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Effects of temperature on photoperiodically induced reproductive development, circulating plasma luteinizing hormone and thyroid hormones, body mass, fat deposition and molt in mountain white-crowned sparrows, *Zonotrichia leucophrys oriantha*

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Abstract

The mountain white-crowned sparrow, *Zonotrichia leucophrys oriantha*, breeds in subalpine meadows throughout many mountainous regions of western North America. Mathematical analysis of 20 years of egg-laying dates at Tioga Pass, California (3030 m elevation) indicated a highly predictable breeding season suggesting that precise environmental cues such as the annual change in day length were important for regulating reproductive function. Additionally, it appeared that there was sufficient yearly variation in the timing of breeding to suggest that other environmental cues may also be important for regulating adjustments in reproductive development and regression. Captive populations of *Z. l. oriantha* showed strong responses in gonadal development following transfer to longs days (15L 9D) and low temperature (5 °C) slowed down photoperiodically induced gonadal growth and subsequent regression, in both males and females. High temperature of 30 °C tended to accelerate gonadal development and regression whereas gonadal development was intermediate in a group exposed to 20 °C. Prior exposure to these temperature regimes while on short days (9L 15D) had no effect on body mass, fat, or plasma levels of luteinizing hormone (LH) and thyroid hormones. Curiously there was no effect of temperature on photoperiodically induced rises in LH in either sex despite marked effects on gonadal growth. Brood patch development was also enhanced in females exposed to 30 °C. Corticosterone levels measured in a subset of plasma samples from this experiment indicated no effect of temperature suggesting that the retarded gonadal development at 5 °C was not a result of thermal stress. Although there was a robust effect of photostimulation on thyroid hormone levels in blood of both sexes, temperature treatment had no effect on tri-iodothyronine (T3) concentrations. However, plasma levels of thyroxine (T4) were lower initially at 5 °C versus 20 and 30 °C treatments. This may be related to the protracted gonadal cycle at 5 °C versus the truncated gonadal cycle at 30 °C. Molt score, an indication of post-reproductive state and onset of photorefractoriness, was delayed in birds exposed to 5 °C. Body mass, and to a lesser extent fat score, tended to be lowest in birds exposed to 5 °C compared with those at 20 and 30 °C. These results demonstrate that ambient temperature significantly affected photoperiodically induced gonadal development and regression in these birds. The endocrine mechanisms underlying these effects require further study.

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1. Introduction

Virtually all vertebrates reproduce intermittently, usually on a seasonal schedule. Although seasonal breeding has been studied for decades, the mechanisms by which environmental cues regulate neuroendocrine and endocrine secretions that orchestrate reproductive development are unclear (e.g., Follett, 1984; Wingfield and Kenagy, 1991). The annual cycle of day length provides very accurate information—(known as initial predictive information—Wingfield, 1983) as to time of year. Changing day length is perceived and transduced into activation of the hypothalamo–pituitary–gonad (HPG) axis in most vertebrates that breed at mid to high latitudes. Mechanisms by which day length stimulates release of gonadotropin-releasing hormone and thus activates the HPG axis has received particular attention in birds (Bentley et al., 2002; Farner, 1986; Follett, 1984; Nicholls et al., 1988). However, the role that other environmental cues (supplementary factors, Marshall, 1960; Wingfield, 1983) play in slowing down photoperiodically induced gonadal growth in, for example, a cold spring, or accelerating it in a warm spring, is equivocal and the mechanisms by which they act are unclear (e.g., Wingfield, 1983; Wingfield et al., 1992, 1993, 1996, 1997; Wingfield and Kenagy, 1991).

The role of supplementary factors has been much less well studied partly because their actions vary tremendously across taxa whereas the effects of photoperiod are obvious and profound in most species studied. The next logical question to ask is why supplementary factors are so variable in their actions. Wingfield et al. (1992) suggested that species breeding in severe environments such as the Arctic have a short time window in which to breed successfully. As a result, their breeding season is highly predictable and only a few weeks in duration. Accurate timing, e.g., by measuring changing day length, is essential and the role of supplementary factors to accelerate or inhibit gonadal development would not confer an advantage by increasing reproductive success. Many other types of breeding cycle in other species may have similar precise timing. On the other hand, for populations that breed in environments in which the period for successful breeding is longer (e.g., 2–4 months at mid-latitudes), photoperiodically induced gonadal development prior to onset of breeding would still be important, but other supplementary factors that allow individuals to adjust breeding according to local conditions may be critical to maximize reproductive success.

By using natural history data such as egg-laying dates, Wingfield et al. (1992, 1993) were able to apply mathematical techniques to describe the plasticity of the breeding season. This analysis raised hypotheses concerning how a population should integrate initial predictive and supplementary environmental cues to regulate gonadal development, onset of nesting, and termination of the breeding cycle. Log-linear analysis of egg-laying dates indicated that most species have a predictable breeding season and should measure an initial predictive cue such as the annual change in day length. Application of Colwell's constancy/contingency model of predictability (Colwell, 1974; Wingfield et al., 1992, 1993) provided further information that may indicate the degree to which supplementary factors are also important. Wingfield et al. (1992, 1993) suggested that the ratio of contingency to constancy in a breeding season indicates the degree to which supplementary cues may influence photoperiodically induced gonadal growth. This ratio has been called the environmental information factor (Ie, Wingfield et al., 1992, 1993). Using this Ie factor, we can now formulate testable hypotheses to investigate how supplementary factors influence photoperiodically induced gonadal growth. Such comparisons may enable us to understand the way in which environmental factors are integrated to regulate life history cycles in general, and then indicate experimental approaches to determine mechanisms.

One problem in these kinds of comparative experiments is the confounding issue of phylogeny. Many species may show different responses to the same experimental paradigms not necessarily because they are adaptive, but simply because they belong to different taxonomic groups. To circumvent this we chose Emberizid sparrows as subjects because they are closely related. White-crowned sparrows (*Zonotrichia leucophrys*) are divided among five subspecies that differ in migratory habits and geographic distribution (see, Cortopassi and Mewaldt, 1965). The Gambel white-crowned sparrow (*Z. l. gambelii*) is a long distance migrant that breeds across much of the boreal and arctic zones of North America (e.g., Blanchard and Erickson, 1949; Cortopassi and Mewaldt, 1965 Wingfield and Farner, 1978b). It has a short (single brooded) and precisely timed breeding season in the Arctic giving it an Ie factor of about 1 (Wingfield et al., 1992). In an earlier study, we showed that photoperiodically induced testicular and ovarian growth in this taxon was not modified by relevant temperature extremes of 5 and 30 °C (Wingfield et al., 1996). In other words this taxon depends mostly on the annual cycle of day length to regulate reproductive maturation. Supplementary factors such as temperature probably have little effect. However, the Puget Sound white-crowned sparrow, (*Z. l. pugetensis*), breeds at mid-latitudes and has a much longer nesting season in which 2–3 broods may be raised (Blanchard, 1941; Cortopassi and Mewaldt, 1965; Lewis, 1975; Wingfield and Farner, 1978a). Mathematical treatment of several years breeding data gave an Ie factor of about 4 (Wingfield et al., 1992). Accordingly, environmentally relevant temperature affected photoperiodically induced gonadal development in this taxon more dramatically. Low temperature of 5 °C tended to

delay ovarian development whereas high temperature of 30 °C tended to enhance it (Wingfield et al., 1997). Testicular development was much less affected. Temperature treatments also influenced the timing of photorefractoriness, a state in which birds no longer respond to long days with GnRH and gonadotropin secretion resulting in gonadal regression and termination of the breeding season (Dawson et al., 2002; Farner, 1986; Follett, 1984; Nicholls et al., 1988).

