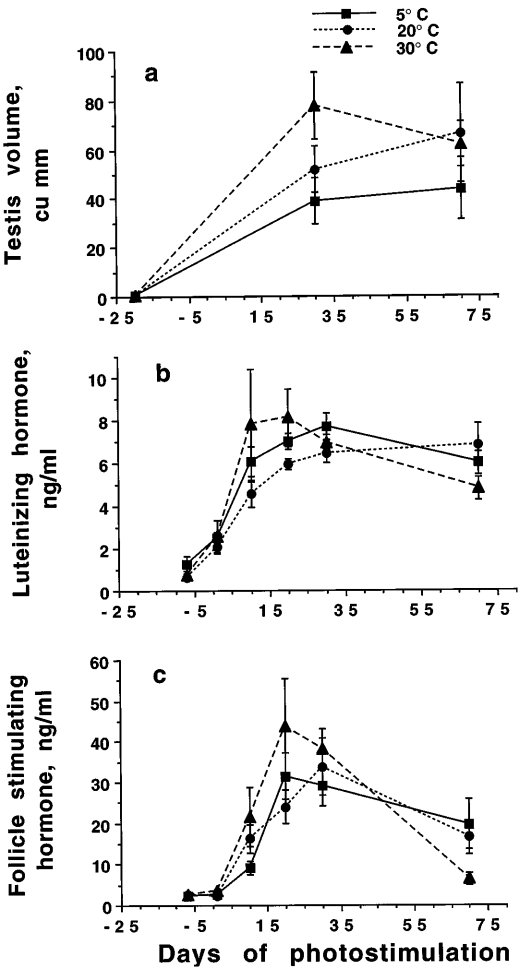

FIG. 1. Effects of temperature and photoperiod on (a) testicular development, (b) plasma levels of luteinizing hormone, and (c) plasma levels of follicle-stimulating hormone in male *Z.l. pugetensis.* Points and bars are means ± standard errors. $N = 10$, 8, and 8 for the 5°, 20°, and 30° groups, respectively.

TABLE 1
Statistical Analysis for Males, Experiment 1

	LH		FSH		Mass		Fat		B		T_4		T_3	
Effect:	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Temperature:	1.642	0.214	0.361	0.701	0.218	0.806	0.934	0.406	4.212	*0.027***	6.061	*0.008***	0.023	0.977
Date:	118.776	*0.001***	89.325	*0.001***	16.450	*0.001***	24.801	*0.001***	4.022	*0.002***	19.800	*0.001***	3.190	*0.010***
Date · temperature:	1.244	0.270	2.712	*0.006***	1.063	0.396	0.745	0.681	2.192	*0.022***	4.654	*0.001***	0.724	0.701
Comparisons within treatments														
Day −5 vs 1														
5°	12.824	*0.001***	0.247	0.623	29.718	*0.001***	15.973	*0.001***	1.135	0.297	0.269	0.609	0.055	0.816
20°	15.049	*0.001***	0.086	0.772	24.456	*0.001***	0.219	0.644	1.776	0.195	0.149	0.703	0.000	0.986
30°	15.623	*0.001***	3.497	0.073	15.214	*0.001***	0.219	0.644	0.097	0.758	0.208	0.653	0.926	0.346
Day 1 vs 10														
5°	18.871	*0.001***	21.049	*0.001***	0.008	0.929	1.138	0.296	0.817	0.375	4.005	0.057	2.249	0.147
20°	11.504	*0.002***	47.936	*0.001***	3.303	0.081	1.264	0.272	1.112	0.302	54.839	*0.001***	0.000	0.994
30°	25.820	*0.001***	36.444	*0.001***	1.302	0.265	0.809	0.377	0.012	0.913	9.695	0.005*	1.616	0.216
Day 10 vs 20														
5°	1.189	0.286	18.614	*0.001***	0.049	0.827	0.277	0.603	9.393	0.005*	2.941	0.100	3.996	0.058
20°	3.364	0.079	1.649	0.211	0.190	0.667	0.308	0.584	0.009	0.925	15.114	*0.001***	10.428	0.004*
30°	1.450	0.240	6.018	0.021*	4.810	0.038*	4.932	0.036*	2.055	0.164	1.008	0.326	4.461	0.046*
Day 20 vs 30														
5°	1.526	0.228	0.051	0.823	11.729	*0.002**	6.196	0.020*	1.008	0.325	0.026	0.874	0.543	0.468
20°	1.088	0.307	1.357	0.255	4.035	0.055*	3.873	0.060	4.935	0.036*	0.190	0.667	10.535	0.004*
30°	1.852	0.186	0.094	0.762	15.219	*0.001***	2.689	0.114	1.001	0.327	6.133	0.021	1.681	0.208
Day 30 vs 70														
5°	5.432	0.028*	5.973	0.022*	6.199	0.020*	12.614	*0.002***	2.641	0.117	0.214	0.648	0.005	0.942
20°	0.010	0.920	7.754	0.010*	12.721	*0.001***	18.307	*0.001***	17.419	*0.001***	10.189	0.004*	1.919	0.179
30°	11.514	*0.002***	39.976	*0.001***	2.887	0.102	7.151	0.013*	0.156	0.696	15.167	*0.001***	0.165	0.689
Comparisons between treatments														
Day −5														
5° vs 20°	—	—	0.098	0.757	—	—	—	—	3.796	0.063	0.496	0.488	—	—
5° vs 30°	—	—	0.704	0.409	—	—	—	—	0.393	0.537	0.039	0.845	—	—
20° vs 30°	—	—	0.264	0.612	—	—	—	—	1.659	0.209	0.236	0.632	—	—
Day 1														
5° vs 20°	—	—	0.281	0.601	—	—	—	—	5.806	0.024*	0.013	0.910	—	—
5° vs 30°	—	—	10.403	0.003*	—	—	—	—	3.239	0.084	0.039	0.845	—	—
20° vs 30°	—	—	13.397	*0.001***	—	—	—	—	0.353	0.558	0.008	0.931	—	—
Day 10														
5° vs 20°	—	—	3.060	0.093	—	—	—	—	0.108	0.746	17.783	*0.001***	—	—
5° vs 30°	—	—	4.947	0.035*	—	—	—	—	0.550	0.465	1.179	0.289	—	—
20° vs 30°	—	—	0.214	0.647	—	—	—	—	0.163	0.690	9.031	0.006*	—	—
Day 20														
5° vs 20°	—	—	0.702	0.410	—	—	—	—	5.197	0.031*	13.535	*0.001***	—	—
5° vs 30°	—	—	0.372	0.547	—	—	—	—	14.232	*0.001***	6.190	0.021*	—	—
20° vs 30°	—	—	1.991	0.171	—	—	—	—	2.117	0.158	1.169	0.291	—	—
Day 30														
5° vs 20°	—	—	0.039	0.846	—	—	—	—	0.823	0.373	2.280	0.145	—	—
5° vs 30°	—	—	1.362	0.254	—	—	—	—	3.280	0.082	9.690	0.005*	—	—
20° vs 30°	—	—	0.895	0.353	—	—	—	—	7.019	0.014*	2.716	0.113	—	—
Day 70														
5° vs 20°	—	—	0.039	0.846	—	—	—	—	2.540	0.124	0.705	0.410	—	—
5° vs 30°	—	—	4.232	0.050*	—	—	—	—	0.701	0.411	1.499	0.233	—	—
20° vs 30°	—	—	3.290	0.082	—	—	—	—	0.544	0.468	4.155	0.053	—	—

Note. Summary of statistical analyses for male *Z. l. pugetensis* housed in the company of females (experiment 1). The 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). The 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 Materials and Methods under *Statistics*) are in italics and marked with double asterisks. Less robust differences (i.e., post hoc comparisons with $P <= 0.05$, but greater than P adjusted for multiple comparisons) are marked with single asterisks. 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 2,23 to 2,25. For date effect, *df* vary from 5,115 to 5,125. For date · temperature effect, *df* vary from 10,115 to 10,125. For post hoc comparisons, *df* vary from 1,23 to 1,25.

