RESEARCH ARTICLE

The Effects of Low Levels of Light at Night Upon the Endocrine Physiology of Western Scrub-Jays (*Aphelocoma californica*)

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ABSTRACT

Florida scrub-jays (*Aphelocoma coerulescens*) in the suburbs breed earlier than jays in native habitat. Amongst the possible factors that influence this advance (e.g., food availability, microclimate, predator regime, etc.), is exposure to artificial lights at night (LAN). LAN could stimulate the reproductive axis of the suburban jays. Alternatively, LAN could inhibit pineal melatonin (MEL), thus removing its inhibitory influence on the reproductive axis. Because Florida scrub-jays are a threatened species, we used western scrub-jays (*Aphelocoma californica*) to investigate the effects of LAN upon reproductive hormones and melatonin. Jays were held under conditions in which the dark-phase of the light:dark cycle was without illumination and then under low levels of LAN. Under both conditions, birds were exposed first to short-days (9.5L:14.5D) that were gradually increased to long-days (14.5L:9.5D). At various times, blood samples were collected during the light part of the cycle to measure reproductive hormones (luteinizing hormone, LH; testosterone, T; and estradiol, E_2). Similarly, samples to assess melatonin were collected during the dark. In males, LAN caused a depression in LH levels and levels were $\sim 4\times$ greater under long- than short-days. In females, there was no effect of LAN or photoperiod upon LH. LAN resulted in depressed T levels in females, although there was no effect on T in males. E_2 levels in both sexes were lower under LAN than under an unlighted dark-phase. Paradoxically, MEL was higher in jays under LAN, and under long-days. MEL did not differ by sex. LAN disrupted the extraordinarily strong correlation between T and E_2 that existed under unlighted nocturnal conditions. Overall, our findings fail to support the hypothesis that LAN stimulates the reproductive axis. Rather, the data demonstrate that LAN tends to inhibit reproductive hormone secretion, although not in a consistent fashion between the sexes. *J. Exp. Zool. 319A:527–538, 2013.* © 2013 Wiley Periodicals, Inc.

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Animals use a great range of environmental information in timing reproduction to maximize productivity, including numerous biotic and abiotic cues. Perhaps the most reliable cue used by animals to predict the onset of favorable conditions is photoperiod. Although photoperiod is certainly predictive of future conditions, it is a rather crude indicator. Whereas the initial predictive nature of photic cues provide key information about conditions over the long-term, supplementary cues, such as temperature, rainfall, or resource availability, that more closely reflect current local conditions are predictive over the short-term and allow animals to time reproduction appropriately (see Wingfield, '80, '83; Wingfield and Farner, '93).

For more than 20 years we have monitored a population of Florida scrub-jays (*Aphelocoma coerulescens*) that resides in a suburban tract with remnant patches of scrub habitat and a nearby population of jays in a natural, or wildland, habitat (Schoech and Bowman, 2001, 2003; Schoech et al., 2004). The suburban birds lay eggs 2–4 weeks earlier and show little annual variation in the onset of breeding relative to the wildland birds. In addition, the suburban birds regularly begin laying eggs in late February, which has been noted only 2–3 times at the wildland site in 43 years. Although considerable study has addressed this issue, the underlying causes remain somewhat obscure. While observational and experimental work point toward the ready access of human-source foods in the suburban environment as the primary driver of the earlier reproduction, numerous other factors might also contribute (Schoech and Bowman, 2001, 2003). Potential factors experienced in a suburban versus a natural habitat that could be stimulatory include increased: (1) temperature due to an "urban heat island" effect (e.g., Fan and Sailor, 2005; for site-specific data see Aldredge et al., 2012); (2) predictability of food resources (i.e., knowing where food can be obtained at any time on a year-round basis, such as a pet food dish, see Bridge et al., 2009); and (3) exposure to artificial lighting at night from street lamps and houses.