This study focuses on the mountain white-crowned sparrow (*Z. l. oriantha*), which breeds primarily in subalpine meadows in the Rocky Mountains and Sierra Nevada of western North America, and winters in Mexico (Cortopassi and Mewaldt, 1965). The breeding biology of this taxon has been the subject of a long term study by Morton (2002) and his associates for over 20 years. Weather in the high mountain habitat of California can vary tremendously from year to year. Both residual winter snow pack and early spring weather can influence timing of onset of breeding. The amount of summer precipitation can determine how long *Z. l. oriantha* may breed. Here we apply mathematical analysis to natural history data from breeding *Z. l. oriantha* and then test whether the Ie factor thus generated indicates how temperature may influence photoperiodically induced gonadal growth. Analyses such as these are important to test theoretical predictions and may provide a firm framework to determine how diverse environmental factors influence neuroendocrine and endocrine secretions.

2. Methods

2.1. Study animals

Juvenile mountain white-crowned sparrows were captured at Tioga Pass, Mono County, CA (37° 50′N, 199° 10′E, 3030 m elevation) in August 1992 and transported to Seattle, WA (47° 30′N, 122° 10′ W, 10 m elevation), where they were held in outdoor aviaries on natural photoperiod and temperature. While in the aviaries, and throughout the remainder of the study, the birds received water and food (mixed wild bird seed and MAZURI small bird diet) ad libitum.

2.2. Natural history data

Egg-laying dates spanning 20 breeding seasons (collected from 1969 to 1993) were arranged in data matrices for each year (rows in Table 1). Each column represented a month of the year in which egg-laying occurred. These matrices were then subjected to log-linear analysis to determine variation in egg-laying date from month to month within years (variation by month—seasonality), year to year variation in egg-laying dates (variation by

Table 1
Breeding data for the mountain white-crowned sparrow at tioga pass

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1	0	0	0	0	0	10	10	0	0	0	0	0
2	0	0	0	0	0	10	10	0	0	0	0	0
3	0	0	0	0	0	10	10	0	0	0	0	0
4	0	0	0	0	0	10	10	0	0	0	0	0
5	0	0	0	0	10	10	10	0	0	0	0	0
6	0	0	0	0	0	10	10	0	0	0	0	0
7	0	0	0	0	0	10	10	0	0	0	0	0
8	0	0	0	0	0	10	10	10	0	0	0	0
9	0	0	0	0	0	10	10	0	0	0	0	0
10	0	0	0	0	0	10	10	10	0	0	0	0
11	0	0	0	0	0	10	10	10	0	0	0	0
12	0	0	0	0	0	10	10	0	0	0	0	0
13	0	0	0	0	10	10	10	0	0	0	0	0
14	0	0	0	0	0	10	10	0	0	0	0	0
15	0	0	0	0	10	10	10	0	0	0	0	0
16	0	0	0	0	0	10	10	0	0	0	0	0
17	0	0	0	0	0	10	10	0	0	0	0	0
18	0	0	0	0	0	10	10	0	0	0	0	0
19	0	0	0	0	0	10	10	0	0	0	0	0
20	0	0	0	0	10	10	10	0	0	0	0	0

0, no egg-laying recorded in that month and year and 10, egg-laying was recorded in that month and year (see Wingfield et al., 1993). Analysis of data in this matrix revealed:

Log-linear analysis: seasonality, U.month = 0.91 (likelihood ratio $\chi^2 = 424$, $p < 0.001$, DF = 68); yearly variation, U.year = 0.1 (likelihood ratio $\chi^2 = 4606$, $p < 0.001$, DF = 220); unpredictability in timing, U.month/year = 0.08 (likelihood ratio $\chi^2 = 380$, $p < 0.001$, DF = 49).

Colwell predictability = 0.89 (Gp = 295.8, $p < 0.005$); contingency = 0.60 (Gm = 200.5, $p < 0.005$); constancy = 0.29 (Gc = 95.32, $p < 0.005$). Ie (the ratio of contingency to constancy) = 2.1.

year) and the interaction of the two (unpredictability and its reciprocal—predictability). If a significant predictability value is obtained for a population, it is hypothesized to indicate that gonadal development is highly responsive to seasonal change in day length (Wingfield et al., 1993). The matrices were also subjected to the contingency/constancy model of predictability devised by Colwell (1974). This model is based on different mathematics from log linear analysis and asserts that predictability has two components, contingency (e.g., the annual cycle has distinct periods when breeding is possible and when it is not), and constancy (e.g., most or all of the year is favorable for breeding, or not). Wingfield et al. (1992, 1993) have suggested that the ratio of contingency to constancy, the environmental information factor, Ie, is an indication of the degree to which supplementary factors in addition to changing photoperiod may regulate gonadal development.

2.3. Data collection

Blood samples (300 μl) were taken by puncturing the alar vein with a 26-G needle and collecting the blood into heparizined microhematocrit tubes (see Wingfield and Farner, 1976). Tubes were immediately sealed with modeling clay and held on ice for at most 6 h before centrifugation (2000 rpm) in a IEC benchtop clinical centrifuge. Plasma samples were harvested with a Hamilton Syringe, and stored in plastic snap-top vials at −20 °C until assay for several different hormones (see below).

Laparotomies were performed on birds under general anesthesia (Metofane, Pittman-Moore, Mundelein, IL). An incision between the last pair of ribs revealed the gonads, and left testis length and width, or diameter of the largest follicle, were measured to the nearest 0.5 mm using forceps. 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. Birds were allowed to recover in their own cage before returning them to their environmental chamber.

Body mass was measured to the nearest 0.1 g using a Pesola spring scale. Subcutaneous fat deposition was scored semi-quantitatively on an arbitrary scale ranging from 0 (no fat) to 5 (gross bulging fat deposits, Wingfield and Farner, 1978a). Separate scores were assigned for furcular and abdominal deposits, and an average of the two calculated.

Presence or absence of molt was determined by observing whether the primary flight feathers were being replaced simultaneously on each wing. The number of primaries, especially the highest primary (from 1 to 9) being replaced were counted on each wing where appropriate.

Two steroid-dependent secondary sex characters were scored. Cloacal protuberance length (a testosterone-dependent organ, e.g., Witschi, 1961) was measured to the nearest millimeter in males, and percent defeathering of the brood patch was estimated in females.

2.4. Hormone assays

Luteinizing hormone (LH), thyroxine (T4), and tri-iodothyronine (T3) were measured by direct radioimmunoassays on unextracted plasma aliquots as follows.

LH was measured by a postprecipitation, double-antibody radioimmunoassay (Follett et al., 1972, 1975; Sharp et al., 1987). The assay utilizes highly purified chicken LH for standard curves and for radio-iodination. Goat anti-rabbit γ globulin precipitating serum was used as second antibody. This assay has been used extensively for measurements of circulating concentrations of LH in a variety of avian species including the genus *Zonotrichia* (Wingfield et al., 1996, 1997). Further details of the LH assay are described by Wingfield et al. (1991), and intra- and interassay variability were similar to those of previous studies.

T4 and T3 were measured by separate RIAs using polyethylene glycol precipitation to separate bound and free hormone (see Tasaki et al., 1986; Wada, 1993). Radio-iodine labeled T3 and T4 were purchased from RadioAmersham. Procedures and assay protocols were described in Wingfield et al. (1996, 1997).

The steroid hormone corticosterone was determined in a subset of plasma samples (i.e., samples with sufficient plasma volume—10 to 20 μl—after completion of RIAs for the hormones above). This was to check whether the temperature treatments resulted in stress that would confound the objectives of this investigation. The assay and summary of the effects of stress on avian reproduction have been summarized in Wingfield et al. (1996, 1997).