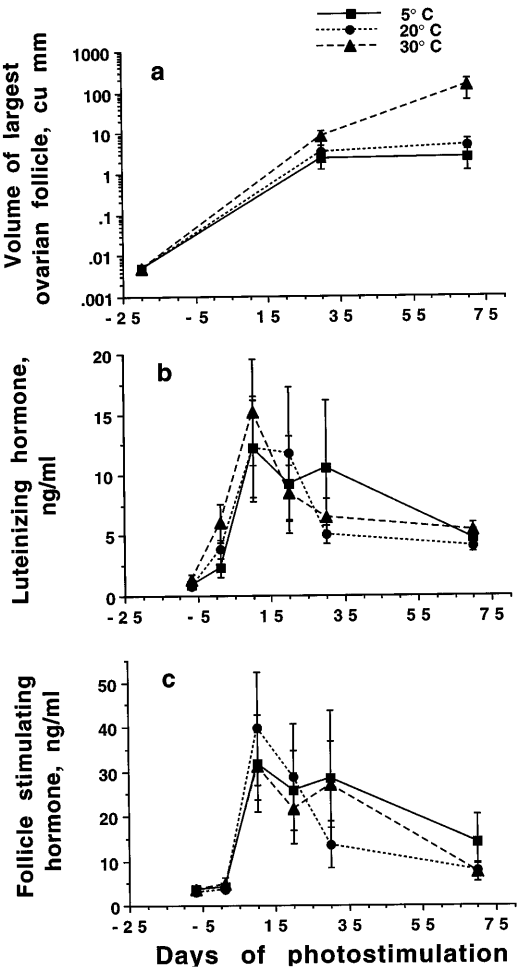

FIG. 2. Effects of temperature and photoperiod on (a) ovarian follicle development, (b) plasma levels of luteinizing hormone, and (c) follicle-stimulating hormone in female *Z.l. pugetensis.* Points and bars are means ± standard errors. $N = 10$ for all groups.

5° and the 20° females (5° vs 30°: $F = 16.683$, $P = 0.001$; 20° vs 30°: $F = 11.470$, $P = 0.004$; both $df = 1,16$). The 5° and 20° birds did not differ at Day 70 ($F = 0.452$, $P = 0.511$, $df = 1,16$).

Photostimulation also induced increased gonadotropin levels in females (Figs. 2b and 2c; Table 2; all further statistics for females in experiment 1 appear in Table 2 unless otherwise noted). Neither LH nor FSH profiles were significantly affected by temperature (Table 2), although low temperature tended to delay the LH increase slightly such that this hormone did not show a really robust increase until Day 10, whereas it did so by Day 1 in both the 20° and 30° groups (Fig. 2b; Table 2). As in males, the FSH increase occurred more slowly than that of LH (Fig. 2c; Table 2).

Effects of temperature and photoperiod on development of secondary sex characters. There was a marked increase in length of the cloacal protuberance (CPL) following photostimulation, but when CPL was compared among treatments at Day 30 there was no effect of temperature (ANOVA, $F = 0.649$, $P = 0.53$, Fig. 3, top).

There was no development of the brood patch at Day 30 of photostimulation; all females showed 0% defeathering at this time. By Day 70, however, females exposed to 30° had significantly greater development of the brood patch (ANOVA, Fig. 3, bottom, $F = 4.124$, $P = 0.031$).

Effects of temperature and photoperiod on body mass and fat score. Body mass and fat scores declined gradually in both males (Figs. 4a and 4b; Table 1) and females (Figs. 5a and 5b; Table 2), with no pronounced effects of temperature in either sex (Tables 1 and 2).

Effects of temperature on circulating levels of corticosterone following transfer to long days. Corticosterone levels changed significantly over time in both sexes. In males, the changes were fairly complex, with significant effects of temperature treatment (Fig. 6a; Table 1). Most conspicuously, males held at 5° had relatively high levels of corticosterone on Day 20 of photostimulation (Fig. 6a; Table 1). In females, only the sampling date effect was significant, apparently reflecting a prompt increase between Day −5 and Day 1 in all three treatments (Fig. 6b; Table 2).

Effects of temperature and photoperiod on T_4 and T_3 levels. Photo-induced changes in plasma T_4 in males were significantly influenced by temperature treatment (Fig. 7a; Table 1). The increase following photostimulation was most pronounced in the 20° birds and was conspicuously damped in the 5° birds. In fact, there were no significant changes between adjacent sampling dates within the 5° group, while the 20° birds showed a robust peak in T_4 levels at Day 10 (Fig. 7a; Table 1). T_3 levels in males also changed over time, but were not significantly affected by temperature (Fig. 7b; Table 1). Most of the sampling date effect appears to be due to increases between Days 10 and 20, followed by decreases by Day 30 (Fig. 7b; Table 1).

