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Apparent dissociation of photoperiodic time measurement between vernal migration and breeding under dim green light conditions in Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*

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Abstract In seasonally breeding birds, the annual cycle of photoperiod is a principal environmental cue for temporal arrangement of different life-history stages, such as migration and breeding. In the past, most research has focused on the mechanisms of photoperiodic control of breeding with less attention paid to migration. In Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii* (GWCS), photoreceptors for induction of breeding are known to reside in the basal hypothalamus. However, it is unknown whether the sites of photoperiodic reception for vernal migration are the same as those for breeding. Therefore, we hypothesized that they may be controlled separately. In this study, we exposed photosensitive GWCSs to low-penetration green light (wavelength at 510 nm) under a regime of 1 lux during the day and <0.1 lux at night, and switched the photoperiodic conditions from short day (10 h daytime) to long day (18 h daytime). The results showed that the experimental birds developed traits associated with vernal migration including mass increase, fat deposition and migratory restlessness behavior when transferred from short day to long day green light cycles, while control birds maintained continuously on short day green light conditions did not express any migration related characteristics. Neither experimental nor control groups showed gonadal recrudescence under either green light cycles. In support of our hypothesis, we were able to apparently dissociate the photoperiodic responses regulating vernal migration and breeding, which suggests separate mechanisms of photoperiodic time measurement. Such distinct photoperiodic mechanisms may drive the fine-tuned temporal arrangement of the two life history stages [*Current Zoology* 59 (3): 349–359, 2013].

Keywords Gambel's white-crowned sparrow,Vernal migration, Gonadal recrudescence, Photoperiodic time measurement

In seasonal animals, the life-history stages (LHSs) are sequentially aligned at different times of the year to constitute an annual cycle of seasonal events. The evolution of the sequentially expressed LHSs is considered a response to the demands of variation in environmental conditions over the year (Dawson, 2008; Wingfield, 2008). Seasonal changes in the environment such as food availability, predator pressure and weather, impose constraints for the timing and durations of LHSs (Dawson, 2008). Among all of the stages, breeding is particularly confined within a certain time window, to insure adequate food availability, raise young and maximize reproductive success (Jacobs and Wingfield, 2000). Each LHS includes three sequential phases: development, mature capability or onset, and termination, and each phase involves differentiation of cells, tissues, and sometime organs, as well as changes in physiology and behavior (Jacobs and Wingfield, 2000; Ramenofsky et al., 2003; Wingfield et al., 2004; Wingfield, 2008). Transitions between LHSs can involve the overlap of the mature capability and termination phases of the previous LHS and development of the next to expedite transitions of morphology, physiology and behavior (Ramenofsky and Wingfield, 2006). Therefore, it is critical for seasonal animals to be able to predict and prepare for a subsequent life history stage particularly if the developmental phase is time-consuming. For many migratory species, the annual change in day length, photoperiod, provides a reliable predictable cue to initiate vernal migration as well as breeding (e.g., Dawson, 2008). However, actual migration (mature capability) precedes that of breeding.

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In avian systems, seasonal timing of reproduction and migration has been investigated since the early twentieth century (Rowan, 1925). The timing of both LHSs has been considered either under the direct regulation of photoperiod or under the regulation of circannual clock which can be entrained by photoperiod as well as other non-photic cues (Gwinner, 1996a; Jacobs and Wingfield, 2000; Dawson et al., 2001). Although the underlying mechanisms apparently are different between photoperiodic and circannual regulation, both are involved with a circadian system (Follett et al., 1974; Gwinner, 1996b). Recently, the physiological and molecular bases of avian photoperiodic regulation of reproduction have been discovered involving encephalic photoreceptors, basal hypothalamic pacemaker and a thyroid hormone signaling system (Halford et al., 2009; Nakane et al., 2010; Kang et al., 2010). Meanwhile, the physiological machinery for the timing of migration, also under the control of the vernal increase in photoperiod, is still poorly understood (Coppack and Bairlein, 2011).

