

Effect of Polar Day on Plasma Profiles of Melatonin, Testosterone, and Estradiol in High-Arctic Lapland Longspurs

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Accepted December 20, 2001

In polar habitats, continuous daylight (polar day) can prevail for many weeks or months around the summer solstice. In the laboratory, continuous light conditions impair or disrupt circadian rhythms in many animals. To determine whether circadian rhythms are disrupted under natural polar day conditions in a species that is only a summer resident in polar regions we analyzed diel rhythms in plasma concentrations of melatonin, testosterone (T), and 17- β estradiol (E_2) during the summer solstice in Arctic-breeding Lapland Longspurs (*Calcarius lapponicus*). We compared these profiles to those of conspecifics housed in outdoor aviaries at a mid-latitude site in Seattle, Washington, during spring, summer, fall, and winter. Under polar day conditions plasma melatonin concentrations of Lapland Longspurs were strongly suppressed, but still showed a significant diel rhythm. Likewise, plasma T in males, and E_2 in females, showed significant diel changes in Arctic birds. Lapland Longspurs housed at mid-latitude in Seattle showed high-amplitude melatonin cycles at all times of the year, and the duration of the nightly melatonin secretion was positively correlated with the duration of the dark phase. We found no

diel changes in plasma T in Seattle males in May, but Seattle females showed significant day/night differences in plasma E_2 in May. The data suggest that even under polar day conditions diel rhythms can persist. The maintenance of hormone rhythms could provide a physiological basis to reports of rhythmic behavior in many birds during the Arctic summer. © 2002 Elsevier Science (USA)

Key Words: polar summer; light suppression; circadian system; steroid hormone; melatonin.

INTRODUCTION

The circadian system is responsible for the diel organization of behavioral and physiological tasks in organisms (Pittendrigh, 1981; Daan, 1987; Aschoff, 1989). To properly function as an endogenous clock, the circadian system needs to be synchronized with the natural 24-h day. This is usually achieved by entrainment of circadian rhythmicity with the diel changes in light and dark (e.g., Aschoff, 1981; DeCoursey, 1989).

Polar regions are challenging environments for circadian systems because they lack diel light-dark changes twice each year: Above the Arctic and Antarctic Circle ($>66^{\circ}33'$), continuous light (polar day) or darkness (polar night) prevails for many weeks

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around the summer and winter solstices, respectively. In the absence of exogenous timing cues circadian systems usually continue to cycle, with a period deviating from 24 h (e.g., Aschoff, 1981). However, exposure to constant bright light can suppress or even disrupt circadian function in many animals (McMillan *et al.*, 1975; Binkley *et al.*, 1980; Yamada *et al.*, 1988; Honma *et al.*, 1992) and the disruption of the circadian system can impair survival (e.g., Daan and Aschoff, 1982; DeCoursey *et al.*, 2000).

Lapland Longspurs (*Calcarius lapponicus*) are small passerine birds with an Arctic circumpolar breeding distribution (Bent, 1968). North American Lapland Longspurs typically arrive on their breeding grounds in Alaska in mid May (e.g., Hunt *et al.*, 1995). Throughout their short, 6-week reproductive season, these birds can experience continuous daylight. We examined how the polar day affects the circadian system of adult Lapland Longspurs at a time of year that is crucial for reproductive success. Are these birds able to maintain circadian rhythmicity or does their circadian system become disrupted?

The functioning of the circadian system can be assessed by measuring its physiological output such as diel rhythms in plasma hormone concentrations. We determined diel profiles of plasma melatonin, testosterone (T), and 17- β estradiol (E_2) in captive Lapland Longspurs around the summer solstice in Alaska. We compared these rhythms to those of conspecifics housed in outdoor aviaries at Seattle, Washington, approximately the latitude of their wintering grounds.

The nightly melatonin secretion is a critical part of the circadian system of birds (Binkley, 1990; Cassone, 1990; Gwinner and Hau, 2000). Pineal melatonin production can be inhibited within less than 1 h after illumination during the night (e.g., Binkley *et al.*, 1980; Honma *et al.*, 1992), and disruption of the diel melatonin pattern can dramatically alter or even abolish circadian rhythms in many avian species (Cassone, 1990; Gwinner *et al.*, 1997). The steroid hormones T and E_2 also show robust diel changes in plasma concentrations in birds (Schanbacher *et al.*, 1974; Balthazart, 1976; Aschoff, 1979; Bachman *et al.*, 1987). Presence or absence of diel changes in these two hormones were determined to assess whether the polar day has a direct inhibitory effect only on melatonin secretion or whether it also affects other diel hormone rhythms.