In females, T_4 increased following photostimulation,

TABLE 2
Statistical Analysis for Females, Experiment 1

	LH		FSH		Mass		Fat		B		T_4		T_3	
Effect:	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Temperature:	0.726	0.495	0.150	0.861	0.239	0.790	0.153	0.859	0.083	0.921	3.993	*0.035***	2.071	0.154
Date:	33.067	*0.001***	21.178	*0.001***	10.571	*0.001***	22.227	*0.001***	4.806	*0.001***	20.354	*0.001***	2.781	*0.022***
Date · temperature:	0.692	0.730	0.496	0.889	0.271	0.986	1.290	0.245	0.386	0.950	1.130	0.348	3.556	*0.001***
Comparisons within treatments														
Day −5 vs 1														
5°	5.663	0.026*	0.409	0.529	9.012	0.007*	0.212	0.650	9.372	0.006*	1.115	0.304	0.050	0.825
20°	26.702	*0.001***	0.273	0.607	3.211	0.088	5.300	0.031*	10.080	0.004*	0.069	0.795	0.007	0.933
30°	28.018	*0.001***	0.000	0.998	4.606	0.044*	4.711	0.041*	9.071	0.006*	0.071	0.793	5.411	0.031*
Day 1 vs 10														
5°	13.641	*0.001***	12.750	0.002*	0.303	0.588	0.000	1.000	2.701	0.115	10.914	0.004*	0.028	0.868
20°	4.439	0.047*	19.477	*0.001***	0.365	0.552	5.500	0.028*	0.550	0.466	14.856	*0.001***	3.501	0.077
30°	6.675	0.017*	23.786	*0.001***	0.095	0.761	6.188	0.021*	0.024	0.879	42.700	*0.001***	0.123	0.730
Day 10 vs 20														
5°	1.302	0.266	0.124	0.728	0.291	0.595	0.150	0.702	2.397	0.136	0.681	0.419	0.128	0.725
20°	0.463	0.503	2.052	0.167	0.028	0.569	1.352	0.257	0.308	0.584	4.668	0.043*	1.189	0.289
30°	3.950	0.059	7.021	0.015*	0.033	0.858	16.158	*0.001***	0.151	0.701	1.768	0.199	0.660	0.427
Day 20 vs 30														
5°	0.079	0.782	0.677	0.420	3.677	0.069	12.283	*0.002***	2.178	0.154	0.106	0.749	0.052	0.822
20°	2.779	0.110	2.015	0.170	5.880	0.024*	1.965	0.175	1.695	0.206	4.782	0.041*	1.748	0.202
30°	1.206	0.284	1.077	0.311	0.467	0.502	0.109	0.744	0.692	0.415	0.994	0.331	3.626	0.072
Day 30 vs 70														
5°	4.057	0.056	0.840	0.370	2.202	0.153	3.980	0.059	0.333	0.570	5.314	0.032*	0.794	0.384
20°	0.698	0.413	0.215	0.648	6.334	0.020*	3.980	0.059	0.002	0.969	7.764	0.011*	0.312	0.583
30°	0.050	0.825	5.255	0.032*	7.114	0.014*	10.834	*0.003***	0.097	0.759	7.396	0.013*	6.027	0.024*
Comparisons between treatments														
Day −5														
5° vs 20°	—	—	—	—	—	—	—	—	—	—	1.709	0.206	0.387	0.541
5° vs 30°	—	—	—	—	—	—	—	—	—	—	0.648	0.430	3.864	0.064
20° vs 30°	—	—	—	—	—	—	—	—	—	—	0.210	0.652	1.931	0.181
Day 1														
5° vs 20°	—	—	—	—	—	—	—	—	—	—	0.167	0.687	0.021	0.886
5° vs 30°	—	—	—	—	—	—	—	—	—	—	0.000	0.999	0.786	0.387
20° vs 30°	—	—	—	—	—	—	—	—	—	—	0.157	0.696	1.043	0.032*
Day 10														
5° vs 20°	—	—	—	—	—	—	—	—	—	—	0.791	0.384	2.019	0.172
5° vs 30°	—	—	—	—	—	—	—	—	—	—	6.705	0.018*	1.785	0.197
20° vs 30°	—	—	—	—	—	—	—	—	—	—	2.994	0.099	0.000	0.984
Day 20														
5° vs 20°	—	—	—	—	—	—	—	—	—	—	5.803	0.026*	0.732	0.403
5° vs 30°	—	—	—	—	—	—	—	—	—	—	2.347	0.141	5.421	0.031*
20° vs 30°	—	—	—	—	—	—	—	—	—	—	0.632	0.436	2.360	0.141
Day 30														
5° vs 20°	—	—	—	—	—	—	—	—	—	—	1.715	0.205	3.797	0.066
5° vs 30°	—	—	—	—	—	—	—	—	—	—	5.936	0.024*	0.015	0.903
20° vs 30°	—	—	—	—	—	—	—	—	—	—	1.372	0.255	2.824	0.109
Day 70														
5° vs 20°	—	—	—	—	—	—	—	—	—	—	0.719	0.406	22.880	*0.001***
5° vs 30°	—	—	—	—	—	—	—	—	—	—	3.672	0.070	3.943	0.062
20° vs 30°	—	—	—	—	—	—	—	—	—	—	1.203	0.286	41.141	*0.001***

Note. Summary of statistical analyses for male *Z. l. pugetensis* housed in the company of males (experiment 1). See Table 1 caption for detailed explanation. 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 2,22. For date effect, *df* vary from 5,95 to 5,110. For date · temperature effect, *df* vary from 10,95 to 10,110. For post hoc comparisons, *df* vary from 1,19 to 1,22.

especially between Days 1 and 10 (Fig. 8a; Table 2). Levels then tended to decline again by Day 70. There also was a significant main effect of temperature, apparently attributable to the consistent tendency for 5° birds to display reduced T_4 levels (Fig. 8a; Table 2). Female T_3 levels also fluctuated over time (Fig. 8b; Table 2). The significant interaction of date and temperature appears to come from the greatly reduced levels of plasma T_3 by Day 70 in the 30° group (Fig. 8b; Table 2).

Experiment 2: Sexes Isolated

Long-term interaction of temperature and photoperiod on gonadal recrudescence. As expected, there were highly significant effects of photostimulation on testis and ovary development in male and female *Z.l. pugetensis* held in single sex groups (upper panels of Figs. 9a and 10a, $F = 103.114$, $P = 0001$, $df = 1,36$ for males and $F = 6.283$, $P = 0.006$, $df = 2,24$ for females). In males, temperature significantly affected the testis profiles (main temperature effect: $F = 4.317$, $P = 0.029$, $df = 2,28$; temperature by date interaction: $F = 4.393$, $P = 0.005$, $df = 4,36$). Testis size in 5° birds was marginally reduced at Day 30 compared with the other groups (5° vs 20°: $F = 6.390$, $P = 0.021$; 5° vs 30°: $F = 5.314$, $P = 0.033$, both $df = 1,18$). By Day 116, all three groups had regressed their testes significantly, indicative of development of photorefractoriness (Day 75 vs 116 within groups, all $F > 28.0$, all $P < 0.001$, all $df = 1,18$). However this process appeared to be accelerated in the 30° birds (Fig. 9a); testes of the 30° birds had already begun to regress by Day 75 (Day 30 vs 75, $F = 5.100$, $P = 0.037$, $df = 1,18$), and those of the 5° birds were still

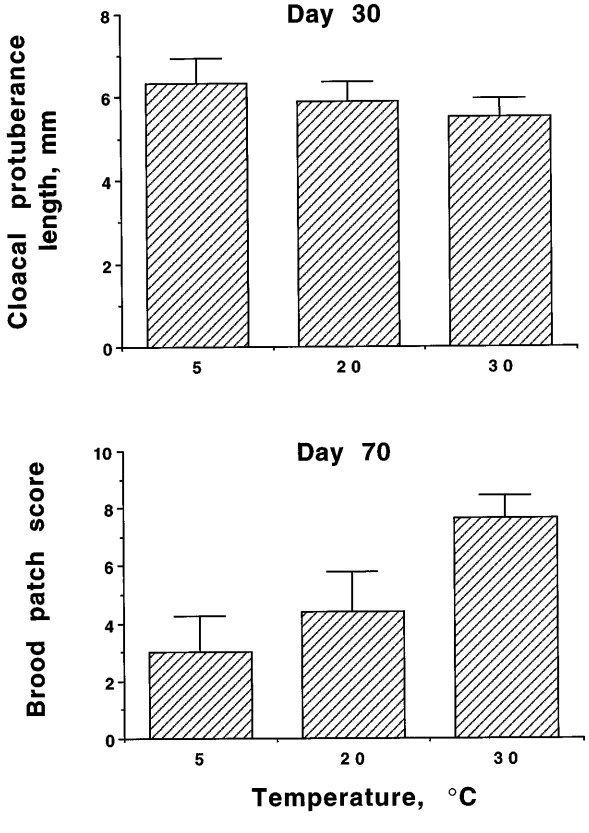

FIG. 3. Effects of temperature on length of the cloacal protuberance in males at Day 30 of photostimulation and temperature treatment (top) and development of the brood patch (bottom) in female *Z.l. pugetensis* exposed to 16L 8D. Bars are means ± standard errors. Sample sizes as for Figs. 1 and 2.