Early studies (summarized in Follett et al., 1974) illustrated that vernal migration is induced by the increasing production of gonadal hormones in response to long photoperiods. The inhibitory effect of gonadectomy was demonstrated on either vernal premigratory fattening or migratory restlessness, *Zugunruhe* or both, when the surgeries were performed before any photoperiodic stimulation (i.e., before the winter solstice) in white-throated sparrows *Zonotrichia albicollis*, golden-crowned sparrow *Zonotrichia atricapilla*, and white-crowned sparrow *Zonotrichia leucophrys* (Weise, 1967; Stetson and Erickson, 1972; Mattocks et al., 1976; Morton and Mewaldt, 1962; Schwabl and Farner, 1989ab). Implants of small quantities of testosterone can reinstate vernal migratory fattening in gonadectomized male and female white-crowned sparrows further indicating that exposure to testosterone prior to increasing daylength may be important in the regulation of vernal migration (Mattocks, 1976; Boswell et al., 1995). Moreover, in free-living white-crowned sparrows, there are elevations in circulating levels of testosterone in males and 5α-dihydrotestosterone (DHT) in females in late winter and early spring (Wingfield and Farner, 1978a). It was concluded that androgens (testosterone and perhaps its metabolites) have a role in the development of premigratory events (hyperphagia, fattening and *Zugunruhe*) for vernal migration (Wingfield et al., 1990). However, it is unknown whether the role of gonadal hormones is just permissive or actually serves as a photoperiodic endocrine signal for the timing of vernal migration. In wild *Zonotrichia leucophrys gambelii* (GWCS), the development phase of vernal migration is initiated earlier than gonadal recrudescence (Wingfield and Farner, 1978a and 1978b; Hahn et al., 1995), and activation of the hypothalamo-pituitary-gonad (HPG) axis is not required for initiation of vernal migration (Weise, 1967; Schwabl and Farner, 1989a), which suggest that the timing mechanism of vernal migration is not fully dependent on photoperiodic induction of HPG axis and the increasing production of gonadal hormones. In addition the pineal gland with its hormone melatonin does not appear to have any effect on photoperiodic regulation of gonadal development (Kumar, 1997; Bentley, 2001), while it has been suggested to play a role in the timing of avian migration (Gwinner, 1996b; Fusani and Gwinner, 2005; Underwood et al., 2001; Kumar et al., 2010). Overall, it remains to be solved whether photoperiodic time measurement (PTM) of avian migration is regulated by the same mechanism as that of reproduction.

In birds, the photoreceptive pathway for photoperiodic gonadal response is different from that in mammals studied to date (Doyle and Menaker, 2007), and the involvement of extraocular photoreceptors has been indicated in various avian species (Menaker et al., 1970; Homma and Sakakibara, 1971; Gwinner et al., 1971; Yokoyama et al., 1978; Wilson, 1990; Wilson, 1991). As for the photoperiodic timing of migration, only a few studies have addressed the role of extraocular photoreceptors (Gwinner et al., 1971; Yokoyama and Farner, 1976; Yokoyama and Farner, 1978). In photosensitive GWCS, bilateral enucleation does not affect the long photoperiod-induced pre-migratory weight increase, fat deposition and gonadal development, which suggests that the retinal photoreceptors are not required for photostimulation of these vernal functions (Yokoyama and Farner, 1976). In the Gwinner et al (1971) study, reducing the amount of light entering the brain significantly inhibited but did not abolish the photoperiodic responses of gonadal recrudescence and *Zugunruhe* activity in both white-crowned sparrow and golden-crowned sparrow. These results suggested that extraocular photoreceptors are involved in the development of vernal migration, although it was not clear whether the initiation of migration was mediated by the same photoreceptors of photoperiodic activation of the HPG axis.

In this study, we focused on the possible dissociation of PTM between vernal migration and reproduction in GWCS. The rationale of our experimental design is based on the observation that the threshold light intensity for photoperiodic induction of gonadal development is 6-10 lux (Bartholomew, 1949; Menaker and Underwood, 1976) while entrainment of locomotor activities is 0.1 lux in sparrows (Menaker, 1968). The assumption has been that *Zugunruhe* and locomotor activity, indeed migration and reproduction in general, are regulated by the same circadian system. The studies outlined in this communication intend to test these assumptions by determining whether migratory traits can be stimulated by thresholds of light that are insufficient for development of breeding. We hypothesize that a long photoperiod (18 h) with daytime light intensity less than 6 lux will induce development and onset of the migration LHS but not gonadal development in photosensitive GWCS. In order to be consistent with previous studies (Menaker and Underwood, 1976; Menaker, 1968) favoring short wavelength light with less capability of penetrating the brain, we applied only green light for both daytime lighting (1 lux) and nighttime (0.1 lux) lighting conditions and performed the following two sets of experiments. In Experiment I, we exposed photosensitive birds held on short day conditions (10 h bright white light and 14 h very dim green light (<0.1 lux)) (10 L/14 vDmD) to dim short day conditions (10 h dim green light (1 lux) and 14 h very dim green light (<0.1 lux) (10 DmL/14 vDmD), and then transferred the birds from 10 DmL/14 vDmD to dim long day conditions (18 h dim green light (1 lux) and 6 h very dim green light (<0.1 lux)) (18 DmL/6 vDmD). In Experiment II, we repeated the Experiment I with an additional group maintained in 10 Dm/14 vDm conditions serving as a control. We predict: (1) the pre-migratory hyperphagia associated mass increase, fattening and *Zugunruhe* will be induced in the group transferred to 18 DmL/6 vDmD in both Experiments I and II, but not in the group maintained in 10 DmL/14 vDmD of Experiment II and (2) that no gonadal recrudescence will occur under either regime of dim green light.