METHODS

Animals

Lapland Longspurs used in this study came from a population that migrates to Alaska in spring to breed. Birds were caught in potter traps and housed singly or in pairs in cages in an indoor room at the Toolik Lake Research Station, University of Alaska (68°38'N, 149°38'W). Alaska birds were allowed to become accustomed to the cages for 3–4 days before the first samples were taken. Cages were situated directly at the window, to provide natural light conditions. Day-length in Toolik Lake at this time of year is 24 h, but light intensity can vary throughout the day (see Table 1), due to changes in the azimuth of the sun (e.g., Benelbaz *et al.*, 1976) and the occurrence of fog (L.M.R., pers. obs.). Another group of birds was transported to Seattle (47°40'N, 122°20'W), initially for other experiments on aggressive behavior. Seattle birds were housed in groups of 6–12 birds in two large outdoor aviaries on the roof of the Zoology Department of the University of Washington (which, due to its height, was not reached by city lights). They were exposed to natural temperature and light conditions for a few months (range: 1–6 months) prior to the experiment. At both locations food and water were supplied *ad libitum* and birds received exactly the same diet.

Hormone Samples

Plasma samples were obtained at six different times of day (“bleeding times”). In Alaska, birds were bled at 0:00, 3:00, 6:00, 12:00, 18:00, and 21:00 h and in Seattle at 2:00, 6:00, 10:00, 14:00, 18:00, and 22:00 h. As our main aim was to examine diel rhythmicity in plasma hormones in Alaska birds, sampling occurred at shorter intervals between 21:00 and 6:00 h in Alaska birds and thus at different intervals than in Seattle birds. Although these differences in sampling schedules prevent a direct comparison between Alaska and Seattle hormone profiles, it maximized the detection of small and short-term increases in hormone concentrations in Alaska birds.

Alaska birds were bled without using an artificial light source in June 1996 (for sampling dates, times, and number of birds see Table 1). For each bleeding

TABLE 1
Sampling Schedule and Ambient Light Intensity at Alaska (Toolik Lake) during the Polar Day

Date	Sampling time	Light intensity (W/m ²)	Light intensity (lux) ^a	Average light intensity (W/m ²) ^b	Average light intensity (lux) ^b
6/1/96	00:00	6	4,100	16 (±6)	11,000 (±4,400)
6/10/96	03:00	0 ^c	0 ^c	1.3 (±1.6)	900 (±1,100)
	06:00	50	34,000	75.6 (±13)	52,000 (±8,600)
6/14/96	09:00	404	275,000	277 (±32)	190,000 (±22,000)
	12:00	654	445,000	443 (±49.2)	303,000 (±34,000)
6/6/96	18:00	70	48,000	334.5 (±49.8)	230,000 (±34,000)
6/5/96	21:00	103	70,000	105.3 (±17.1)	72,000 (±12,000)

^a 1 lux = 1 lumen/m², 1 lumen = 0.0014705882 W; so 1 lux = 0.0014705882 W/m².

^b Mean (± SE) of 15 days, May 31–June 14, 1996.

^c The 0 light intensity on day 6/10/96 remains unexplained but perhaps was due to a failure in the recording system. At this time it was light outside (L.M.R., pers. obs.). Light intensity data from the Toolik Lake Research Station were collected by Dr. G. Shaver and the Ecosystems Center at the Marine Biological Laboratory at Woods Hole, Massachusetts, as part of the Long Term Ecological Research Program in the Arctic. Data are averages per hour and reflect light intensity at the time that the samples were taken.

time individuals were used at random. Only a limited number of birds could be caught and kept in captivity; thus to achieve good sample sizes most individuals were bled repeatedly for different bleeding times (between two and five times). Because of the small body size of Lapland Longspurs (average body mass 27.5 g; L.M.R., unpublished data), the same individual was not bled more than once in every 4–5 days (previous experiments have used similar bleeding intervals on small birds without adverse affects; e.g., Wingfield *et al.*, 1982; Beldhuis *et al.*, 1988). Data collection for a complete diel hormone profile at each time of year thus took about 2 weeks (photoperiodic conditions remaining similar).

In Seattle, for each bleeding time birds from one aviary were used. To complete a diel hormone profile for each time of year, most birds were used repeatedly (two to three times) for different bleeding times. Repeated sampling of the same individual was spaced at least 4 days apart (see above). Daytime samples were taken without artificial light; nighttime sampling was done with a headlamp that emitted very dim blue light directed at the wing, the head of the bird being in the dark (short wavelengths do not penetrate the skull well in vertebrates; Hartwig and van Veen, 1979). For most nighttime bleeding times, birds were caught at dusk, kept in a bird holding container in the dark, bled at a specific time, and then released back into their aviaries at dawn. Bleeding times and times of year are listed in Tables 1 and 2. Blood samples (about 200 μl)

TABLE 2
Sampling Schedule for Seattle Birds (Males and Females Combined)