2.5. Experimental protocol

On August 28, 1992 the birds were assigned at random to three different groups and transferred to individual cages (55 × 25 × 25 cm) in three identical light- and temperature-controlled chambers at 20 °C. Day length (photoperiod) was decreased by three

increments to 9 h light, 15 h dark (9L:15D) by October 2. They were held in these conditions for 20 days. On October 20 and 21 all birds were laparotomized to determine sex and gonadal state. On November 6 birds were exposed to the temperature on which they would eventually be photostimulated (one group at 5 °C, one at 20 °C, and another at 30 °C) for 6 days. Blood samples collected during this period were used to determine whether temperature affected circulating hormones independent of a change in photoperiod. Body mass and fat score were also recorded. All birds were then returned to 20 °C for 24 days prior to photostimulation. On December 7 all birds were transferred to long days of 15L:9D. At this time, one group was switched to a constant temperature of 5 °C, another to 30 °C, and the third was left at 20 °C.

On day 5 relative to the first long day, blood samples were collected and the birds weighed and scored for molt, fat deposition, brood patch, and cloacal protuberance development. These samples and measurements were taken again from all birds on days 1, 10, 31, 68, and 96 relative to transfer to long days (day 0 being the first long day).

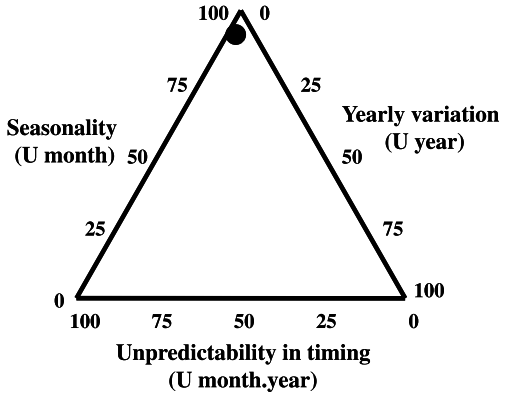

Fig. 1. Predictability of the breeding season in mountain white-crowned sparrows as determined from 20 years of egg-laying dates from Tioga Pass. The triangle represents the relative contribution of seasonality (U.month), year to year variation (U.year) and unpredictability, the interaction of seasonality and yearly variation (Umonth.year). The black circle indicates the intersection of all three variables. Predictability this is the converse of Umonth.year and is very high (0.92). See Wingfield et al. (1993) for details of the analysis.

2.6. Statistics

Except where indicated below, all data were analyzed by repeated measures ANOVA in Systat. When appropriate, post-hoc comparisons between adjacent sampling dates within temperature treatments, or between temperature treatments at particular dates, were compared using univariate F tests built into Systat's Multivariate General Linear Models (MGLH) module. Differences were only considered significant if they survived adjustment for multiple comparisons (i.e., had a p value < 0.05 divided by the number of contrasts). LH values and gonad size measures (both sexes) were log transformed prior to ANOVA to normalize distributions. This was unnecessary for all other data. Note that the ANOVAs on testis and follicle volumes did not include data from the laparotomy at day 20. This laparotomy was performed only to determine sex of individuals and to confirm that all birds had regressed gonads. No quantitative estimates of difference in gonad condition were performed at that time.

Molt score at day 96 was analyzed by Kruskal–Wallis ANOVA followed by Dunn's non-parametric multiple comparisons.

3. Results

3.1. Seasonality of the breeding period

Log-linear analysis of the egg-laying dates for female *Z. l. oriantha* at Tioga pass over a 20 year period revealed a highly significant effect of seasonality (U.month = 0.91) with less yearly variation (U.year = 0.01). Interaction of the two (Umonth.year), or unpredictability, was 0.08. Predictability (the converse of Umonth.year) was thus very high (Table 1, Fig. 1). The

Table 2
Effects of six days of exposure to various temperatures on circulating levels of hormones, body mass and fat score in mountain white-crowned sparrows held on short days (9L:15D)

	Males			Females		
	5 °C	20 °C	30 °C	5 °C	20 °C	30 °C
LH	0.60 ± 0.12	0.75 ± 0.12	0.68 ± 0.08	0.68 ± 0.7	0.77 ± 0.15	0.57 ± 0.1
T3	0.92 ± 0.26	1.29 ± 0.23	0.93 ± 0.24	0.84 ± 0.2	0.82 ± 0.18	0.61 ± 0.15
T4	2.79 ± 0.56	4.20 ± 0.54	2.92 ± 0.42	3.33 ± 0.5	3.73 ± 0.52	4.03 ± 0.22
Mass	33.9 ± 2.03	34.3 ± 0.96	37.2 ± 1.13	28.9 ± 0.7	31.4 ± 1.21	31.2 ± 1.52
Fat	3.42 ± 0.47	3.79 ± 0.34	4.19 ± 0.13	3.17 ± 0.2	3.70 ± 0.26	3.38 ± 0.26

LH, T3, and T4 expressed as ng/ml. Body mass is expressed in grams, and fat score on an arbitrary score from 0 to 5 (see Wingfield and Farner, 1978b). All values means ± standard errors. $N = 6$, 7, and 8 for males on 5, 20, and 30 °C, respectively. $N = 9$, 10, and 8 for females on the same respective temperature regimes.

Table 3
Statistical comparisons of hormone, body measures, and temperature treatments in male mountain white-crowned sparrows, *Z. l. oriantha*

Effect	LH		T4		T3		Mass		Fat		CP		Testis	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Temperature	0.163	0.851	0.094	0.911	2.713	0.099	4.044	0.041*	2.979	0.081	0.016	0.984	0.037	0.964
Date	***72.751***	***0.001*****	***10.098***	***0.001*****	2.616	0.031*	***24.174***	***0.001*****	***29.981***	***0.001*****	***84.331***	***0.001*****	***58.670***	***0.001*****
Date * Temperature	1.745	0.088	2.407	0.015*	1.023	0.432	0.966	0.480	1.091	0.380	2.922	0.017*	***13.640***	***0.001*****
Comparisons within treatments														
Day −5 vs 1														
5°	5.419	0.035*	3.130	0.097	4.090	0.061	0.098	0.759	11.020	0.005*				
20°	3.053	0.102	1.772	0.203	0.628	0.441	8.079	0.013*	0.306	0.588				
30°	***15.218***	***0.002*****	3.811	0.070	0.068	0.797	0.024	0.879	0.306	0.588				
Day 1 vs 10														
5°	5.384	0.036*	0.000	0.987	0.128	0.726	3.775	0.072	2.155	0.163				
20°	***18.829***	***0.001*****	***25.720***	***0.001*****	0.281	0.604	2.042	0.175	1.379	0.259				
30°	2.448	0.140	8.248	0.012*	0.029	0.868	0.023	0.882	0.086	0.773				
Day 10 vs 31														
5°	**38.870**	**0.001****	0.065	0.803	6.106	0.026*	0.649	0.434	1.067	0.318	3.333	0.088		
20°	7.977	0.014*	***18.543***	***0.001*****	0.764	0.396	10.390	0.006*	***13.067***	***0.003*****	***13.333***	***0.002*****		
30°	6.814	0.021*	7.382	0.016*	0.020	0.889	9.651	0.008*	11.267	0.004*	***40.833***	***0.001*****		
Day 31 vs 68														
5°	0.025	0.875	0.234	0.635	6.638	0.021*	0.773	0.394	***14.639***	***0.002*****	***37.835***	***0.001*****	2.585	0.129
20°	3.308	0.090	0.020	0.889	0.005	0.944	1.168	0.298	***30.542***	***0.001*****	***19.055***	***0.001*****	2.293	0.151
30°	0.096	0.762	0.015	0.903	0.532	0.477	7.124	0.018*	***14.639***	***0.002*****	***15.748***	***0.001*****	0.000	1.000
Day 68 vs 96														
5°	***34.332***	***0.001*****	1.043	0.323	0.034	0.857	2.977	0.106	0.399	0.537	***22.975***	***0.001*****	0.958	0.343
20°	***27.175***	***0.001*****	4.742	0.046*	1.672	0.216	0.048	0.830	0.399	0.537	***42.722***	***0.001*****	***46.964***	***0.001*****
30°	***33.337***	***0.001*****	4.160	0.059	8.369	0.011*	0.586	0.457	0.178	0.679	***48.608***	***0.001*****	***58.364***	***0.001*****