1 Materials and Methods

1.1 Animals and experiment design

Experiment I In the autumn of 2007, female Gambel's white-crowned sparrows were captured during autumn migration at the Sunnyside Game Refuge in Central Washington State (46.1°N, 119.5°W), transported to Seattle and held in outdoor aviaries at University of Washington. On Feb 4th 2008, photosensitive birds (n=8) were moved to an indoor environmental chamber (25 °C) and housed one per registration cage under short day conditions, 10 h bright white light (550 lux) and 14 h very dim green light (<0.1 lux) (10 L/14 vDmD). On Feb 9th, the light-dark cycles were switched to dim short day conditions, 10 h dim green light (1 lux) and 14 h very dim green light (<0.1 lux) (10 DmL/14 vDmD) for a week, and then to dim long day conditions, 18 h dim green light (1lux) and 6 h very dim green light (<0.1 lux) (18 DmL/6 vDmD) on Feb 17th for 68 days (Table 1). The light intensities under different conditions were measured at the cage top level, and the wavelength is around 510 nm under both dim and very dim green light conditions. The purpose of using very dim green light (<0.1 lux) for the night phase is that previous studies showed that birds held under complete darkness remain still and display only limited movement (Ramenofsky et al., 2003; Landys, et al., 2004). All experiments were approved by the Animal Care and Use Committee at the University of Washington, Seattle.

Table 1 Experimental setup in both Experiment I and II

Experiment I	Period 1	Period 2	Period 3
Schedule	Feb 4th –Feb 8th	Feb 9th – Feb 16th	Feb 17th – April 25th
Light condition	10 L/14 vDmD	10 DmL/14 vDmD	18 DmL/6 vDmD
Duration (days)	5	7	68
Experiment II	**Period 1**	**Period 2**	**Period 3**
Schedule	Feb 3rd – Feb 20th	Feb 21st – March 6th	March 7th – April 6th
Light condition	10 L/14 vDmD	10 DmL/14 vDmD	10 DmL/14 vDmD (con.) 18 DmL/6 vDmD (exp.)
Duration (days)	18	14	30

Experiment I and II were performed separately in 2007 and 2009, and birds were sequentially subjected to specific light conditions from period 1, period 2 to period 3. Abbreviations: 10 L/14 vDmD, 10 h bright white light and 14 h very dim green light (<0.1 lux) short day conditions; 10 DmL/14 vDmD, 10 h dim green light (1lux) and 14 h very dim green light (<0.1 lux) short day conditions; 18 DmL/6 vDmD, 18 h dim green light (1 lux) and 6 h very dim green light (<0.1 lux) long day conditions. Note: con., control group; exp., experimental group.

Experiment II In order to confirm whether the results from experiment I were a response to photoperiodic change or exposure to dim light conditions, Experiment II was performed by repeating Experiment I with an additional dim short day control group, which was maintained under 10 DmL/14 vDmD condition while experimental group was switched from 10DmL/ 14 vDmD to 18 DmL/6 vDmD conditions (Table 1).

On Feb 2nd, 2009, wintering GWCS were captured with walk-in traps in West Putah Creek on the UC Davis campus Yolo County, California. Female birds (*n*=16) were captured and then transferred to individual cages in experimental rooms where temperature was maintained at 25 °C. From Feb 3rd to Feb 20th, 2009, all birds were exposed to 10 L/14 vDmD conditions. On Feb 21st, the light-dark cycles were switched to 10 DmL/14 vDmD. On March 7th after 17 days under 10 DmL/14 vDmD, the light-dark cycles were changed to 18 DmL/6 vDmD for half of the birds (experimental group, *n*=8) but maintained under 10 DmL/14 vDmD for the left half (control group, *n*=8). One bird from experimental group died under 10 L/14 vDmD conditions and was removed from the study. All birds were sacrificed a month later on April 6th, 2009. The light intensities for different light-dark cycles applied were identical to the previous study except that the day time light intensity under 10 L/14 vDmD was lower (range from 120 to 400 lux for different cages) but consistent for each cage. All animal care protocol was approved by Animal Care and Use Committee at the University of California, Davis.

1.2 Measurement of body mass, fat score and ovarian follicle size

For both of the experiments, morphological measures were monitored during the daytime period without exposure to extra light. Mass was measured by placing the birds in a cotton sock before placement on an electronic scale (Ohaus Scout Pro, model SP202) to the nearest 0.1 g, and fat score was determined as the mean of the deposits in the furcular fossa and abdominal cavity using an arbitrary scale from 0 to 5 (see Wingfield and Farner, 1978b). In Experiment I, ovarian follicle size was examined by laparotomy, and the diameter of the largest follicular of the left ovary was recorded at about 1.5 months after birds were transferred to dim light/dark cycles (in the middle of 18 DmL/6 vDmD period). The protocol for laparotomy was modified from a previous study (Wingfield and Farner, 1976), which has been in routine use for more than 40 years. Briefly, just prior to this surgical procedure, birds were transferred in light tight cloth bags into a surgery room under dim light conditions, and then anesthetized with a commercial vaporizer that delivered 2%–4% isoflurane mixed with oxygen. Then the left flank was exposed, and single incision was made between the last pair of ribs on the left side. The diameter of the largest follicle on the left ovary was measured. The whole procedure was done with a laser green light directly focused on the incision opening in the room with ambient dim green light less than 0.1 lux. In Experiment II, the diameter of the largest follicle on the left side was examined upon sacrifice at the end of the experiment.