Date	Sampling time	N (birds)
May (daylength 14.5 h)		
4/30/96	02:00	9
	14:00	11
5/5/96	10:00	10
	22:00	10
5/10/96	06:00	10
	18:00	9
August (daylength 15.0 h)		
7/27/96	02:00	10
	14:00	10
8/1/96	22:00	10
8/2/96	10:00	9
8/8/96	18:00	10
8/9/96	6:00	9
October (daylength 11 h)		
10/16/96	10:00	8
	22:00	8
10/21/96	14:00	9
10/22/96	2:00	6
10/25/96	18:00	7
10/26/96	6:00	7
January (daylength 8 h) ^a		
1/4/97	14:00	5
1/6/97	2:00	7
1/20/97	18:00	5
1/21/97	6:00	4

^a In January 1997 no samples for 10:00 and 22:00 h could be taken.

were taken by venipuncture of the alar vein. Blood was kept at 4° until centrifugation; plasma was then aspirated off and frozen at -20°. For analysis, samples were transported to Germany on dry ice.

Hormone Analysis

Hormones were analyzed after separation on diatomaceous earth columns based on the methods of Wingfield and Farner (1976), with modifications (Van't Hof, 2000; T. J. Van't Hof, unpublished). Briefly, for estimation of recovery we added to all samples 10 μl of tritiated label for each hormone measured (i.e., 3000 dpm for melatonin, 800 dpm for T, 500 dpm for E_2). Samples were then extracted twice with 2 ml redistilled dichloromethane and twice with 2 ml chloroform. We eluted the following fractions from diatomaceous earth columns by adding the following mixtures of ethyl acetate (EA) in isooctane (IO): 2 ml pure IO (fraction discarded), 1.5 ml of 10% EA in IO (discarded), 2 ml of 20% EA in IO (contained T), 2 ml of 40% EA in IO (contained E_2), 2 ml of 50% EA in IO (discarded), and 3.5 ml of 65% EA in IO (contained melatonin). Fractions were dried down and T and E_2 fractions were redissolved in 200 μl PBSG buffer. The melatonin fraction contained some glycols, which were removed by adding 500 μl distilled water and extracting twice with 2 ml chloroform. Dried melatonin extracts were redissolved in 200 μl tricine buffer. From each sample, 45 μl of the buffer solution was used to determine recoveries; the rest was used for radioimmunoassay in duplicates. A total of seven assays were conducted. For melatonin, average (mean \pm 95% CI) detection limit was at 29 ± 9.3 pg/ml, average recovery was $69.9 \pm 2.9\%$, intraassay variation was $8.9 \pm 4.3\%$ (coefficient of variation for a total of three standards of different concentrations), and interassay variation was $28.1 \pm 4.3\%$ (coefficient of variation for a total of three standards of different concentrations). For T, average detection limit was at 7.5 ± 2.7 pg/ml, average recovery was $85.7 \pm 1.8\%$, intraassay variation was $5.8 \pm 2.4\%$, and interassay variation was $19.4 \pm 9.9\%$. For E_2 , average detection limit was at 8.0 ± 2.7 pg/ml, average recovery was $79.1 \pm 2.5\%$, intraassay variation was $6.8 \pm 4.6\%$, and interassay variation was $19.8 \pm 2.8\%$. To eliminate bias owing to interassay variation, hormone samples were arranged so that representative samples from each bleeding

time, time of year, and location were included in a single assay. Given the high interassay variation, this evenly distributed the introduced error over all samples. The high interassay variation in our hormone analyses was unexpected, yet diel rhythmicity of plasma hormone concentration exceeded interassay variation and thus could still be detected.

Data Analysis

Hormone profiles consisted of a mixture of dependent and independent data and bleeding times differed between Alaska and Seattle birds. Therefore, to analyze diel hormone differences and compare profiles between different times of year and locations we first averaged "daytime" and "nighttime" values for each individual for each time of year and location and used those in further analyses. In Seattle birds, "day" and "night" times were taken from photoperiod charts (from the "Seattle Times"). "Day" in May and August included bleeding times from 6 a.m. through 6 p.m. and "night" from 10 p.m. through 2 a.m. In October, "day" included 10 a.m. through 6 p.m. and "night" 10 p.m. through 6 a.m. In January, "day" was 2 p.m. and "night" was from 6 p.m. through 6 a.m. To compare Alaska and Seattle birds, we divided the Alaska day such that it corresponded to the Seattle "day" and "night" at the same time of year, but termed it "bright" and "dim" instead of "day" and "night." Hence, in Alaska "bright" included all bleeding times from 6 a.m. through 6 p.m. and "dim" from 9 p.m. through 3 a.m. Changes in plasma hormone concentrations of individuals between "day" and "night" or "dim" and "bright" for each time of year and location were analyzed using unbalanced repeated-measures ANOVA models (BMDP Statistical Software, Los Angeles, 1993). This approach derives parameter estimates using maximum-likelihood or restricted maximum-likelihood methods and does not make restrictive assumptions on the structure of the within-subjects covariance matrices. These models allow repeated-measures analyses where one or more measurements (over time) are missing for a given subject by imputing missing data points. The imputation method functions by estimating a condition mean of the missing value, given the values of the response variable that are present for that subject. The numbers of missing data points in each analysis are given under