Comparisons between treatments						
Day −5						
5° vs 20°	1.435	0.250				
5° vs 30°	0.459	0.509				
20° vs 30°	3.516	0.080				
Day 1						
5° vs 20°	2.539	0.132				
5° vs 30°	0.293	0.596				
20° vs 30°	1.107	0.309				
Day 10						
5° vs 20°	2.672	0.031*	2.927	0.108		
5° vs 30°	2.379	0.144	0.183	0.675		
20° vs 30°	0.704	0.415	1.646	0.219		
Day 31						
5° vs 20°	1.844	0.195	6.575	0.022*	4.888	0.043*
5° vs 30°	0.808	0.383	12.432	0.003*	***12.508***	***0.003*****
20° vs 30°	0.211	0.653	0.925	0.352	1.758	0.205
Day 68						
5° vs 20°	0.799	0.385	0.017	0.899	3.851	0.069
5° vs 30°	0.412	0.531	0.000	1.000	2.909	0.109
20° vs 30°	0.064	0.804	0.017	0.899	0.066	0.801
Day 96						
5° vs 20°	0.061	0.808	7.064	0.018*	***13.179***	***0.002*****
5° vs 30°	0.151	0.703	8.721	0.010*	***20.628***	***0.001*****
20° vs 30°	0.020	0.889	0.087	0.772	0.831	0.376

Table 4
Statistical comparisons of hormone, body measures, and temperature treatments in female mountain white-crowned sparrows, *Z. l. oriantha*

Effect	LH		T4		T3		Mass		Fat		BP		Ovary	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Temperature	0.478	0.626	3.114	0.064	0.105	0.901	2.208	0.133	3.299	0.055	8.909	0.001**	24.382	0.001**
Date	122.159	0.001**	23.241	0.001**	4.230	0.002**	33.016	0.001**	35.844	0.001**	10.767	0.003**	98.596	0.001**
Date * Temperature	1.249	0.269	2.322	0.016*	1.191	0.305	4.903	0.001**	3.261	0.001**	0.862	0.436	31.483	0.001**
Comparisons within treatments														
Day −5 vs 1														
5°	1.558	0.226	4.951	0.036*	1.409	0.248	13.059	0.001**	5.750	0.025*				
20°	0.456	0.507	0.090	0.766	7.681	0.011*	6.292	0.020*	1.878	0.184				
30°	9.135	0.006*	3.094	0.092	0.825	0.374	4.982	0.036*	0.000	1.000				
Day 1 vs 10														
5°	89.558	0.001**	1.811	0.191	2.042	0.168	21.448	0.001**	0.833	0.371				
20°	98.828	0.001**	20.193	0.001**	0.138	0.714	9.965	0.004*	0.052	0.822				
30°	58.109	0.001**	33.796	0.001**	5.508	0.029*	6.196	0.020*	14.986	0.001**				
Day 10 vs 31														
5°	5.034	0.036*	0.591	0.450	1.091	0.308	1.551	0.226	0.043	0.838				
20°	8.855	0.007*	2.937	0.100	0.059	0.811	12.249	0.002*	13.855	0.001**				
30°	13.022	0.002**	0.001	0.979	0.908	0.352	15.167	0.001**	30.066	0.001**				
Day 31 vs 68														
5°	5.689	0.027*	3.012	0.096	0.529	0.475	1.898	0.182	0.529	0.048*		11.003	0.003**	
20°	17.752	0.001**	1.273	0.271	0.436	0.516	3.194	0.087	9.265	0.006*		4.222	0.052	
30°	4.419	0.048*	16.127	0.001**	7.201	0.014*	13.344	0.001**	19.707	0.001**		167.306	0.001**	
Day 68 vs 96														
5°	45.897	0.001**	7.080	0.014*	0.890	0.356	2.158	0.155	0.446	0.511	0.821	0.374	8.051	0.010*
20°	5.720	0.026*	5.596	0.027*	4.234	0.052*	0.758	0.393	0.251	0.621	7.393	0.012*	5.672	0.026*
30°	17.414	0.001**	9.804	0.005*	2.584	0.123	3.340	0.081	0.125	0.726	4.206	0.052*	157.136	0.001**

Comparisons between treatments										
Day −5										
5° vs 20°	2.303	0.143	5.674	0.026*	2.205	0.151				
5° vs 30°	0.015	0.903	6.313	0.019*	1.589	0.220				
20° vs 30°	1.819	0.191	0.041	0.842	0.032	0.859				
Day 1										
5° vs 20°	0.000	0.992	4.516	0.045*	10.153	0.004*				
5° vs 30°	0.009	0.923	4.957	0.036*	5.375	0.030*				
20° vs 30°	0.008	0.931	0.027	0.871*	0.597	0.448				
Day 10										
5° vs 20°	9.019	0.006*	5.716	0.025*	11.681	0.002*				
5° vs 30°	19.116	0.001**	18.459	0.001**	23.177	0.001**				
20° vs 30°	2.128	0.158	3.909	0.060	2.245	0.148				
Day 31										
5° vs 20°	0.016	0.901	0.266	0.611	0.745	0.397			0.907	0.351
5° vs 30°	7.990	0.010*	0.775	0.388	1.064	0.313			10.909	0.003**
20° vs 30°	7.318	0.013*	1.905	0.181	0.038	0.848			5.217	0.032*
Day 68										
5° vs 20°	0.514	0.481	1.533	0.228	0.119	0.734	0.058	0.812	0.026	0.873
5° vs 30°	0.553	0.465	1.062	0.314	0.733	0.401	9.314	0.006*	50.117	0.001**
20° vs 30°	0.002	0.962	0.029	0.866	1.417	0.246	10.797	0.003**	49.523	0.001**
Day 96										
5° vs 20°	0.157	0.695	0.471	0.499	0.108	0.746	0.893	0.354	0.072	0.791
5° vs 30°	1.215	0.282	0.252	0.620	0.014	0.906	14.378	0.001**	4.606	0.043*
20° vs 30°	0.515	0.480	1.364	0.255	0.040	0.844	8.265	0.009*	3.332	0.082

contingency/constancy model also gave a significant predictability value (0.89, $G = 295.8$, $p < 0.005$), but the degree of contingency (0.60, $G = 200.5$, $p < 0.005$) was higher than the degree of constancy (0.29, $G = 95.32$, $p < 0.005$) giving a Ie of 2.1.

Effects of temperature on luteinizing hormone (LH), thyroid hormones, body mass, and fat score on short days.

Exposure to 5, 20, or 30 °C for 6 days prior to photostimulation had no effect on circulating LH ($F = 0.48$, DF = 2,20, $p = 0.63$ in males; $F = 0.73$, DF = 2, 26, $p = 0.49$ in females), thyroxine (T4, $F = 2.36$, DF = 2,20, $p = 0.12$ for males; $F = 0.72$, DF = 2,26, $p = 0.49$ for females), tri-iodothyronine (T3, $F = 0.75$, DF = 2,20, $p = 0.48$ for males; $F = 0.51$, DF = 2,26, $p = 0.60$ for females), or body mass ($F = 1.86$, DF = 2,20, $p = 0.19$ for males; $F = 1.40$, DF = 2,26, $p = 0.27$ for females), and fat score ($F = 1.47$, DF = 2,20, $p = 0.26$ for males; $F = 1.10$, DF = 2,26, $p = 0.35$ for females, Table 2). However, there may have been a delayed effect, see below.