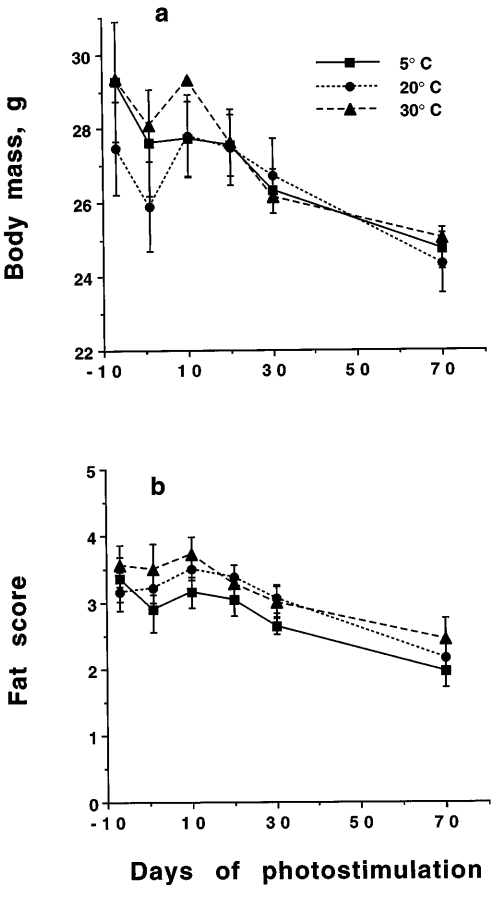

FIG. 4. Effects of temperature on body mass (a) and fat score (b) in male *Z.l. pugetensis* transferred to 16L 8D from 9L 15D. Each point is the mean and vertical lines the standard errors. Sample sizes as for Fig. 1.

relatively large on Day 116 (5° vs 20°: $F = 4.978$, $P = 0.039$; 5° vs 30°: $F = 12.620$, $P = 0.002$, both $df = 1,18$). A similar tendency for acceleration of onset of refractoriness at higher temperatures was apparent in females as well (Fig. 10a); females at 5° showed less complete gonadal regression than those at 20° and 30° (5° vs 20° and 30°: both $F > 5.60$, both $P < 0.035$, both $df = 1,12$).

In both sexes, circulating LH levels increased following photostimulation and then decreased as the birds became photorefractory (Figs. 9b and 10b); temperature treatment did not affect these LH profiles (Tables 3 and 4).

Circulating T_4 levels also changed significantly over time in both sexes (Figs. 11a and 11b; Tables 3 and 4). Males showed a particularly robust increase in T_4 in all three temperature treatments between Days −1 and 31

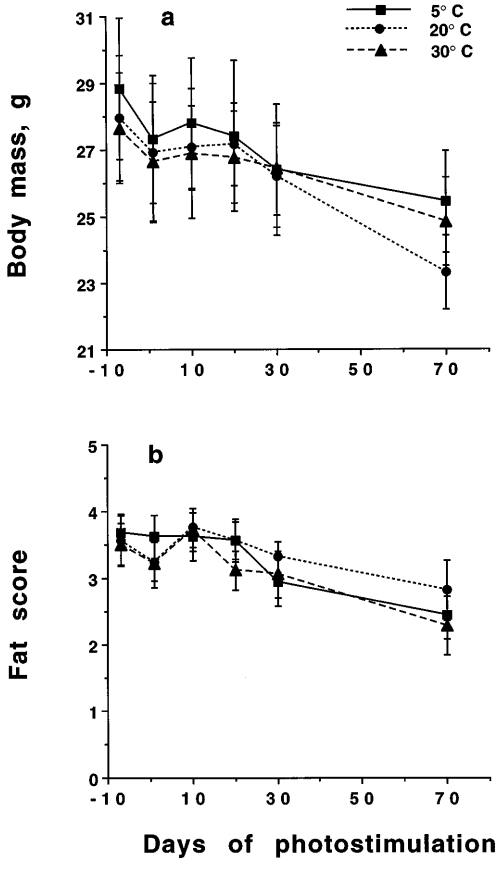

FIG. 5. Effects of temperature on body mass (a) and fat score (b) in female *Z.l. pugetensis* transferred to 16L 8D from 9L 15D. Each point is the mean and vertical lines the standard errors. Sample sizes as for Fig. 2.

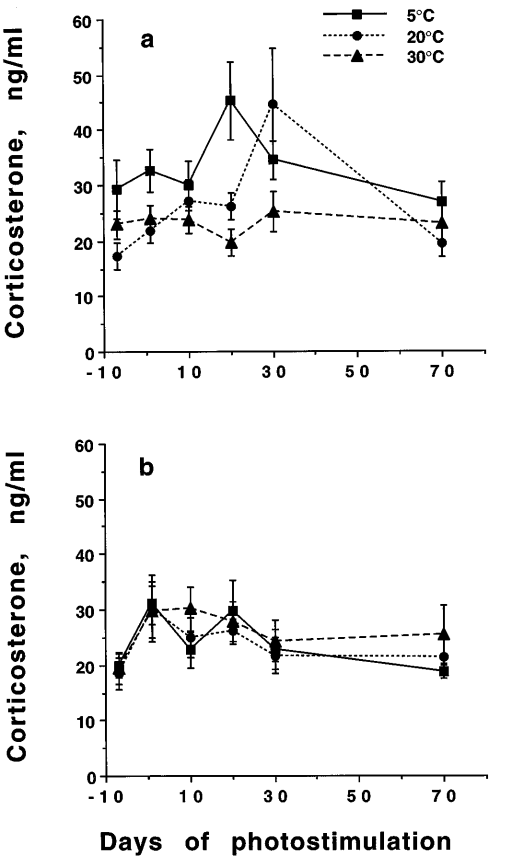

FIG. 6. Effects of temperature on circulating levels of corticosterone in male (a) and female (b) *Z.l. pugetensis* transferred from 9L 15D to 16L 8D at Day 0. Each point is the mean, and vertical lines the standard errors. Sample sizes as for Figs. 1 and 2.

(Table 3). In addition, male T_4 profiles were influenced by temperature (significant temperature by date interaction, Table 3). Specifically, levels in the 5° birds remained steady between Days 31 and 75, while those in the 20° and 30° birds declined, and by Day 116 T_4 levels of the 5° birds were relatively elevated compared to the other two groups (Fig. 11a; Table 3). In females, there was no clear effect of temperature on T_4 profiles; however, the sampling date effect was most evident in the group held at 20° (Fig. 11b, Table 4).