Results. Wald tests were used for hypothesis tests of variation in hormone concentrations between day and night. For melatonin data, results from both sexes were pooled, while for T and E₂, results were analyzed separately for each sex. Because there is a rapid seasonal decline in testosterone in male Alaska Lapland Longspurs in the wild (from May 27 to June 2 males have on average 2–3 ng/ml, while from June 3 to 15 they have only 1–1.5 ng/ml; Soma *et al.*, 1999), we subtracted the mean for the population at that bleeding day in that year from all values before performing analyses (data taken from Soma *et al.*, 1999). This procedure removed potential bias due to the day of the season at which samples for a specific bleeding time were taken. Most data for hormone profiles were not normally distributed because many samples were undetectable and set at the lower detection limit for statistical analysis. Such data are presented in the figures as medians \pm 95% percentiles. All other data are presented as means \pm 95% confidence intervals (CI).

RESULTS

Melatonin Profiles

Alaska birds showed a detectable diel difference in plasma melatonin concentrations, even under the continuous daylight of their high-arctic breeding habitat (Figs. 1a and 2a; $\chi^2 = 5.5$, $P = 0.02$, $n = 14$; missing data for “dim” = 5 and for “bright” = 0, abbreviated below as d5/b0). Also, in eight of nine individuals for which we had both “bright” and “dim” data, melatonin concentrations during the “dim” phase were higher than those during the “bright” phase. However, overall melatonin concentrations were dramatically suppressed and lower than those of Seattle birds during the nighttime in any season. As expected, Seattle birds had a robust diel rhythm in plasma melatonin throughout the year (May 1996: $\chi^2 = 54.6$, $P < 0.0001$, $n = 21$, missing data for “night” = 2 and for “day” = 0, abbreviated below as n2/d0; August 1996: $\chi^2 = 38.6$, $P < 0.0001$, $n = 21$, n1/d1; October 1996: $\chi^2 = 15.7$, $P = 0.0001$, $n = 18$, n3/d1; January 1997: $\chi^2 = 12.3$, $P < 0.0005$, $n = 14$, n3/d8; Figs. 1b–1e and 2b–2e).

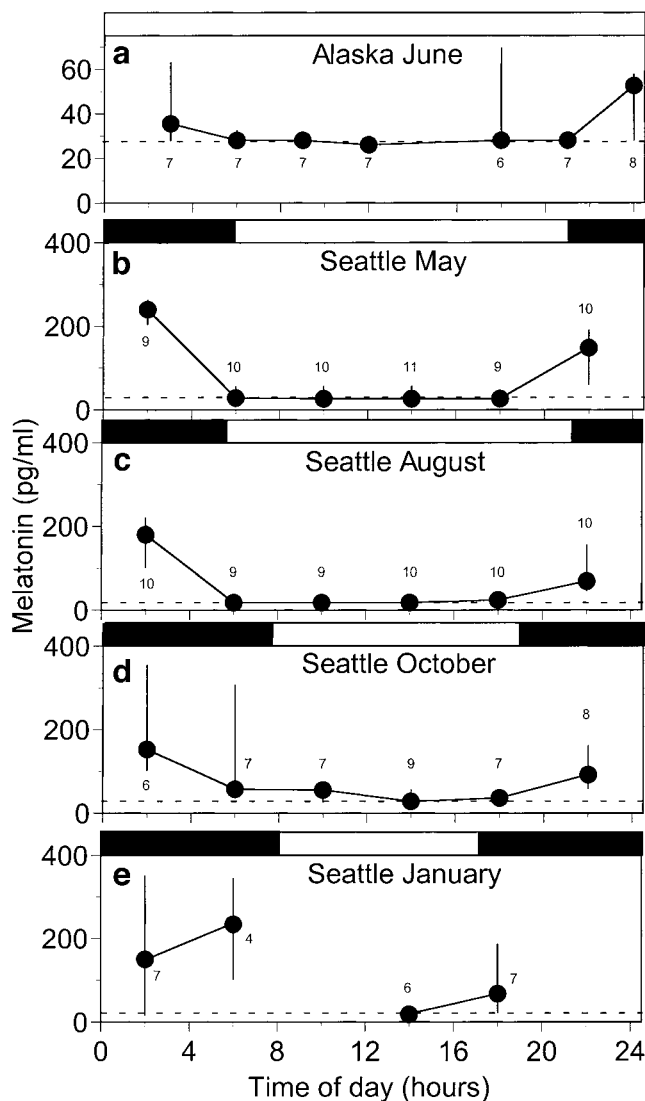


FIG. 1. Plasma melatonin concentrations of captive Lapland Longspurs (a) at their high-arctic breeding site in Alaska in June 1996 during the polar day and in Seattle in (b) May 1996, (c) August 1996, (d) October 1996, and (e) January 1997 during different photoperiods. No complete profile could be obtained for January. Data are median \pm 95% percentiles, as data were not normally distributed. Broken horizontal line indicates lower detection limit of the assay. Numbers above (or below in a) data points are sample sizes. Note that scale of y axis for Alaska data is different from that for Seattle data to better illustrate the small-scale changes in melatonin concentrations. Horizontal bars above each panel indicate photoperiod: black bars, night; open bars, daytime as obtained from sunrise/sunset charts. For statistical analysis of diel rhythms, data presented in Fig. 2 were used.