It is possible that exposure to light at night (LAN) could be stimulatory to the reproductive axis via the same pathways by which increases in photoperiod act. However, in birds the impacts of exposure to LAN have been little studied at any level, especially with regard to how or whether such a stimulus might affect the reproductive axis (but see Partecke et al., 2004; Pandey and Bhardwaj, 2011; Singh et al., 2012; Dominoni et al., 2013). Increasing day length, which by definition results in a decrease in the dark-phase of the daily cycle, is the most robust driver of reproductive axis activation in temperate zone species. The duration of the melatonin (MEL) signal, secreted from the pineal, varies inversely with day length, and in mammals can play a key role in seasonal physiologic changes (see review in Paul et al., 2008). Similarly, MEL is thought to regulate the pulsatile release of gonadotropin-releasing hormone (GnRH), although this is believed to be an indirect effect mediated with the involvement of serotoninergic and dopaminergic neurons (review in Malpaux et al., '99). It is debated whether MEL plays a role in seasonal reproduction in birds (reviews in Cassone et al., 2009; Yoshimura, 2010). However, El Halawani et al. (2009) present evidence that photic cues during the sensitive period preceding the typical breeding period upregulate dopamine and MEL neurons of the hypothalamus in domestic turkeys (*Meleagris gallopavo*) and suggest that this reflects their participation in the "generation and expression of seasonal reproductive rhythms." Additionally, in some bird species, MEL mediates the seasonal upregulation of some reproduction-related physiological changes (e.g., song control regions in European starlings, *Sturnus vulgaris* [Bentley et al., '99] and house sparrows, *Passer domesticus* [Cassone et al., 2008]). Further, MEL receptors have been localized in the testes of several species of birds where MEL binding can have anti-gonadal effects (i.e., inhibit steroid hormone production; Ayre and Pang, '94; Murayama et al., '97). Lastly, Grieve et al. (2011) treated female great tits (*Parus major*) with MEL and found implanted birds delayed clutch initiation when compared to controls.

Recently, the characterization of gonadotropin-inhibitory hormone (GnIH, Tsutsui et al., 2000) and strong evidence that this decapeptide plays a critical role in seasonal down-regulation of the hypothalamic–pituitary–gonadal (HPG) axis in birds (review in Bentley et al., 2009; Tsutsui, 2010) offer new avenues of exploration. For example, McGuire et al. (2011) show that in addition to central GnIH production, GnIH synthesis and receptors occur in the testes of European starlings. Further, they note that in vitro, the inhibition of testosterone secretion may be regulated seasonally through the combined actions of MEL and the gonadal GnIH system. Additionally, two recent reviews make strong cases for links between MEL and GnIH in birds and point toward MEL being a key mechanism in the synthesis and release of GnIH (Tsutsui et al., 2010; Chowdhury et al., 2013). A mammalian homologue of GnIH, RFamide-related peptide (RFRP-3), that exhibits similar effects has been identified as well (Kriegsfeld et al., 2006; Johnson et al., 2007; Murakami et al., 2008).

The links among light exposure, MEL, and the reproductive axis, as well as our observation of early breeding in a population of Florida scrub-jays that are exposed to LAN, led to two hypotheses

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that are not necessarily mutually exclusive. Further, recent studies on "Indian weaver birds" (baya weaver, *Ploceus philippinus*) and European blackbirds (*Turdus merula*) found that LAN lowers circulating MEL levels (Singh et al., 2012; Dominoni et al., In press). We reasoned that exposure to LAN facilitates advancement in the timing of reproduction by: (1) decreasing levels of MEL which, in turn, reduces gonadal inhibition and (2) stimulating the HPG axis leading to increased production of reproductive hormones. Using western scrub-jays (*Aphelocoma californica*) as a proxy for the federally threatened Florida scrub-jay we carried out a captive study to test whether exposure to LAN would result in hormone secretion consistent with our hypotheses.