3.2. Temperature and photoperiod effects

All parameters measured changed significantly over time in photostimulated birds. Statistical results are summarized in Tables 3 (males) and 4 (females).

3.2.1. Males, reproductive measures

Low temperature influenced photoinduced testis development and regression dramatically (Fig. 2, top panel). Exposure to 5 °C inhibited gonadal development by day 31, particularly relative to birds at 30 °C. Whereas testes of males at both 20 °C and 30 °C showed complete gonadal regression by day 96, testes of males at 5 °C did not change size between days 68 and 96. At no time did testes of males at 20 and 30 °C differ significantly.

Low temperature also slowed development of the cloacal protuberance (CP), but did not affect maximum size achieved in all groups on day 68 (Fig. 2, lower panel). CP length increased significantly between days 10 and 31 in both 20 and 30 °C birds, but not in 5 °C birds (within treatments analysis). The differences between treatments at day 31 were only marginally significant after adjustment for multiple comparisons, however. CPs regressed in all three groups between days 68 and 96. This decline was delayed somewhat at 5 °C, as CPs were slightly larger in 5 °C birds than the other groups on day 96.

Despite these differences in development of gonads and CPs, temperature had little effect on photoinduced LH secretion (Fig. 3). There was a tendency for LH to rise more rapidly at higher temperatures (within treatments analysis). A robust increase in LH occurred by day 1 at 30 °C, by day 10 at 20 °C, and by day 31 at 5 °C. LH levels in all groups were steady between days 31 and 68, and dropped by day 96.

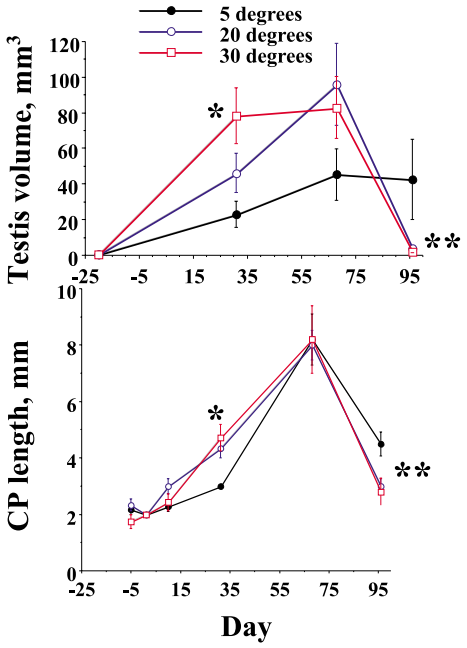

Fig. 2. Patterns of testis volume (upper panel) and cloacal protuberance length (CP length, lower panel) in male mountain white-crowned sparrows at different temperatures following photostimulation. Day 0 is the first long day. Means and standard errors are plotted. See Table 3 for analysis. *$p < 0.04$ and **$p < 0.001$ for temperature effects on photoperiodically stimulated testis size at day 31 and 96, respectively. *$p < 0.02$, **$p < 0.01$ for temperature effects of photoperiodically stimulated CP length at days 31 and 96, respectively.

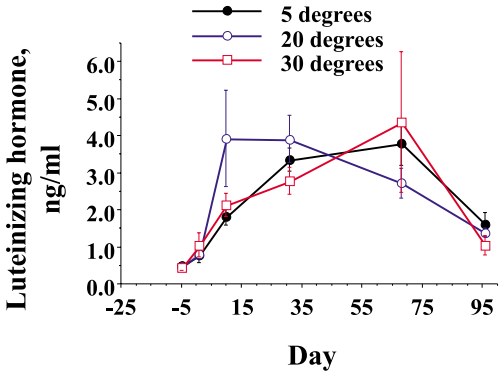

Fig. 3. Patterns of circulating luteinizing hormone (LH) in male mountain white-crowned sparrows at different temperatures following photostimulation. Day 0 is the first long day. Means and standard errors are plotted. See Table 3 for analysis. There were no effects of temperature treatments.

3.2.2. Males, thyroid hormones

Circulating T4 levels of males were affected by temperature (Fig. 4, top panel). A robust increase between days 1 and 10 in birds at 20 °C was absent at 5 °C. There was a marginally significant increase at 30 °C during the same time period (within treatments analysis). T4 levels had declined by day 30 in both of the higher temperature treatments. The only apparent difference between

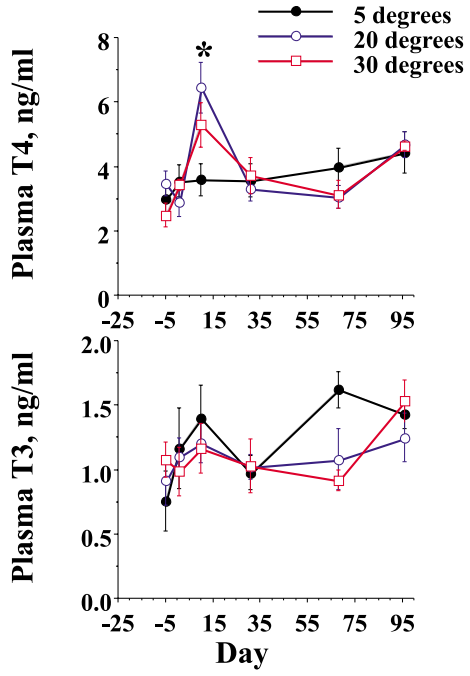

Fig. 4. Patterns of circulating thyroxine (T4, upper panel), and triiodothyronine (T3, lower panel) in male mountain white-crowned sparrows at different temperatures following photostimulation. Day 0 is the first long day. Means and standard errors are plotted. See Table 3 for analysis. $*p < 0.03$ for temperature effect on T4 at day 10. Temperature had no effect on photostimulated increases in T3.

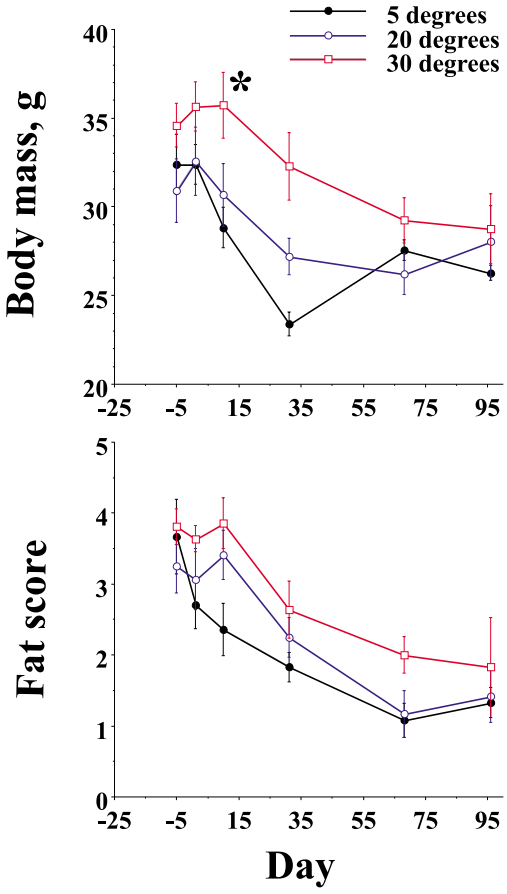

Fig. 5. Patterns of body mass (upper panel) and fat deposition (lower panel) in male mountain white-crowned sparrows at different temperatures following photostimulation. Day 0 is the first long day. Means and standard errors are plotted. See Table 3 for analysis. $*p < 0.04$ for body mass at day 10. There were no effects of temperature on fat score.

treatments was on day 10, when 20 °C birds had marginally higher levels than 5 °C birds.