1.3 Locomotor activity and behavioral monitoring

In Experiment I, locomotor activities were monitored as previously described (Agatsuma and Ramenofsky, 2006; Coverdill et al., 2008). Briefly, within each registration cage, 1 perch was centrally located with one IR photodetector (Radio Shack Invisible Beam Alarm Entry, model #43-311 and #43-312) and a reflector mounted on either end of the perch and perpendicular to the perch. Activity was registered with an infra-red (IR) beam between photodetector and reflector which, when broken by the birds' movement, was catalogued by Labview software (National Instruments Corporation, Austin, TX). The activity records were further refined by D. Baldwin (NMFS Unit, Seattle, WA) calculating locomotor activity in units of average beam breaks per minute per 30-min interval. Actograms and 3-D activity plots were graphed by using MatLab 7.0. In addition to locomotor activity monitoring, actual behavioral displays for each bird was sampled by IR video camera (Sony Handycam Vision, model CCD-TRV87) at different times of the day when night time activities were indicated by the IR-beam motion detecting system. The occurrence of *Zugunruhe*, was confirmed by the videos according to the previously characterized and distinct Beak-Up and Beak-Up Flight in GWCS (Agatsuma and Ramenofsky, 2006; Coverdill et al., 2008).

In Experiment II, the closed circuit television (CCTV) system was applied to behavioral monitoring. The CCTV system was set up by connecting 4 active IR analog video cameras to 1 PCI video recording card installed on the PC with windows 2000, and the CCTV video recording program, Pico 2000, was used to continuously video-record the behavior of birds with 1 frame per second. Each IR camera monitored 4 birds at the same time. To quantify the behavior of *Zugunruhe*, we applied an instantaneous scan method (Martin and Bateson, 1993; Agatsuma and Ramenofsky, 2006; Coverdill et al., 2008) with modification. Specifically, all birds were video-taped continuously throughout the experiment, and 4 h videos at the mid of both day and night time were visually examined at 20-sec intervals continuously for two days on Feb 26th– 27th (after 2 weeks under 10DmL/14vDmD for both control and experimental group, Stage I), March 14th-15th (1 week after the switch to 18DmL/6vDmD for control group, Stage II), March 21st –22nd (2 weeks after the switch, Stage III), and March 30th-31th (3 weeks after the switch, Stage IV). For behavioral identification, Beak-Up and Beak-Up Flight were quantified as a measure of *Zugunruhe* like behavior (Agatsuma and Ramenofsky, 2006) (Supplemental figure), and the percentages of Beak-Up and Beak-Up Flights for each 4-h sampling period were averaged continuously for two days for later statistical analysis.

1.4 Statistical analyses

All data were first tested for normality with the Shapiro-Wilk test and then subjected to either parametric or nonparametric test when appropriate. In Experiment I, body mass and average fat score at each sampling point under 18DmL/6vDmD were compared to initial sampling point under 10L/14vDmD by using paired T test for mass and Wilcoxon matched pair test for average fat score. In Experiment II, body mass was analyzed by One-Way Repeated-Measure ANOVA followed by Bonferroni's multiple comparison test while average fat score was analyzed by Friedman test followed by Dunns post-hoc test separately in control and experimental group. The percentages of migratory-like behavior of all occurring behaviors were first analyzed by the one-sample Wilcoxon signed-rank test for the presence of *Zugunruhe* like behavior at both the middle of day and the middle of night under different light conditions. Then Wilcoxon matched pair test was used to examine the difference of the percentages of *Zugunruhe* like behavior between the middle of day and the middle of night in the photoperiodic conditions when the behavior was present. For all the statistical analysis, data were presented as MEAN±SEM, and significance was considered when P-value was equal to or less than 0.05. All the statistics were done by using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA).

2 Results

2.1 Experiment I

Under both 10L/14vDmD and 10DmL/14vDmD conditions, the daily rhythms of locomotor activities were entrained to the ambient light-dark cycle with typical diurnal pattern and the onset of locomotor activities synchronized to the light-on signal (Fig. 1). About two weeks after the light-dark cycles were switched to 18 DmL/6 vDmD, 7 out of 8 birds developed migratory restlessness (*Zugunruhe*) with onset synchronized to the light-off signal (Fig. 1). Furthermore, the video recordings confirmed the display of Beak-Up and Beak-Up Flight for *Zugunruhe* like behavior (Supplemental Figure). On the 28th day of 18 DmL/6 vDmD conditions, both body mass and fat score were significantly increased from short days (wintering) state (Table 2, Fig. 2). During the period of 68 days under 18DmL/6vDmD conditions, body mass and fat scores were maintained without significant decrease, and the migratory restlessness behavior was sustained throughout (Fig. 1 and 2). On the 45th day of DimLD conditions, laparotomy showed that all birds had completely regressed winter-like (short day) ovaries with the largest ovarian follicle diameter less than 0.1 mm.