METHODS

Animals and Animal Husbandry

Western scrub-jays were captured between September 17 and December 2, 2004 in and around Davis, CA (38°32′42″N, 121°44′21″W: 16 m asl). Birds were subsequently transported to the University of Memphis, and housed in a single room within the Department of Biology Animal Care Facility in the Life Sciences building. All were housed individually in cages (46 cm × 61 cm × 46 cm) and provided ad libitum water and food (see below). All birds were sexed by DNA (see Ellegren, '96) or by discriminate function analysis using a suite of morphometric data (Schoech, unpublished data). During the experimental periods (see below), jays were fed Roudybush™ maintenance diet (Roudybush, Inc., Woodland, CA, USA). This diet was supplemented with dried dog food or high-protein bird food (Mazuris parrot breeder diet, PMI Nutrition International, St. Louis, MO, USA) during non-experimental periods in which the birds underwent an "annual" molt (note that with acceleration of the light regimen, a "year" took approximately 8.5 months). Temperature was maintained at approximately 20°C throughout. Birds were occasionally handled for cage cleaning and were blood-sampled for other experiments that were separate from this study. The study began with 20 male and 26 female jays; by the conclusion the respective sample sizes were 15 and 12. Based upon annual mortality rates of Florida scrub-jays (~23%), the losses in our study are within the expected range. All methodologies were approved and monitored by the University of Memphis Institutional Animal Care and Use Committee.

Experimental Schedule, Photoperiod Manipulation, and Blood Sampling

Birds were initially held on short-days (9.5 hr light and 14.5 hr dark, 9.5L:14.5D), a period roughly equivalent to winter day length at their capture site. The first stage of the experiment in which birds were exposed to no nocturnal light during the dark-phase of their daily cycle began on Jan 21, 2005. The second stage, in which birds were exposed to low levels of nocturnal lights, was initiated on Dec 19, 2006. In the intervening period, during which other experiments were undertaken (e.g., Bridge et al., 2009), the birds were processed through a gradual photoperiod transition from short- (9.5L:14.5D) to long-days (14.5L:9.5D), with a minimum of 3 months on long-days, followed by a gradual transition from long- to short-days, and a minimum of 3 months on short-days. During these transitions, photoperiod was increased or deceased by 10 min per day. Hence, in the intervening period between stages, the birds had experienced two simulated "years," during which feather molt during the transition from long- to short-days verified appropriate responses to the changing photic cues.

During both experimental stages, blood samples for sex steroid (T and E_2) and luteinizing hormone analyses were collected between 3 and 5 hr after the lights had been turned on (control of the lights was by a programmed automated system). To minimize the time from initial entry into the room until sample collection, a team of two to four people collected blood samples as rapidly as possible. The elapsed time from first entry into the room, as well as the time of sample collection relative to first light were noted and were statistically controlled in all analyses. Blood samples for MEL measurement were collected beginning 3 hr after lights out. During nighttime blood collection, a similar procedure was used (i.e., multiple individuals collecting blood samples, noting both elapsed time and time since lights out); however, low level red lights were used to facilitate removal of birds from their cages and collection of blood samples. Because of concerns about the frequency of sample collection and the health of the birds, sometimes MEL samples were collected several days after daytime sampling (see Table 1). In all instances, birds were removed from the housing room to an adjacent room where blood samples were collected in heparinized microhematocrit tubes following venipuncture with a 25 g needle. All samples were centrifuged and the plasma fraction collected and frozen within 1–2 hr of sample collection. The sample collection schedule is in Table 1. Following blood sample collection, birds were weighed with a spring balance.

Nocturnal Light Levels

Because the nocturnal illumination during stage two was intended to approximate that experienced by Florida scrub-jays in the suburban population, S.J.S. and R.B. took numerous readings of light levels throughout the suburban study site on the night of Mar 9, 2000. Beginning at 900 PM we visited two sites at each of 53 territories. We qualitatively assessed each territory and took a series of readings from the perceived brightest and dimmest sites in each territory. We used a LI-188B, Li-Cor, Inc. (Lincoln, NE, USA) photometer for all readings. At each site we took five readings, utilizing a 10 sec integration time for each, one in each of the four cardinal directions and one straight up. We used the highest value (3.2 lux) for the experimental nocturnal illumination.

We illuminated the experimental room with 50 W incandescent bulbs strung evenly throughout the room and controlled by an

Table 1. Blood sample collection schedule for sex steroid and luteinizing hormone (daytime samples) and melatonin (nighttime samples) analyses.