To estimate the duration of the nighttime melatonin peak we fitted quadratic or cubic regression models to the raw data, depending on the best fit. From this we

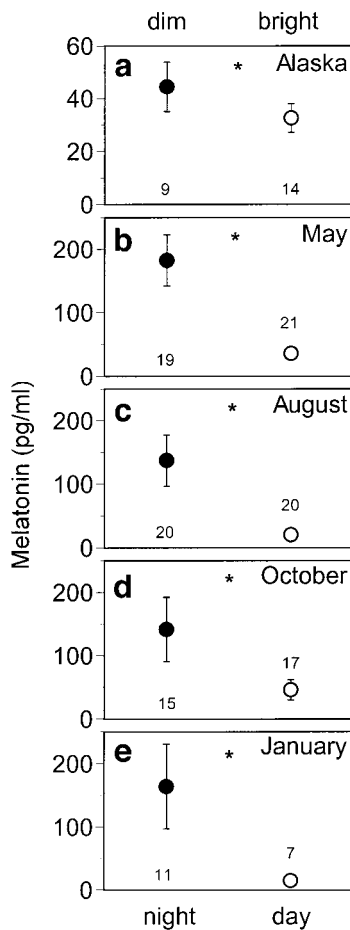


FIG. 2. Comparison of "dim" and "bright" or "day" and "night" concentrations (mean \pm 95% CI) of plasma melatonin concentrations in Lapland Longspurs from (a) Alaska during the polar day and from Seattle during (b) May, (c) August, (d) October, and (e) January. Numbers indicate sample sizes; each data point represents an average of all nighttime or daytime hormone concentrations of an individual bird. *Indicates a significant diel difference ($P < 0.05$).

determined the time at which melatonin was at half-maximal concentration in the morning and evening. This was then used to bracket the duration of melatonin secretion. We then correlated these data with the photoperiods in Seattle. The duration of the melatonin peak decreased almost linearly with photoperiod ($r^2 = 0.95$, $P = 0.005$; Fig. 3b). However, there was no effect of photoperiod on the height of the melatonin peak (regression of the highest mean melatonin value per season against photoperiod; $r^2 = 0.64$, $P > 0.1$).

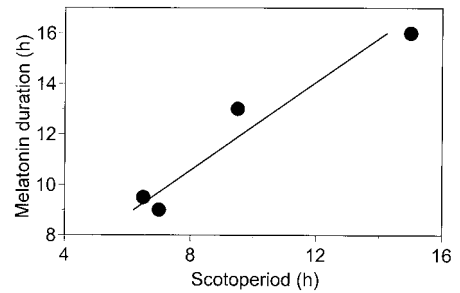


FIG. 3. Linear regression of the mean duration of melatonin secretion (when birds reached half-maximal concentrations of melatonin) against the scotoperiod at that time of year for Seattle birds ($r^2 = 0.95$, $P = 0.005$).

Testosterone Profiles

Male Toolik Lake Lapland Longspurs showed significant diel differences in plasma T concentration ($\chi^2 = 9.56$, $P < 0.002$, $n = 16$, $n1/d1$; Fig. 4a). Male Seattle birds did not show significant diel changes in plasma T at any time of year (May 1996: $\chi^2 = 0.1$, $P > 0.7$, $n = 9$, $n4/d0$; August 1996: $\chi^2 = 1.9$, $P > 0.15$, $n = 15$, $n0/d0$; October 1996: $\chi^2 = 0.2$, $P > 0.6$, $n =$

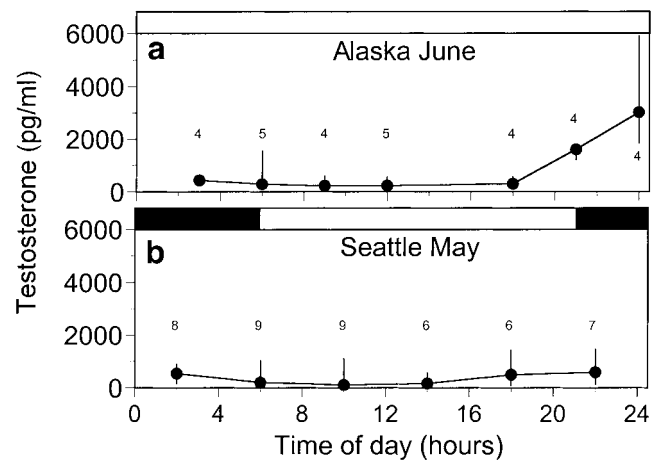


FIG. 4. Plasma testosterone concentrations of captive male Lapland Longspurs (a) at their high-arctic breeding site in Alaska in June during the polar day and (b) in Seattle in May. Data from other times of the year are not shown as concentrations were very low and did not vary significantly. Data are median \pm 95% percentiles, as data were not normally distributed. Numbers above data points are sample sizes. Horizontal bars above each panel indicate photoperiod: black bars, night; open bars, daytime as obtained from sunrise/sunset charts. For statistical analysis of diel rhythms, data presented in Fig. 5 were used.