Effects of temperature on prebasic moult. In both males and females, there were variations in moult score at Day 116 (Fig. 12, top, $F = 10.89$, $P = 0.0008$, $df = 2,20$ for males; bottom, $F = 6.28$, $P = 0.012$, $df = 2,15$ for females). Post hoc tests revealed that in males, the 30° group was more advanced in moult than the 5° and 20° groups (Scheffe F tests, $P < 0.05$). In

females, the 5° group was significantly delayed compared with the 30° group (Scheffe F test, $P < 0.05$), but not different from the 20° group.

Comparison of gonadal development in isolated versus mixed sex groups. Comparisons (two-way ANOVA) of gonadal condition as a function of temperature treatment and social arrangement (sexes together vs sexes separate, experiment 1 vs 2) at Days 30/31 and 70/75 after photostimulation help to illustrate more clearly the differences between the sexes in temperature-mediated effects on long-day-induced gonadal development (Fig. 13). In males, there was a significant main effect of temperature at Day 30/31 ($F = 4.864$, $P = 0.012$, $df = 2,46$), but no effects of social arrangement or its interaction with temperature (both $F < 0.80$, both $P > 0.45$, $df = 1,46$ and 2,46, respectively). At Day 70/75 there were no effects of temperature, social arrangement, or the interaction of the two on testis volume (all $F < 1.50$, all $P > 0.25$, $df = 2,41$; 1,41, and 2,41, respectively). In contrast, females showed main temperature effects at both Days 30/31 and 70/75 ($F = 5.711$, $P = 0.007$, $df = 2,40$, and $F = 5.669$, $P = 0.008$, $df = 2,29$, respectively). Social arrangement also contributed a main effect at both dates (Day 30/31: $F = 6.470$, $P = 0.015$, $df = 1,40$; Day 70/75: $F = 7.132$, $P = 0.012$, $df = 1,29$). There was no interaction of temperature with social arrangement at Day 30/31 ($F = 0.053$, $P = 0.949$, $df = 2,40$). However, this interaction effect was significant at Day 70/75 ($F = 6.238$, $P = 0.006$, $df = 2,29$), reflecting the great enhancement of follicular development in the 30° females housed with males (Fig. 13; Tukey HSD pair-

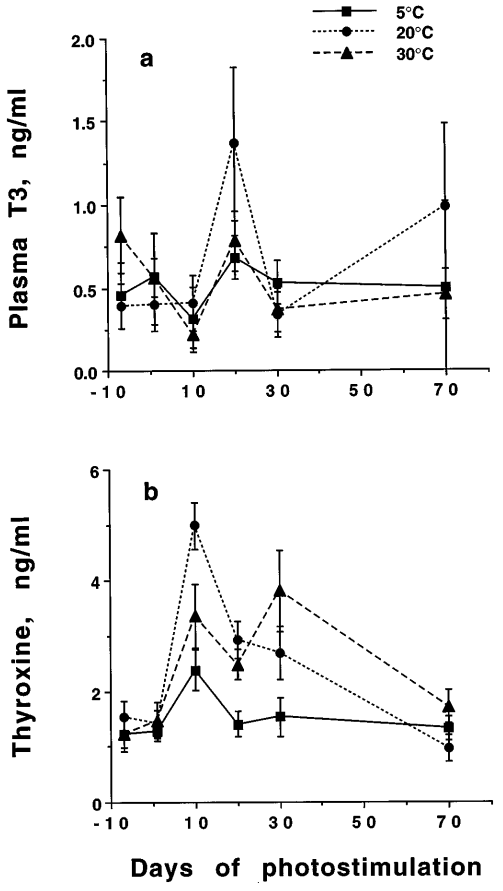

FIG. 7. Effects of temperature on plasma levels of tri-iodothyronine (a) and thyroxine (b) in male *Z.l. pugetensis* transferred to 16L 8D from 9L 15D. Each point is the mean and vertical lines the standard errors. Sample sizes as for Fig. 1.

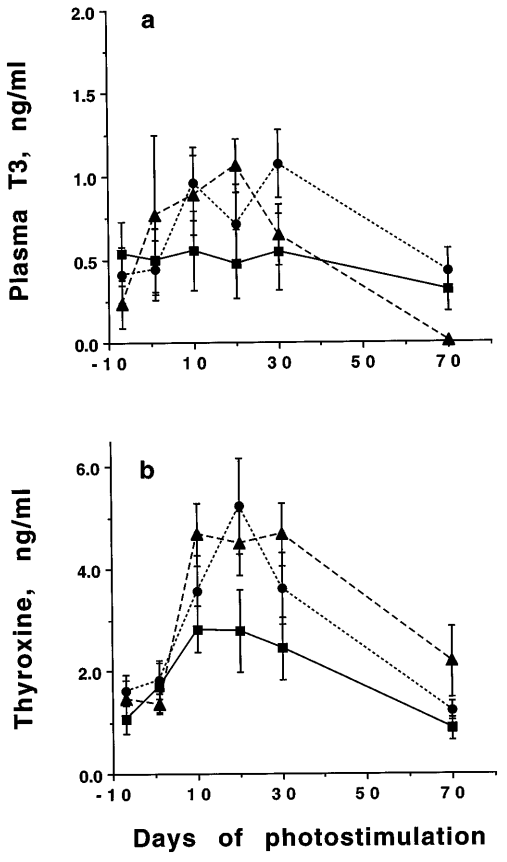

FIG. 8. Effects of temperature on plasma levels of tri-iodothyronine (a) and thyroxine (b) in female *Z.l. pugetensis* transferred to 16L 8D from 9L 15D. Each point is the mean and vertical lines the standard errors. Sample sizes as for Fig. 2.

wise comparisons, all $P < 0.007$ for 30° females housed with males compared with all other treatments).

DISCUSSION

Effects of Supplementary Cues on the Hypothalamo–Pituitary–Gonad Axis

Low environmental temperature failed to inhibit photoperiodically induced gonadal development, and high temperature did not enhance it in either sex of *Z.l. gambelii* exposed to long days (Farner and Mewaldt, 1952; Lewis and Farner, 1973; Wingfield *et al.*, in press). However, in this study of *Z.l. pugetensis*, ovarian development was increased by treatment with 30°, though not inhibited by a low temperature of 5°. A similar trend is seen in males at Day 30 but this was not significant. These findings are consistent with the predictions of Wingfield *et al.* (1992a, 1993) that a population such as *Z.l. pugetensis*, with a high I_e factor (in this case about 4.0) should integrate both initial predictive and supplementary information. *Z.l. gambelii*, on the other hand, has a lower I_e value (about 1.0) and appears to be insensitive to effects of temperature, at least on photoperiodically induced gonadal development. It is very interesting that in the present study, the only effect of temperature on plasma gonadotropin levels in either sex was the slightly faster increase in FSH in males, and this subtle difference did not