Although T3 levels changed significantly over time (Fig. 4, lower panel), there were no effects of temperature on photoperiodically induced T3 levels. There were differences between adjacent sampling dates (within treatments analysis). Birds at 5 °C had low T3 at day 31 and 30 °C birds showed an increase from day 68 to 96. The apparent elevation of T3 on day 68 in birds at 5 °C was not significant.

3.2.3. Males, body mass and fat deposition

Temperature had a significant effect on body mass in males (Fig. 5, top panel). This was a main effect (not an interaction of date and temperature) and was already evident prior to photostimulation and modification of temperature. Recall, however, that the birds in each treatment had experienced a short exposure to the planned temperature treatment several days earlier. The tendency was for mass of all groups to decline similarly over the course of the experiment.

There was no robust effect of temperature on fat deposition in males (Fig. 5, lower panel). A decline in fat score in birds at 5 °C by day 1 was marginally significant, but during the same time interval fat score in the other two treatments tended to rise slightly. Fat levels declined more dramatically between days 10 and 31 at the higher temperatures than at 5 °C, and continued to do so in all three treatments between days 31 and 68.

3.2.4. Females, reproductive measures

Exposure to 30 °C enhanced ovarian development in females (Fig. 6, top panel). Size of largest follicle was greater in 30 °C females than 5 °C females on day 31, and greater than both lower temperature treatments on day 68. Ovaries began regression by day 96 in all groups (within treatments analysis), but this decline was significant only in the 30 °C birds. Note that on day 68, some females at 30 °C had follicles containing yellow yolk indicating that final maturation of the ovary had begun. This final maturation process can lead to ovulation.

Brood patch (BP) development was also enhanced in females at 30 °C (Fig. 6, lower panel). No BP development had occurred in any group by day 31. On day 68, females at 30 °C showed greater loss of feathers on the

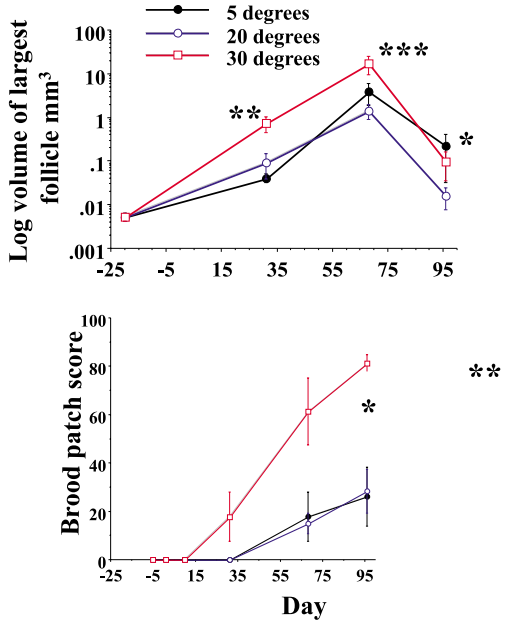

Fig. 6. Patterns of largest ovarian follicle volume (upper panel) and brood patch development (lower panel) in female mountain white-crowned sparrows at different temperatures following photostimulation. The brood patch score varies from 0 (features covering it completely) to 1.0 (patch 100% bare of feathers); in no case did edema develop. Day 0 is the first long day. Means and standard errors are plotted. See Table 4 for analysis. *$p < 0.04$, **$p < 0.003$, ***$p < 0.001$ for temperature effects on photoperiodically stimulated follicle growth at days 96, 31, and 68, respectively. *$p < 0.003$, **$p < 0.001$ for temperature effects on photoperiodically stimulated brood patch score at days 68 and 96, respectively.

BP than those at 20 and 5 °C. By day 96, some females on 30 °C had begun growing new feathers on their BPs, but the overall percent loss of feathers was greater than in females held at 5 and 20 °C. Note that the temperature effect appears as a main effect, rather than a date/temperature interaction, because only data from days 68 and 96 were used. Prior to day 68, there was no variation in BP development so these data could not be incorporated in the ANOVA. By day 68 the differences between treatments were already large, leading to the appearance of a main effect of temperature in the ANOVA (see Table 4).

As in males, photoinduced LH secretion in females was little affected by temperature (Fig. 7). Within 1 day there was a marginally significant trend for LH to increase most rapidly in the 30 °C birds (within treatments analysis). Levels rose dramatically and similarly in all three groups between days 1 and 10. LH tended to decline more slowly at lower temperatures. A significant decline first occurred between days 10 and 31 at 30 °C, between days 31 and 68 at 20 °C, and between days 68 and 96 at 5 °C (within treatments analysis). Overall, however, photoinduced LH secretion patterns were strikingly similar in the three temperature treatments.

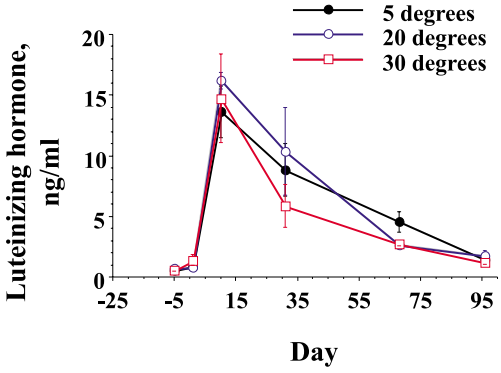

Fig. 7. Patterns of circulating luteinizing hormone (LH) levels in female mountain white-crowned sparrows at different temperatures following photostimulation. Day 0 is the first long day. Means and standard errors are plotted. See Table 4 for analysis. There were no effects of temperature treatments on photoperiodically stimulated LH release.

3.2.5. Females, thyroid hormone levels

Circulating T4 levels increased dramatically in both warmer treatments between days 1 and 10, but did not change at 5 °C (Fig. 8, lower panel, within treatments analysis). The subsequent decline by day 68 was more gradual at 20 ° than at 30 °C. There was a significant difference in circulating T4 between 5 and 30 °C birds

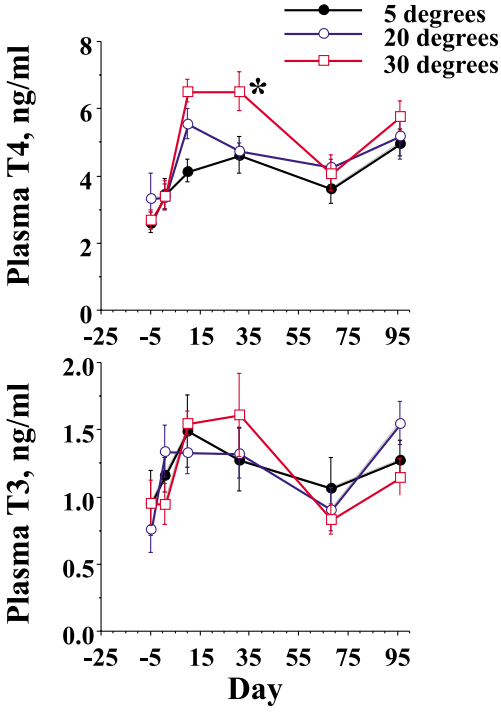

Fig. 8. Patterns of circulating thyroxine (T4, upper panel) and triiodothyronine (T3, lower panel) in female mountain white-crowned sparrows at different temperatures following photostimulation. Day 0 is the first long day. Means and standard errors are plotted. See Table 4 for analysis. *$p < 0.001$ for temperature effects on photoperiodically stimulated T4 levels at day 31.

on day 10, but only marginally significant between 20 and 30 °C birds. All three treatments showed similar levels of T4 between days 68 and 96 (within treatments analysis).