Table 2 Values and statistics of body mass and average fat score in Experiment I and II

Experiment I		10 L/14 vDmD	18 DmL/6 vDmD	Statistics
	Mass	26.76 ± 0.79	30.44 ± 1.53*	$n = 8$, $t = 2.99$, $P = 0.017$
	Fat	2.25 ± 0.23	4.38 ± 0.34*	$n = 8$, $W = -28$, $P = 0.021$
Experiment II		10 L/14 vDmD	18 DmL/6 vDmD	
	Mass (con.)	22.26 ± 0.545	24.13 ± 0.73	$n = 8$, $t = 0.13$, $P > 0.05$
	Fat (con.)	1.75 ± 0.25	2.19 ± 0.51	$n = 8$, rank difference = - 7, $P > 0.05$
	Mass (exp.)	22.78 ± 0.75	27.20 ± 2.38*	$n = 7$, $t = 3.81$, $P < 0.05$
	Fat (exp.)	1.83 ± 0.38	3.75 ± 0.68*	$n = 7$, rank difference = - 22.5, $P < 0.05$

In Experiment I, data were listed for the 2nd day under 10 L/14 vDmD conditions and the 28th day under 18 DmL/6 vDmD. In Experiment II, data were listed for the 2nd day under 10 L/14 vDmD for both groups, 5 weeks under 10 L/14 vDmD for control group and 3 weeks under 18 DmL/6 vDmD for experimental group. Note: con., control group; exp., experimental group.

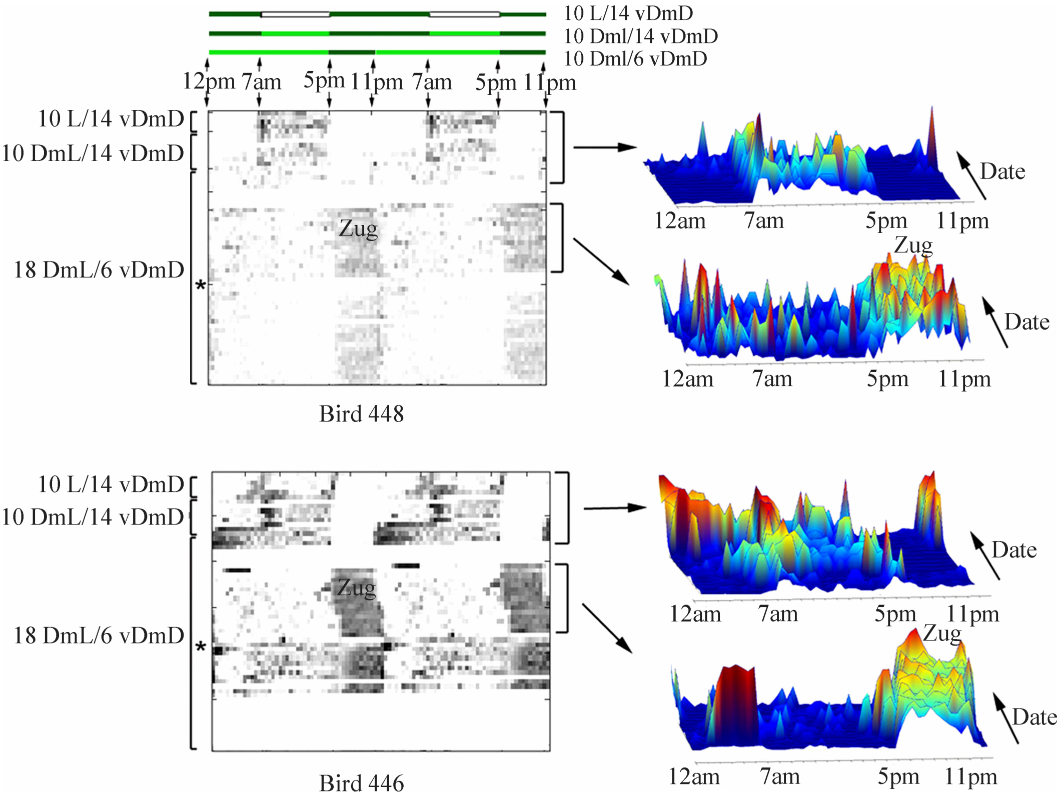


Fig. 1 Actograms and 3-D graphs of locomotor activities from two representative birds in Experiment II

Actograms (left) were double-plotted for the entire experimental period. The photoperiodic and light manipulations (illustrated on top of the actograms by colored bars) were indicated on the left of the actograms. Part of the locomotor activity data were plotted in 3-D graphs (right) indicated by the arrows on the right of the actograms in both birds. In 3-D graphs, three axes indicate the time of day (x-axis), the date (y-axis) and the amount of locomotor activity (z-axis) respectively. Zug (*Zugunruhe*) indicates the migration-like Beak-Up and Beak-Up Flight behaviors were confirmed with video. The asterisks in the actograms indicate the day when laparotomy was performed. The blank areas in the actograms indicate that data are not available during those periods.

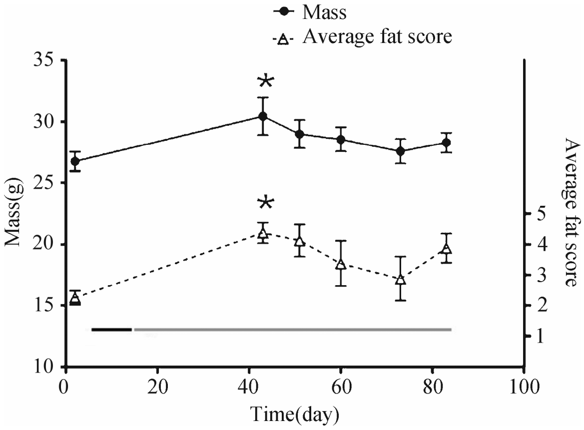


Fig. 2 Changes in body mass (black dot) and average Fat scores (open triangle) over days in Experiment I

Photoperiodic conditions of 10 DmL/14 vDmD and 18 DmL/6 vDmD were indicated by black bar and gray bar respectively. Significance ($p < 0.05$) was indicated by the asterisk.