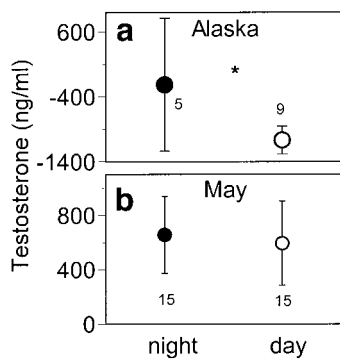


FIG. 5. Comparison of “dim” and “bright” or “day” and “night” concentrations (mean \pm 95% CI) of plasma testosterone concentrations in Lapland Longspurs from (a) Alaska during the polar day and from (b) Seattle during May. Because there is a rapid seasonal decline in T in Alaska birds, we subtracted the mean for the population at that date (Soma *et al.*, 1999) from the data presented here. Numbers indicate sample sizes; each data point represents an average of all nighttime or daytime hormone concentrations of an individual bird. *Indicates a significant diel difference ($P < 0.05$).

10, n2/d0; January 1997: $\chi^2 = 0.2$, $P > 0.6$, $n = 7$, n3/d2; Figs. 4 and 5; data for August to January not shown).

Plasma T concentrations in high-arctic female Lapland Longspurs were much lower than those of males and did not differ between day and night ($\chi^2 = 2.0$, $P > 1.5$, $n = 5$, n1/d0; Table 3). Female birds kept in Seattle showed a trend for a diel rhythm in plasma T in May, but not at other times of the year (May 1996: $\chi^2 = 3.7$, $P = 0.055$, $n = 5$, n1/d0; August 1996: $\chi^2 = 0.4$, $P > 0.5$, $n = 6$, n1/d1; October 1996: $\chi^2 = 1.4$, $P > 0.2$, $n = 8$, n0/d1; January 1997: $\chi^2 = 0.01$, $P > 0.9$, $n = 7$, n0/d6; Table 3).

Estradiol Profiles

For diel changes in estradiol we only statistically analyzed the data from Alaska birds in June and Seattle birds in May (Table 3). At all other times of year, E_2 concentrations were undetectable. Alaska males had no detectable E_2 rhythm ($\chi^2 = 0.12$, $P > 0.7$, $n = 9$, n4/d0), whereas females did show significant day/night differences ($\chi^2 = 13.41$, $P < 0.0005$, $n = 5$, n1/d0). E_2 concentrations of Seattle males in May did not vary significantly ($\chi^2 = 1.4$, $P > 0.2$, $n = 16$, n2/d0) but females again showed a significant rhythm ($\chi^2 = 8.14$, $P < 0.005$, $n = 5$, n1/d0).

DISCUSSION

Exposure to natural continuous daylight at their high-arctic breeding grounds strongly suppressed melatonin secretion in Lapland Longspurs (Fig. 1). However, even under polar day conditions, Alaska Lapland Longspurs showed significant diel changes in plasma melatonin concentrations (Fig. 2). Also, males showed diel differences in plasma T concentrations and females in plasma E_2 concentrations (Figs. 4 and 5, Table 3). Hence, while in the laboratory constant bright illumination can eliminate diel hormonal and behavioral rhythms, hormone rhythms can persist in birds during exposure to natural constant light conditions.

In the current study, there was some methodological variation in the treatment of Alaska and Seattle birds but we believe that those differences did not affect the main outcome of the study. For example, Alaska birds were sampled in June while Seattle birds were sampled in May. However, birds at both places were in breeding condition, as indicated by high steroid concentrations (Table 3) and song activity (L. M. Romero and M. Hau, pers. obs.), and thus were sampled in a similar seasonal phase. Housing and environmental conditions differed to some extent but the main difference was in photoperiod. Ambient temperatures may have been lower in Alaska, but have generally only very slight effects on hormone concentra-

TABLE 3

Plasma Testosterone and Estradiol Concentrations of Male and Female Lapland Longspurs [(pg/ml); mean \pm 95% CI (n)]