As in males, T3 levels of females varied significantly and in a manner similar to T4 over the course of the experiment (Fig. 8, lower panel). However, apart from general increases following photostimulation, changes were variable.

3.2.6. Females, body mass and fat deposition

Body mass of females increased by day 1 and continued to do so through day 10 in birds at 30 °C (Fig. 9, top panel). In contrast, mass declined between days 1 and 10 in the two cooler treatments, dramatically so at 5 °C. Mass subsequently declined in birds at 30 °C. The only significant difference between treatments was on day 10, when birds at 30 °C were heavier than birds at 5 °C.

Fat deposition appeared to be inhibited by low temperature (Fig. 9, lower panel). Gradual declines in fat depot at 5 °C, and increases at the higher temperatures, resulted in reduced fat levels at 5 °C on day 10. By day 31, fat levels of birds on the warmer treatments had declined.

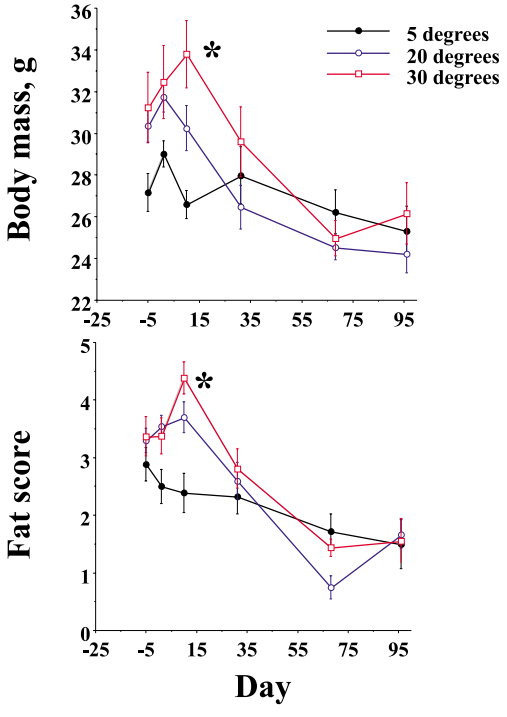

Fig. 9. Patterns of body mass (upper panel) and fat deposition (lower panel) in female mountain white-crowned sparrows at different temperatures following photostimulation. Day 0 is the first long day. Means and standard errors are plotted. See Table 4 for analysis. *$p < 0.001$ for temperature effects on photoperiodically stimulated changes in body mass and fat score at day 10.

3.3. Effects of temperature and photoperiod on molt

As expected, both males and females on all treatments began molt after about 70–90 days of photostimulation (Fig. 10) as the gonads began regressing (Figs. 2 and 6). Molt was most advanced at day 96 in the 30 °C groups of males and females, most delayed in the 5 °C groups and intermediate in the 20 °C groups (Kruskal–Wallis test: $H = 7.59$, $p = 0.02$).

3.4. Effects of temperature and photoperiod on plasma levels of corticosterone

Corticosterone levels increased following photostimulation in males (Fig. 11, $F = 5.14$, 5,10, $p = 0.0012$) but not in females ($F = 1.67$, 5, 10, $p = 0.16$). There were no differences among temperature treatments (Fig. 11, $F = 1.25$, 2,7, $p = 0.34$ for males; $F = 3.4$, 2,8, $p = 0.085$ for females), nor was there a significant interaction of photostimulation and temperature treatments ($F = 1.59$, 10,35, $p = 0.15$ for males; $F = 0.65$, 10,40, $p = 0.76$ for females).

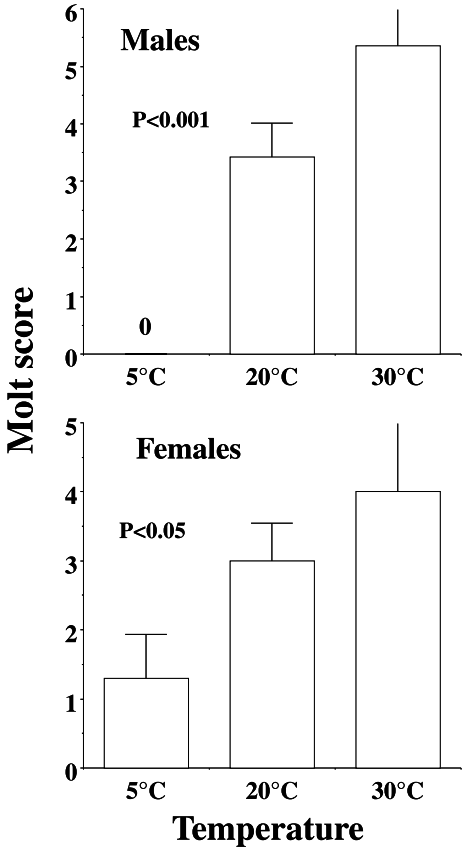

Fig. 10. Patterns of molt in male (upper panel) and female (lower panel) mountain white-crowned sparrows. Note that at the low temperature onset of molt was retarded and rate of molt slowed in both sexes compared with birds exposed to higher temperatures.

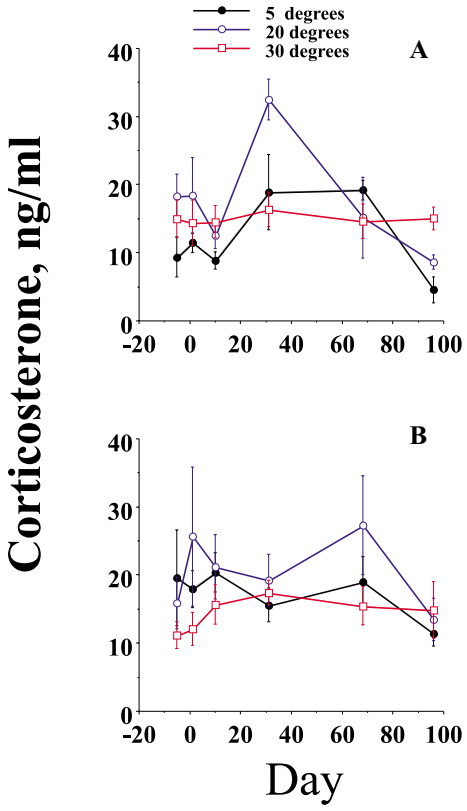

Fig. 11. Plasma levels of corticosterone in male and female mountain white-crowned sparrows exposed to different temperature regimes following photostimulation. Points are means ± standard errors. There were no effects of temperature on photoperiodically induced changes in corticosterone.

4. Discussion

The mountain white-crowned sparrow (*Z. l. oriantha*) breeds throughout the Sierra Nevada of California, the Rocky Mountains, and other montane regions in the western United States (Cortopassi and Mewaldt, 1965). It breeds in alpine meadows to above the tree-line (e.g., Morton et al., 1990). At these elevations environmental conditions permitting onset of nesting can be delayed, especially when snow pack is deep or when spring weather is severe. Thus breeding is restricted to late spring and summer months but the timing of onset of breeding is likely to vary because of snow etc. (Morton et al., 1972). Log-linear analysis of egg-laying dates from female mountain white-crowned sparrows recorded from 20 breeding seasons to define when the nesting period begins and ends in each year, suggested a highly predictable breeding season. The robust response of gonadal growth following photostimulation is thus to be expected, as was also found in Gambel's (*Z. l. gambelii*), and Puget Sound white-crowned sparrow (*Z. l. pugetensis*) populations.