2.2 Experiment II

Under both 10 L/14 vDmD and 10 DmL/14 vDmD conditions, no significant changes were observed for either body mass and fat score in either control or experimental group (Fig. 3). Video analysis on Stage I (2 weeks under 10 DmL/14 vDmD conditions) did not reveal any obvious *Zugunruhe* like behavior during the 4 h sampling period at either mid-day or mid-night (Fig. 4, Supplemental video). After the light-dark cycles were switched to 18 DmL/6 vDmD in the experimental group, both body mass and fat score were significantly increased after 3 weeks under 18DmL/6vDmD conditions, while no significant increases were observed for either body mass or fat score in control birds maintained under 10 DmL/14 vDmD conditions (Table 2, Fig. 3). After 1 week under 18 DmL/6 vDmD conditions (Stage II), experimental birds started to display small amount of *Zugunruhe* like behavior at either the mid of day or the mid of night or both while significantly more *Zugunruhe* like behavior was displayed at the mid of day than at the mid of night (Fig. 4). Until 3 weeks after 18 DmL/6 vDmD conditions, significant amounts of *Zugunruhe* like behavior (> 50%) were observed only at mid-night in experimental birds. Control birds held under 10 DmL/14 vDmD conditions did not display any

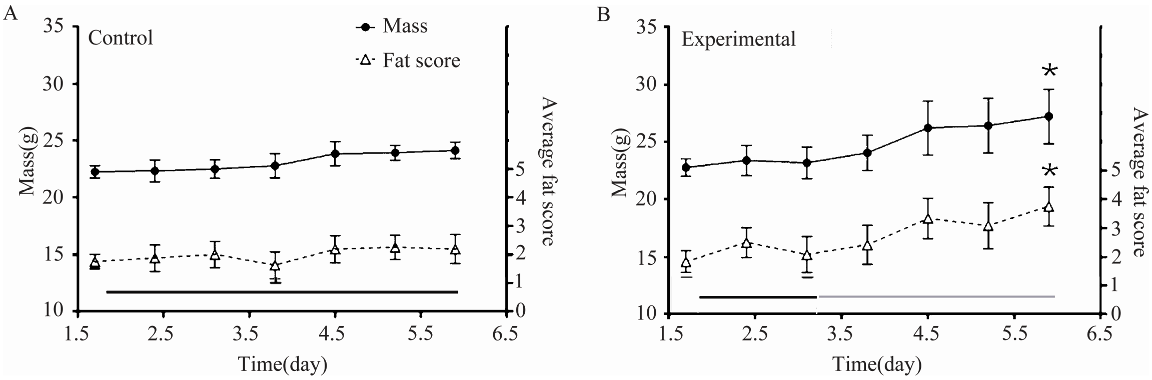
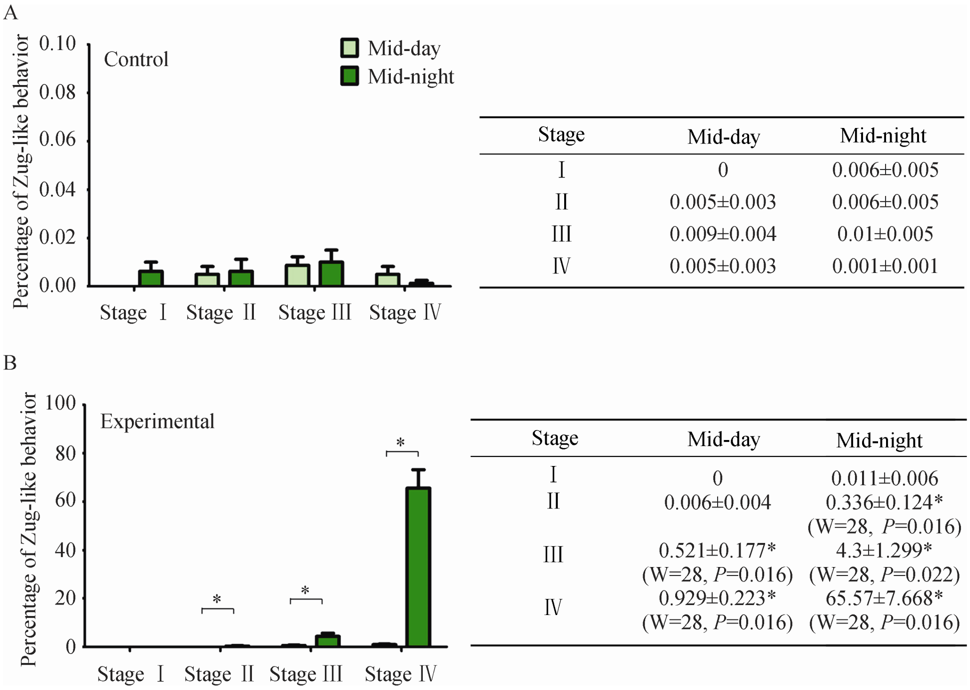


Fig. 3 Changes in body mass (black dot) and average fat scores (open triangle) over days of control group (A) and experimental group (B) in Experiment II

Photoperiodic conditions of 10 DmL/14 vDmD and 18 DmL/6 vDmD were indicated by black bar and gray bar respectively. Significance was indicated by either single asterisk ($P < 0.05$), double asterisks ($P < 0.01$) or triple asterisks ($P < 0.001$). Significance ($P < 0.05$) was indicated by the asterisk.