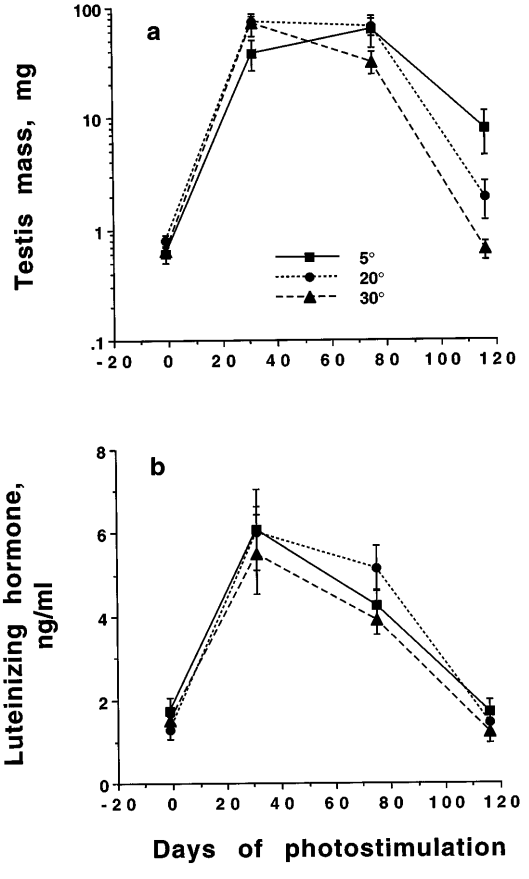

FIG. 9. Effects of photostimulation and temperature treatment on testis volume and plasma levels of luteinizing hormone in male *Z.l. pugetensis* isolated from females. Points are means and vertical bars standard errors. $N = 8$, 6, and 7 for the 5°, 20°, and 30° groups, respectively.

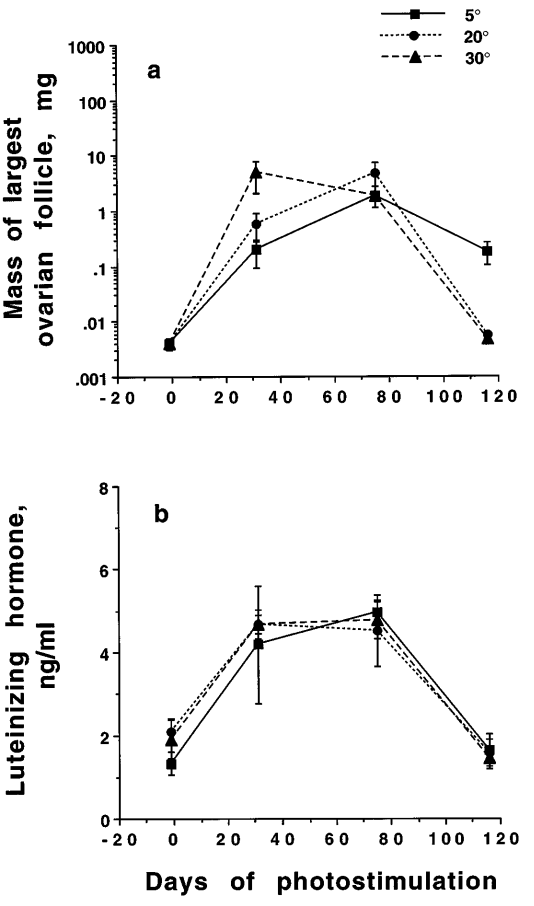

FIG. 10. Effects of photostimulation and temperature treatment on volume of the largest ovarian follicle and plasma levels of luteinizing hormone in female *Z.l. pugetensis* isolated from males. Points are means and vertical bars standard errors. $N = 6$, 5, and 5 for the 5°, 20°, and 30° groups, respectively.

translate into any significant differences in gonadal development. Despite the enhanced follicular development in females housed with males at 30° in experiment 1, no differences in gonadotropin secretion in females were apparent.

In other species it has been shown that low temperatures may or may not inhibit testicular development in spring (or induced by long days) and that this may be related to migratory status (see Wingfield *et al.*, 1992a, for review). For example, in migratory species that have a highly predictable breeding season, low ambient temperature had no effect on testicular recrudescence (Kendeigh, 1941; Engels and Jenner, 1956; Rowan, 1925). However, sedentary species with longer or more flexible breeding seasons apparently showed reductions in rates of testicular recrudescence at low temperatures (e.g., Suomalainen, 1937; Jones, 1986; Storey and Nicholls, 1982). Data for ovarian development are sparse but support the results for males (Suomalainen, 1937; Jones, 1986; Storey and Nicholls, 1982). Photostimulation of willow tits, *Parus montanus*, at 20° resulted in advanced maturation up to 2 weeks earlier than in willow tits held at 10° or 4° (Silverin and Viebke, 1994), although maximum testis size attained was rather small and below that seen in free-living birds at maturity. Similar treatment of great tits, *P. major* (Silverin and Viebke, 1994), revealed no effects of temperature on photoperiodically induced gonadal

TABLE 3
Statistical Analysis for Males, Experiment 2

Effect:	LH		T_4	
	F	*P*	*F*	*P*
Temperature:	0.574	0.573	1.470	0.255
Date:	109.826	*0.001***	12.801	*0.001***
Date · temperature:	1.271	0.285	5.885	*0.001***
Comparisons within treatments				
Day −1 vs 31				
5°	37.134	*0.001***	15.492	*0.001***
20°	45.032	*0.001***	13.110	*0.002***
30°	33.650	*0.001***	17.730	*0.001***
Day 31 vs 75				
5°	7.095	0.015*	0.151	0.702
20°	1.542	0.229	21.949	*0.001***
30°	5.958	0.025*	10.470	0.004*
Day 75 vs 116				
5°	61.343	*0.001***	7.157	0.015*
20°	90.005	*0.001***	0.909	0.352
30°	91.140	*0.001***	0.594	0.450
Comparisons between treatments				
Day −1				
5° vs 20°	—	—	2.618	0.122
5° vs 30°	—	—	5.069	0.036*
20° vs 30°	—	—	0.218	0.646
Day 31				
5° vs 20°	—	—	3.192	0.090
5° vs 30°	—	—	6.001	0.024*
20° vs 30°	—	—	0.232	0.636
Day 75				
5° vs 20°	—	—	10.223	0.005*
5° vs 30°	—	—	0.032	0.859
20° vs 30°	—	—	9.184	0.007*
Day 116				
5° vs 20°	—	—	8.714	0.008*
5° vs 30°	—	—	8.304	0.010*
20° vs 30°	—	—	0.081	0.779

Note. Summary of statistical analyses for male *Z. l. pugetensis* housed isolated from females (experiment 2). See Table 1 caption for detailed explanation. 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*) are 2,19. For date effect, *df* are 3,57. For date · temperature effect, *df* are 6,57. For post hoc comparisons, *df* are 1,19.