Log-linear analysis does not appear to be highly sensitive in assessing both seasonal and yearly variation (Wingfield et al., 1993). Application of the constancy/contingency model of predictability (Colwell, 1974) revealed again a highly predictable breeding season consistent with a strong effect of photoperiod on reproductive development and regression. However the degree of contingency (the change from times of year when conditions are suitable for breeding compared with times of year when they are not) was higher than the degree of constancy to give an Ie of about 2. This is higher than in *Z. l. gambelii*, (Ie = 1) the arctic migrant with a short and highly predictable breeding season, but lower than in *Z. l. pugetensis* (Ie = 4) which has the longest breeding season and is most flexible (see Wingfield et al., 1992, 1993, 1996, 1997). Thus we predicted from the Ie values that unlike in *Z. l. gambelii, Z. l. oriantha* should be sensitive to cues other than day length but perhaps less so than in *Z. l. pugetensis*. The rationale is that the higher the Ie value, the more environmental cues a population of birds should use to time breeding. Those populations with more flexible breeding seasons should respond to as many environmental variables as possible to regulate gonadal growth and onset of breeding so that reproductive success in a variable habitat is maximized. In contrast, populations with a short breeding season that occurs at the same time each year have lower Ie values and should be less sensitive to environmental cues other than photoperiod (see Wingfield et al., 1992, 1993). This study corroborates these predictions because temperature modulated photoperiodically induced gonadal growth in *Z. l. oriantha*, whereas it did not in *Z. l. gambelii* (Wingfield et al., 1996). Although *Z. l. oriantha* had a lower Ie value than *Z. l. pugetensis*, it appears that the effects of temperature on gonadal recrudescence were marked.

We used temperature as a supplementary factor because it is an environmental cue that all populations are likely to assess in some way. It is highly relevant to *Z. l. oriantha* nesting at high elevation where persistently low spring temperatures are likely to lead to delayed snow melt and retarded appearance of conditions suitable for onset of nesting. It is important to note here that the experimental temperature range (5, 20, and 30 °C) is relevant to those *Z. l. oriantha* experience in their natural habitat in spring, although the 30 °C temperature is probably at the extreme upper end that they experience while breeding. Thus the temperatures used are pertinent to the question asked and unlikely to be stressful since plasma levels of corticosterone, an indicator of stress, were similar in all treatments. Similarly, temperature regimes in other white-crowned sparrow taxa had no effect on corticosterone levels consistent with a non-stressful experimental treatment (Wingfield et al., 1996, 1997).

In this study, males as well as females responded to temperature treatment whereas in *Z. l. pugetensis*, only females showed significant effects (Wingfield et al.,

1997). This suggests that although the mathematical models are useful to determine the degree of predictability in the breeding season, and whether individuals should be sensitive to initial predictive cues such as changing day length, they may not be useful for detailed comparisons of populations and taxa. However, the Ie factor does appear to be useful in predicting whether individuals in a given population should rely mostly on initial predictive cues, and whether supplementary cues are also important (such as temperature). The sensitivity of the models may preclude further conclusions. Nonetheless, analysis of natural history data such as egg-laying dates in this manner may allow us to predict neuroendocrine and endocrine responses to initial predictive versus supplementary cues.

The mechanisms by which temperature inhibits or accelerates photoperiodically induced gonadal growth remains a mystery. There were no obvious effects of temperature treatment on plasma levels of LH in either sex. These results are consistent with those from *Z. l. pugetensis* in which females showed significant responses of ovarian development to temperature treatment without any apparent effect on LH or FSH (Wingfield et al., 1997). It also seems highly unlikely that low environmental temperatures were sufficiently stressful to elevate circulating corticosterone levels (Fig. 11). It is possible that the delayed secretion of T4 in birds exposed to 5 °C may explain the protracted gonadal cycle, and that enhanced secretion of T4 in the 30 °C group may explain the truncated cycle (i.e. early gonadal regression and onset of molt). Thyroid hormones may regulate termination of reproductive function, particularly onset of photorefractoriness (a condition in which animals become insensitive to the stimulatory effects of long days) and onset of molt, has been implicated in several avian and mammalian species (e.g., Nicholls et al., 1988; Wilson and Reinert, 1993). Although it is now thought that T4 may not be required for expression of the photorefractory state, at least in European starlings (*Sturnus vulgaris*, Bentley et al., 1997), temperature-modulated thyroid hormone secretion may regulate the rate at which photorefractoriness develops which in turn affects the onset of molt. It should be noted that temperature treatment on short days had no effect on thyroid hormone levels suggesting that the experimental treatments did not induce a massive metabolic response. Temperature affected thyroid hormones only after photostimulation. Whether the hypothalamo–pituitary–thyroid axis may regulate variation in termination of the breeding season deserves further study.

Although we found no effects of temperature on circulating LH levels following photostimulation, the data should be treated with caution because the chicken LH antibody probably cross reacts with thyroid-stimulating hormone (TSH) that in turn regulates secretion of T4 and thus production of T3 (Follett et al., 1972, 1975). However, in both sexes, plasma levels of LH dropped markedly by day 96 even though plasma levels of T4 and T3 remained high. Thus we feel it is unlikely that TSH cross-reactivity confounded the results, but nonetheless, further investigation is necessary to rule out a possible effect on LH. Note also that in previous experiments, temperature had no effect on photoperiodically induced increases in the other gonadotropin, follicle-stimulating hormone (FSH) further suggesting that temperature effects on gonadal development may involve a separate neuroendocrine pathway (Wingfield et al., 1996, 1997).

There were no significant increases in body mass and fat score following photostimulation unlike *Z. l. gambelii* (Wingfield et al., 1996) and similar to *Z. l. pugetensis* (Wingfield et al., 1997). *Z. l. oriantha* and *Z. l. pugetensis* are relatively short-distance migrants and the marked change in photoperiod from 9L to 15L may have proved to be such a strong stimulus for gonadal development that any migratory-related changes in body mass and fat were masked. A long distance migrant, *Z. l. gambelii*, did express pre-migratory fattening when transferred to 20L 4D (Wingfield et al., 1996). In this study it was clear that body mass and fat score slowly declined in all groups but were lower at 5 °C. In females, however, body mass was lower in the 5 °C group at the point of photostimulation, suggesting they differed prior to onset of the experiment. Reasons for this are unclear but it is possible that prior exposure to temperature treatments while on short days may have had a delayed effect that was then continued following photostimulation and further temperature treatment. Effects of temperature on body mass and fat following photostimulation need further investigation, especially in relation to degree of migratory activity. As with reproductive function, the role of supplementary environmental factors on regulation of vernal and autumnal migration, particularly the endocrine mechanisms, remains poorly understood.

This study supports a role for mathematical analysis of natural history data in studies of the control of reproduction and other life history events. Flexibility in nesting periods is important for many species in low to mid-latitudes and a method to estimate this objectively should be useful. The combination of log-linear analysis and the constancy/contingency model of Colwell (1974) allows us to predict whether initial predictive information (day length) is important AND to what extent other factors may be involved (Ie factor). This in turn results in design of specific experimental manipulations to test physiological and morphological responses to relevant environmental cues. Comparing taxa that are closely related but with presumably adaptive responses to the same suite of environmental cues is a particularly powerful way to focus on possible neuroendocrine and endocrine pathways. Studies on three taxa of *Z. leucophrys*

indicate also that the traditional hypothalamo-pituitary-gonadal axis (gonadotropin-releasing hormone and gonadotropins) may not be the only hormonal cascade that transduces environmental information that influences reproductive function.

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