	Night	Day
Testosterone (Females)		
Alaska June	250.9 \pm 261.4 (4)	112.2 \pm 21.2 (5)
Seattle May	842.6 \pm 920.8 (4)	153.8 \pm 225.2 (5)
Seattle August	29.1 \pm 20.8 (5)	24.3 \pm 11.8 (5)
Seattle October	21.7 \pm 16.4 (8)	57.8 \pm 64.8 (7)
Seattle January	10.03 \pm 1.8 (7)	10.2 (1)
Estradiol		
Males		
Alaska June	7.3 \pm 1.8 (5)	7.8 \pm 3.0 (9)
Seattle May	12.6 \pm 6.8 (4)	8.8 \pm 1.0 (5)
Females		
Alaska June	20.4 \pm 10.6 (4)	6.2 \pm 0.8 (5)*
Seattle May	22.3 \pm 12.2 (4)	7.6 \pm 1.2 (5)

* $P < 0.05$.

tions in Lapland Longspurs (Romero *et al.*, 2000). Alaska birds were kept singly or in pairs in cages while Seattle birds were kept in groups in large aviaries. These social conditions may have influenced T and E₂ concentrations (Wingfield *et al.*, 1990) but at both locations birds could see, hear, and interact with other conspecifics. Seattle birds had been in captivity much longer than Alaska birds but it is unlikely that the stress of captivity affected diel patterns of hormone secretion. Instead, differences in absolute concentrations, which were not critical to this study, are to be expected. Finally, sample sizes for the steroid hormones were limited and may have obscured the detection of rhythmicity, especially in Seattle birds, but we were still able to detect a trend for T and E₂ rhythms in Alaska birds.

Persistence of Diel Melatonin Rhythms under Polar Day Conditions

The dramatic reduction in the amplitude of the melatonin rhythm during the polar day in these passerine birds is consistent with previous results on nonpasserine birds and mammals. However, contrary to our results, polar day conditions completely abolished melatonin rhythms in penguins (*Pygoscelis* spp, Benelbaz *et al.*, 1976; Cockrem, 1991; *Aptenodytes forsteri*, Miché *et al.*, 1991), ptarmigans (*Lagopus mutus hyperboreus*, Reierth *et al.*, 1999), reindeer (*Rangifer tarandus tarandus*, Stokkan *et al.*, 1994; Eloranta *et al.*, 1995), and Weddell seals (*Leptonychotes weddellii*, Barrell and Montgomery, 1989; but see Griffith *et al.*, 1986). In the laboratory, plasma melatonin rhythms also disappear after exposure to constant bright light in different bird species, either immediately (Meyer and Millam, 1991) or within a few days or weeks (Ralph *et al.*, 1975; Binkley, 1979; Yamada *et al.*, 1988).

How could a diel melatonin rhythm persist in Arctic Lapland Longspurs while in other passerine birds continuous light of intensities higher than about 10 lux eliminates circadian rhythms (e.g., Wever, 1980)? Three nonexclusive mechanisms are conceivable. First, elevated plasma melatonin concentrations during the night may have been caused by lower ambient light concentrations (Table 1), permitting increased melatonin production. In this scenario, the resulting melatonin rhythm may have been due to a masking effect rather than a circadian rhythm (Aschoff, 1960). Sec-

ond, even though light intensities during both the "dim" and the "bright" phases of the polar day were high enough to cause arrhythmicity alone, rhythmic alternation between higher and lower light intensities may have acted as a zeitgeber, entraining the circadian system of the birds (Wever, 1980). Third, other zeitgebers such as changes in the azimuth of the sun, light intensity, and spectral composition of the light (Demmelmeier and Haarhaus, 1972; Krüll 1976a,b,c; Pohl, 1999), nonphotic zeitgebers such as food (e.g., insect availability; Hau and Gwinner, 1992; Reierth and Stokkan, 1998), or social cues (Gwinner, 1966) may also have kept the circadian system entrained. In the present study, circadian rhythms of Arctic Lapland Longspurs could also have been free-running instead of being synchronized to 24 h. However, as data collection occurred over a period of 10 days, more variability in the data would be expected if rhythms were not synchronized (see Fig. 1).

It remains to be investigated whether the low-amplitude variations in plasma melatonin concentrations in Arctic Lapland Longspurs are functionally significant. Other birds (barn owls, *Tyto alba*, Van't Hof *et al.*, 1998; Nazca boobies, *Sula granti*, E. Tarlow *et al.*, in preparation) and humans (Waldhauser and Dietzel, 1985) can have comparatively low or undetectable concentrations of plasma melatonin under natural conditions. The finding of diel rhythms in the two steroid hormones; however, suggests that at least some parts of the Lapland Longspur circadian system were functional under polar day conditions. Interestingly, recent studies have suggested that a reduction or abolishment of the melatonin amplitude leads to a weakening of the avian circadian system, which in turn facilitates synchronization with weak photic and nonphotic zeitgebers such as the ones mentioned above (Hau and Gwinner, 1994; Pohl, 1996; Abraham *et al.*, 2000). Thus, light suppression of the melatonin rhythm during polar day could have a function in enabling synchronization with "alternative" zeitgebers (Gwinner *et al.*, 1994, 1997). Regardless of the potential mechanism, our data indicate that 24-h rhythms can persist under polar day conditions. Future experiments need to investigate whether the low-amplitude melatonin rhythm is causally related to T and E₂ rhythms.