TABLE 4
Statistical Analysis for Females, Experiment 2

Effect:	LH		T_4	
	F	*P*	*F*	*P*
Temperature:	0.198	0.823	0.109	0.898
Date:	36.136	*0.001***	6.337	*0.001***
Date · temperature:	0.817	0.564	1.638	0.165
Comparisons within treatments				
Day −1 vs 31				
5°	16.318	*0.002***	2.422	0.146
20°	6.517	0.025*	14.150	*0.003***
30°	9.797	0.009*	4.311	0.060
Day 31 vs 75				
5°	0.612	0.449*	1.724	0.214
20°	1.080	0.319	3.121	0.103
30°	0.004	0.949*	2.018	0.181
Day 75 vs 116				
5°	50.289	*0.001***	5.203	0.042*
20°	18.315	*0.001***	0.673	0.428
30°	44.494	*0.001***	0.013	0.912

Note. Summary of statistical analyses for female *Z. l. pugetensis* housed isolated from males (experiment 2). See Table 1 caption for detailed explanation. 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*) are 2,12. For date effect, *df* are 3,36. For date · temperature effect, *df* are 6,36. For post hoc comparisons, *df* are 1,12.

development. However, the mechanisms by which temperature may modulate gonadal recrudescence remain unknown.

It is of considerable interest that the patterns of LH secretion in willow tits were identical despite differences in temperature-modulated photostimulation of testis growth (Silverin and Viebke, 1994). These data are very similar to our findings in *Z.l. pugetensis* in this study and in *Z.l. gambelii* (Wingfield *et al.*, 1996). On the other hand, LH levels in great tits exposed to 20° were markedly higher than those in groups held at 10° and 4°, even though there was 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

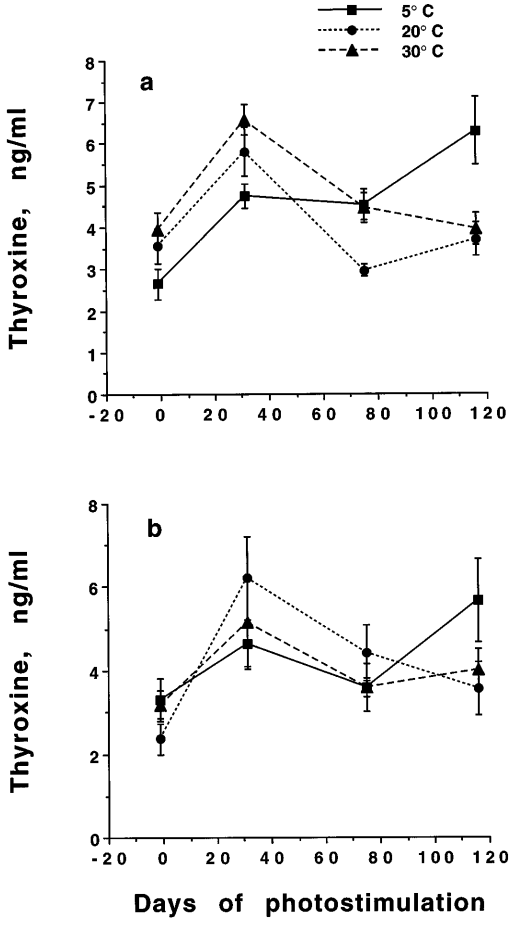

FIG. 11. Effects of photostimulation and temperature treatment on plasma levels of thyroxine in male (a) and female (b) *Z.l. pugetensis* isolated from members of the opposite sex. Points are means and vertical bars standard errors. Sample sizes as for Figs. 9 and 10.

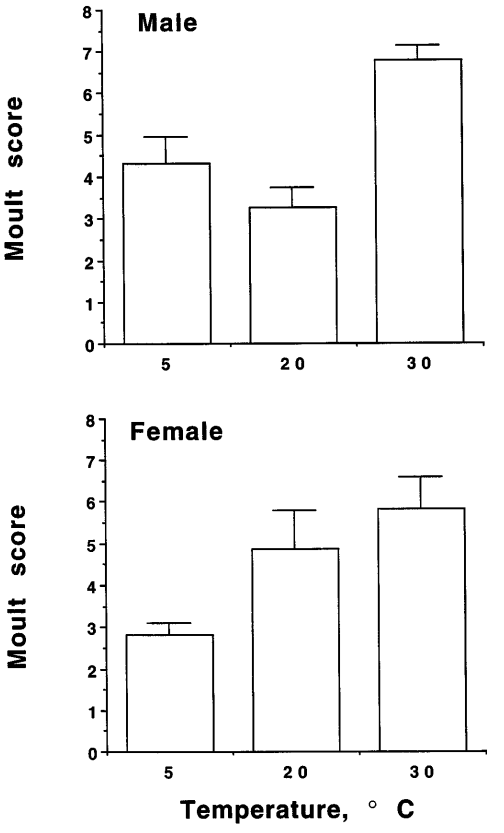

FIG. 12. Effects of temperature on moult score at Day 116 of photostimulation in male (top) and female (bottom *Z.l. pugetensis*. Bars are means plus or minus standard errors. Sample sizes as for Figs. 9 and 10.

behaviors that occur early in the breeding season of this species. The overall trend appears to suggest that effects of temperature on seasonal maturation of the gonads in wild birds may not be mediated through altered luteinizing hormone secretion, although it is possible that there was a slight effect in FSH secretion, but this was not paralelled with differences in gonadal growth in males. More experiments are required to resolve this.

Role of the Hypothalamo–Pituitary–Adrenal Axis

We were careful to select low and high temperatures that were within the normal (i.e., predictable) range that *Z.l. pugetensis* would experience during spring (see Wingfield *et al.*, 1992a). Exposure to temperatures outside this range may elicit a stress response and gonadal development would be retarded by a different

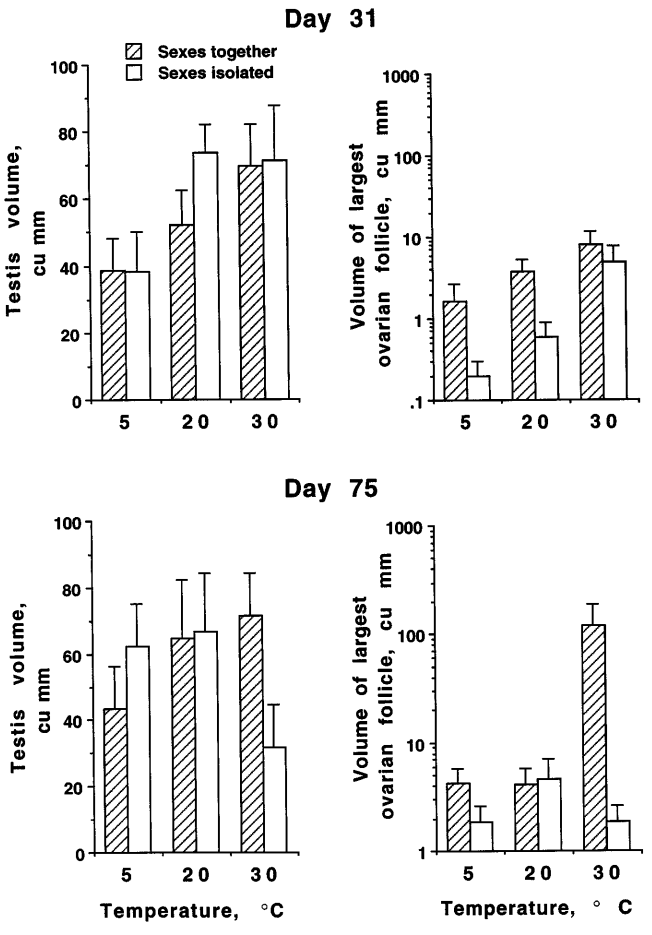

FIG. 13. Comparisons of the effects of photostimulation and temperature on testis volume and volume of largest ovarian follicles in *Z.l. pugetensis* housed either in visual and auditory contact with members of the opposite sex or isolated from them. Bars are means plus or minus standard errors. $N = 9$ and 7 for males exposed to or isolated from females, respectively, and $N = 8$ and 5 for females exposed to or isolated from males, respectively.