Stage	Mid-day	Mid-night
I	0	0.006±0.005
II	0.005±0.003	0.006±0.005
III	0.009±0.004	0.01±0.005
IV	0.005±0.003	0.001±0.001

Stage	Mid-day	Mid-night
I	0	0.011±0.006
II	0.006±0.004	0.336±0.124* (W=28, *P*=0.016)
III	0.521±0.177* (W=28, *P*=0.016)	4.3±1.299* (W=28, *P*=0.022)
IV	0.929±0.223* (W=28, *P*=0.016)	65.57±7.668* (W=28, *P*=0.016)

Fig. 4 Percentage of observed *Zugunruhe* like behavior (Beak-Up and Beak-Up Flight) in a 4 h sampling period during both mid-day (light green bar) and mid-night (dark green bar) in different stages (I, II, III, IV) in both control (A) and experimental group (B) in Experiment II

The detailed values and statistics were listed in the table on the right. The asterisks in the graphs indicates the significant difference between the mid of day and the mid of night, and the asterisks in the tables indicates the significant difference from zero. Stage I, 2 weeks under 10 DmL/14 vDmD conditions in both control and experimental group; Stage II, 3 weeks under 10 DmL/14 vDmD conditions in control group and 1 weeks under 18 DmL/6 vDmD conditions in experimental group; Stage III, 4 weeks under 10 DmL/14 vDmD conditions in control group and 2 weeks under 18 DmL/6 vDmD conditions in experimental group; Stage IV, 5 weeks under 10 DmL/14 vDmD conditions in control group and 3 weeks under 18 DmL/6 vDmD conditions in experimental group. Note: In control group, the percentages of *Zugunruhe* like behavior were extremely low so that the scale of y-axis (0–0.1%) was different from the scale (0–100%) in experimental group.

obvious *Zugunruhe* like behavior (Fig. 4, Supplemental video). At the end of the experiment, no development was observed for ovarian tissue in both control and experimental birds, and the diameter of largest ovarian follicle was regressed (< 0.1 mm) for all birds.

3 Discussion

The timing of migration has been assumed to be closely associated with the photoperiodic regulation of the hypothalamo-pituitary-goand (HPG) axis (Ramenofsky et al., 2012), but it is unknown whether migration and breeding life history stages are expressed under identical photoperiodic machinery. Instead, it has been suggested in GWCS that all vernal functions (pre- migratory hyperphagia, migratory restlessness, prenuptial molt and gonadal recrudescence) can be potentially separated under different photoperiodically-induced mechanisms (Moore et al., 1982). This study is the first to experimentally dissociate the photoperiodic regulation of migration-related vernal functions from gonadal recrudescence.

Consistent with previous studies (Bartholomew, 1949; Menaker and Underwood, 1976), dim light conditions with daytime light intensity at 1 lux and night time intensity <0.1 Lux, do not effectively serve to photostimulate gonadal development, irrespective of photoperiod. In contrast, photoperiodic increase from 10 h to 18 h dim green light (1 lux) with <0.1 lux dim green light at night initiated vernal migration-related functions including mass increase, fattening and migratory restlessness (*Zugunruhe*). Birds maintained under 10 DmL/14 vDmD conditions for more than 1 month did not progress beyond the short day or wintering stage in Experiment II. Our results indicate that switching low intensity illumination (1 lux) from short to long days was sufficient to stimulate development of the vernal stage of migration but not breeding. The apparent dissociation between vernal migration and breeding under dim long days suggests the different phototransduction mechanisms of photoperiodic time measurement between the two life history stages. In another study, where centrally implanted fiber optics were used to illuminate specific brain areas in GWCS, Yokoyama and Farner (1978) demonstrated that illumination of the basal hypothalamus with 20 L 4 D long day conditions resulted in the development of nocturnal, *Zugunruhe*-like activity in comparison with short day controls (8 L 16 D). But it is arguable from their activity data whether the nocturnal activities displayed in the ambient scotophase are associated with subjective day or subjective night when the basal hypothalamus was illuminated. Especially when considering the experimental birds' 4-h quiet period with neither ambient light nor brain illumination, suggests that this nocturnal activity may be related to regular daily locomotor activity rather than specifically *Zugunruhe*. Unfortunately, no video record is available from this study for identification of the specific behaviors. In the same study (Yokoyama and Farner, 1978), significant testicular growth was observed before the development of nocturnal activity in the brain illuminated birds, which is unusual for GWCS in either the natural or ambient light conditions. As a result, the photoreceptors within the basal hypothalamus may not be involved in the timing of migration. In our study, 18 DmL/6 vDmD long day conditions successfully induced migratory related functions without stimulating gonadal recrudescence while extended 10 DmL/14 vDmD short day conditions did not do so in photosensitive GWCS. Our results suggest that activation of the encephalic photoreceptors involved in activating and driving the HPG axis are not required for initiation of vernal migration. Although there may be a sex difference in the gonadal responsiveness of males and females, we did not observe any ovarian development in our female birds under dim long day conditions in this study, which suggests that the lack of gonadal development is unlikely due to lesser responsiveness of ovarian tissue. Combined with Gwinner et al. (1971) and the Yokoyama and Farner (1976 and 1978) studies, our findings can point to a potential photoreceptor for timing vernal migration that may reside in the retina, pineal or other brain areas in addition to the basal hypothalamus.