Seasonal Changes in Melatonin Secretion

The duration of melatonin secretion in Seattle birds changed linearly with the duration of the scotoperiod (or period of darkness; Fig. 3). Similar data have been obtained for a wide variety of species and exemplify how the night length is internally represented by melatonin secretion (Reiter, 1991), at least within a certain range of scotoperiods. In the present study, we could not detect differences in maximal melatonin concentrations of Seattle birds between different seasons and scotoperiods. Likewise, in Atlantic salmon (*Salmo salar*) and horses a change in the amplitude of plasma melatonin secretion with night length did not occur (Guerin *et al.*, 1995; Randall *et al.*, 1995). This differs from findings on other vertebrates (humans: Stokkan and Reiter, 1994; mammals: Bubenik and Smith, 1987; Stokkan *et al.*, 1994; Eloranta *et al.*, 1995; birds: Binkley, 1988, Miché *et al.*, 1991; Reierth *et al.*, 1999; Brandstätter *et al.*, 2001; reptiles: Vivien-Roels *et al.*, 1988; fish: Iigo and Aida, 1995; frogs: D'Istria *et al.*, 1994), but in some of these studies photoperiodic changes were more extreme. In the present study, Seattle birds may have been secreting melatonin at a maximal rate in each season. Although, unlike in mammals, seasonal changes in the duration of melatonin secretion are not essential for the avian annual system (e.g., Follett *et al.*, 1985), new evidence suggests that melatonin nevertheless may be involved in regulating some seasonal phenomena in birds (Bentley *et al.*, 1999).

Diel and Seasonal Changes in Steroid Hormones

Like melatonin, plasma concentrations of T in male, and E₂ in female, Arctic Lapland Longspurs were higher during the "dim" than during the "bright" phases of the polar day. Interestingly, in male chickens exposed to constant light, in some individuals diel cycles of T and dihydrotestosterone were still apparent after 4 weeks (Bachman *et al.*, 1987). In other bird species, T is also elevated around nighttime and the early morning (Schanbacher *et al.*, 1974; Balthazart, 1976; Aschoff, 1979; Balthazart *et al.*, 1981; Bachman *et al.*, 1987). Likewise, in human males, T peaks in the early morning hours (e.g., Rose *et al.*, 1972; Aschoff, 1979; Schulz *et al.*, 1995). An opposite pattern can be found in nocturnal mammals (e.g., Aschoff, 1979; Lerchl and Nieschlag, 1995). A diel difference in

plasma T concentrations was apparent in Arctic male Lapland Longspurs in June but not in Seattle males in May (Figs. 4 and 5). The lack of a T rhythm in Seattle birds could be due to two nonexclusive reasons: First, Seattle males perhaps had not yet achieved full breeding condition as samples were taken almost 1 month earlier than in Alaska birds. Second, plasma steroid concentrations may have been suppressed in Seattle males due to long-term captivity (Wingfield and Farner, 1993) and thus did not reach peak concentrations like Alaska birds (Fig. 4).

Possible Function of Hormone Rhythms in the High Arctic

The persistence of hormone rhythms in birds living under polar day conditions may provide a physiological basis to the reports of continuing behavioral rhythmicity in some high-arctic animals during the polar day. Diel differences in song, locomotor activity, or feeding of young during the polar day have been observed for a number of birds (Palmgren, 1935; Franz, 1949; Hoffmann, 1959; Haarhaus, 1968), including Lapland Longspurs (Palmgren, 1935). Most of these species cease to feed their young for a couple of hours each "night" and show an increase in song activity very early in the morning. This pattern fits well with the diel changes in steroid hormones reported here, which are involved in regulating many reproductive behaviors such as song and sexual displays (Balthazart, 1976; Wingfield *et al.*, 1990).

CONCLUSIONS

Data on diel hormone levels of animals living under natural lighting conditions are generally rare. The current results suggest that laboratory conditions may be devoid of cues that are important for free-living animals. Hence, studies of animals in their natural environment are needed to understand more fully the properties and behavior of the circadian system and to evaluate the adaptive significance of circadian functions.

ACKNOWLEDGMENTS

We thank John Wingfield and Kiran Soma for help in bleeding Alaska birds and Martin Wikelski for help with Seattle birds. Kathleen Hunt generously provided Seattle birds and advice. We thank Ingrid Schwabl for her expert help with the hormone assays and Martin Wikelski, Eberhard Gwinner, and two anonymous reviewers for their valuable comments on previous versions of the manuscript. G. Shaver kindly provided the Toolik Lake weather data. This study was supported by NSF OPP9530826 (to John C. Wingfield, University of Washington) and NSF BIR-9406842 (to L.M.R.).

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