mechanism. 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 this study it appears that although temperature treatments had slight effects on corticosterone levels in males, those in females were unaffected, suggesting that these birds were not stressed. The highest corticosterone levels were well below those induced by a variety of stresses (Wingfield *et al.*, 1982). These data suggest that temperatures that have dramatic effects on gonadal recrudescence in at least female *Z.l. pugetensis* need not act through the adrenocortical axis.

Role of Social Stimulation

A noteworthy result of this study was the observation that exposure to long days and 30° stimulated final ovarian maturation (yolk deposition) in females that could see and hear males. Normally this process culminates in egg-laying. Again there were no differences in circulating levels of LH and FSH, which leaves the mechanism involved uncertain. It appears that social stimulation, rather than temperature per se, could have enhanced ovarian development (see Wingfield *et al.*, 1994), since in experiment 2 (sexes isolated), the same treatments of photostimulation and temperature were much less effective in enhancing ovarian development (Fig. 13). Perhaps male behavior (e.g., singing) was affected by temperature and the behavioral stimulation was a factor influencing ovarian development. There is considerable evidence that females of several avian species show increased ovarian development when exposed to male displays—especially vocalizations (e.g., Brockway, 1965; Lehrman, 1965; Hinde, 1965). This effect has also been demonstrated in photostimulated female *Z.l. gambelii* (Morton *et al.*, 1985). However, we have some indication that there was no straightforward relationship between amount of exposure to male song and degree of reproductive development of females in the present study. The total number of songs counted during the first hour following "lights-on" in each temperature group (Fig. 14; recordings made with a microphone permanently installed in each chamber without disturbance of the birds) showed that although males in the 30° group sang more over the first few days of transfer to long days, by Day 25 total numbers of songs given by males were similar in all treatments. Thereafter, the 5° group sang less, but the 20° and 30° groups were identical (Fig. 14). We feel that it is unlikely that the effects of temperature on ovarian development at Days 30 and 75 are purely a result of behavioral stimulation from males. Nevertheless, these data indicate a strong influence of the presence of males on responsiveness to temperature in female *Z.l. pugetensis* (or, alternatively, a strong influence of temperature on responsiveness to males). It is intriguing that females should be able to

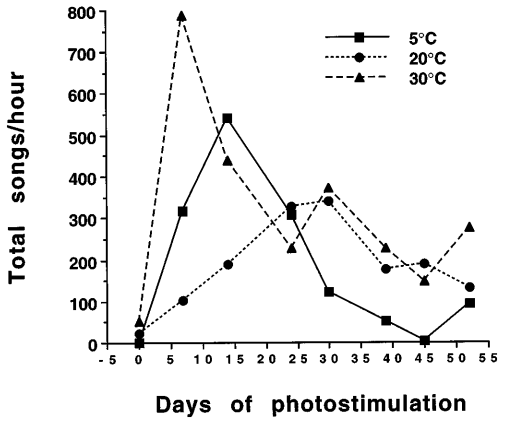

FIG. 14. Total songs given per hour after "lights-on" in male *Z.l. pugetensis* exposed to long days and various temperature treatments.

modulate their photoperiodically induced ovarian growth in relation to ambient temperature. That this is also dependent upon the presence of males adds a new level of complexity to possible neural pathways by which these environmental cues are transduced into neuroendocrine secretions. Furthermore, given that the effects on LH and FSH appear absent, the mechanisms by which these social and temperature signals may be transduced presents an exciting challenge.

Possible Role of the Hypothalamo–Pituitary–Thyroid Axis

Low environmental temperature has been shown to activate the hypothalamo–pituitary–thyroid axis, resulting in elevation of circulating T_3 and/or T_4 (e.g., Assenmacher, 1973; Smith 1982). Thus it is possible that temperature effects on gonadal function could be transduced through thyroid hormone secretion. In the present study, whereas photostimulation increased plasma concentrations of T_4, the low temperature group (at 5°) actually had lower levels. These data suggest that the range of temperatures used indeed had an effect on thyroid hormone secretion and may have retarded effects of photostimulation on T_4 release. If true, then it is possible that T_4-mediated effects of temperature may be on the timing of termination of reproduction (see also Nicholls *et al.*, 1988) and not on gonadal development. The effects of temperature treatment on T_3 levels were rather more complex. However, it is noteworthy that at Day 75 of photostimulation, T_3 levels of females housed at 30° in the company of males—the only group to show advancement to the yolk-deposition stage of follicular development—had dropped to nearly undetectable levels. This result is consistent with the hypothesis that circulating T_3 levels might play a role in regulating gonadal sensitivity to gonadotropins. Further experiments testing this idea are warranted.

Effects of Temperature on Other Seasonal Processes

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 information to begin preparations for migration and then short-term predictive cues 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 of the control of body mass and fat depots for migration by photoperiod in several avian species (Wingfield *et al.*, 1990; Ramenofsky, 1990), but none, as far as we are aware, 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 significantly less accumulation of fat and lower body mass than the other groups, suggesting that low temperature may indeed inhibit preparations for migration. Furthermore, the low temperature group had significantly higher circulating levels of corticosterone soon after transfer to long days and temperature treatments, also suggesting a possible mechanism (Wingfield *et al.*, 1996). It is unlikely that these females were stressed since the highest corticosterone levels were still well below those measured in chronically stressed females (Wingfield *et al.*, 1982), and note that ovarian development was not affected. These data indicate that preparations for migration may be influenced more by temperature than is ovarian development in *Z.l. gambelii* (Wingfield *et al.*, 1996).

Z.l. pugetensis is a short-distance migrant and premigratory hyperphagia and fattening are much less intense than those in *Z.l. gambelii*. There were minimal

effects of photostimulation and temperature on body mass and fat score in both male and female *Z.l. pugetensis.* Both sexes were relatively fat at the onset of the experiment and gradually lost mass and fat as treatments progressed and regardless of temperature. As far as we are aware this is the first comparison of temperature effects on photoperiodically induced premigratory events in long- and short-distance migrants.

Clearly there is great flexibility of the control systems for reproductive and associated cycles and the mechanisms by which they integrate environmental signals. Additional tests on other taxa with varying predictability and I_e factors will indicate whether the models for integration of environmental signals will prove to be useful in general. Furthermore, it appears likely that the effects of temperature on photoperiodically induced reproductive functions may vary according to stage in the breeding cycle. Whether the mechanisms underlying these responses to environmental cues also vary with stages in the cycle is currently under investigation.

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