Allowing for the diverse migration and breeding strategies, the independent temporal regulation of both LHSs may offer more flexibility in response to different environmental situations. In the three subspecies of white-crowned sparrows, GWCSs (a long-distance migrant), Puget Sound White-crowned sparrows (*Z. l. pugetensis*, a short-distance migrant), and the completely resident Nuttall's White-crowned sparrows (*Z. l. nuttalli*) there is large variation of the temporal coupling of photoperiodic responses to migration and breeding (Blanchard and Erickson, 1949; Farner and Lewis, 1973; Wingfield and Farner, 1978a; Ramenofsky and Wingfield, 2006; Coverdill et al., 2011). Our study suggests independent photoperiodic regulation between vernal migration and breeding in GWCSs, the subspecies with highest extent of temporally coupling under natural conditions. Based on our study, we hypothesize that the photoperiodic machinery of vernal migration is separable from that of breeding in *Z. l.* species, and different organization and threshold for inducible photoperiod (including light intensity) of both mechanisms result in the different extent of temporally coupling between vernal migration and breeding in different subspecies.

The photoperiodic response systems in birds appear to be composed of multiple dissociable parameters, which are independently susceptible to natural selection (Hahn and MacDougall-Shackleton, 2008; MacDougall-Shackleton et al., 2009). These kinds of multi-dimensional reaction norms of the regulatory systems offers enough flexibility of photoperiodic responses for different LHS strategies and environmental conditions, so that a modification of a few such parameters is potentially possible to transform a strictly seasonal breeding schedule into an almost entirely opportunistic one (Hahn et al., 2009). The results of our study are consistent with this hypothesized multi-dimensional reaction norm of photoperiodic response systems. Furthermore, our study suggests that the photoperiodic responses for breeding and migration may have evolved independently with natural selection acting on different sets of dissociable parameters. It is possible that by varying degrees of sharing of these parameters comprising the photoperiodic response mechanisms of breeding and vernal migration, may allow more or less dissociation/association.

In summary, the present study apparently dissociated the PTM between vernal migration and reproduction in captivity under dim green light conditions in GWCS. Different regulation of photoperiodic responses between vernal migration and breeding LHSs may provide temporal flexibility of arrangement between these two stages under different environmental conditions. In addition, the dissociated PTM systems suggest and independent evolution of photoperiodic response systems between migration and breeding.

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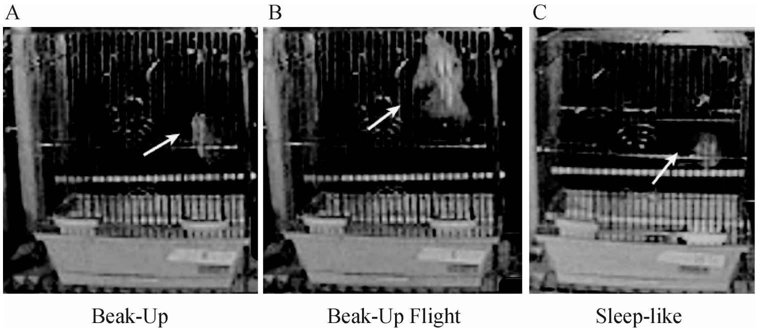
Supplemental Materials



Beak-Up Beak-Up Flight Sleep-like

Supplemental figure. Representative photos showing *Zugunrhe like* behavior (Beak-Up and Beak-Up Flight) during migratory stage and sleep like behavior at night time during non-migratory stage.

Supplemental video. Representative video recording during both mid-day and mid-night under either dim short day (DimSD) conditions (10 DmL/14 vDmD) and 3 weeks after dim long day (DimLD) conditions (18 DmL/6 vDmD) were provided.

Corrigendum

The authors of Rolán-Alvarez et al. paper [Can sexual selection and disassortative mating contribute to the maintenance of a shell color polymorphism in an intertidal marine snail?" Current Zoology 58(3): 463474; 2012] wish to correct both supplementary Table S2 and Tables 1-4 due to some errors detected in the published version of this work. Nevertheless, the main results and conclusions of this paper are unaffected. Corrected versions of these tables are displayed at http://www.currentzoology.org/issuedetail.asp?volume=58&number=3&issue_id=527