	Daytime samples				Nighttime samples			
	Date	Year	L:D	*n* (♂, ♀)	Date	Year	L:D	*n* (♂, ♀)
Stage 1	21	2005	9.5:14.5	11, 9	31	2005	10.33:13.66	10, 9
	22	2005	9.5:14.5	10, 10	96	2005	14.5:9.5	5, 12
	49	2005	12:12	9, 11				
	50	2005	12:12	8, 12				
	84	2005	14.5:9.5	8, 9				
	85	2005	14.5:9.5	9, 13				
Stage 2	353	2006	9:15	7, 7	353	2006	9:15	8, 5
	361	2006	9:15	8, 5	361	2006	9:15	7, 7
	8	2007	11.33:12.66	15, 12	2	2007	10.33:13.66	15, 12
	18	2007	13:11	14, 13	33	2007	14.5:9.5	15, 13
	28	2007	14.5:9.5	14, 13				

Stage 1 was the period with no nocturnal illumination, whereas stage 2 represents the period during which jays were exposed to low levels of light during the nocturnal phase.
Date is day-of-year and L:D is the hours of light and dark.

adjustable dimmer switch. Light intensity was adjusted in a trial and error fashion until readings at multiple sites in the room were at 3.2 ± 0.2 lux. Although we did not take a reading of light levels during the "daylight" stage in the bird room, the full spectrum florescent lighting is estimated to be equivalent to that of "office lighting" (i.e., 320–500 lux, http://en.wikipedia.org/wiki/Lux). Levels during the dark stage of the unlighted stage of the experiment were 0.01 lux.

Hormone Analyses

Testosterone and estradiol were measured in four radioimmunoassays following separation with column chromatography. This assay, adapted from the protocol followed by Wingfield, has been in use in the Schoech laboratory for 12 years (for details see Wingfield and Farner, '75; Ball and Wingfield, '87; Wingfield et al., '91; Schoech et al., '96, 2004). Intra-assay variation for T ranged from 5.43% to 8.56% and from 4.76% to 7.52% for E_2, whereas inter-assay CV was 10.63% and 14.10%, respectively.

Plasma levels of luteinizing hormone (LH) were determined with a post-precipitation, double antibody radioimmunoassay (Follett et al., '72; Sharp et al., '87). This assay uses purified chicken LH as a standard and rabbit-reared antisera against LH that were kindly provided by Dr. Peter Sharp (Agricultural Research Council, Roslyn, Scotland). All samples were assayed in duplicate using 20 μL of plasma. Inter- and intra-assay variation were within acceptable limits, less than 15% and 10%, respectively.

Plasma levels of melatonin were determined by two radioimmunoassays following the procedures described in Goymann et al. (2008). All samples were assayed in duplicate, standard curve and sample concentrations were calculated with Immunofit 3.0 (Beckman, Inc., Fullerton, CA, USA), using a four parameter logistic curve fit. The lower detection limit of the assay was determined as the first value outside the 95% confidence intervals for the zero standard (Bmax) and ranged between 1.4 and 1.5 pg/tube. The intra-assay coefficients of variation were 6.1% and 8.6% and the inter-assay coefficient of variation was 4.6%. Melatonin concentrations were adjusted for individual recoveries (mean $\pm$ SD $= 88.0 \pm 3\%$).

Statistical Analyses

We tested for the effects of LAN using linear mixed models. Because it was not possible to sample all birds on the same days due to logistical reasons and consideration of the overall health of the birds, our sampling schedule is somewhat imbalanced (see Table 1). Therefore, daytime samples over the course of the study (i.e., those collected for measurement of T, E_2, and LH), were assigned to one of three groups: short days—9 and 9.5 hr of light; intermediate days—11.33, 12, and 13 hr; and long days—14.5 hr (see Figs. 1 and 2). However, for the nocturnal samples and for examination of the effects of the light treatments on MEL, we use the actual hours of daylight to which birds were exposed as a continuous variable, that is, 9, 10.33, and 14.5 hr. We then used photoperiod (either the grouped variable as explained above or the actual daylight hours), along with sex and "night light" (whether or not birds were exposed to LAN) as fixed factors in the models to explore their effects on the dependent variables LH, MEL, T, and E_2. In all cases, to control for potential disturbance effects of researcher activities during sample collection, elapsed time (from time zero—opening of the bird room door) was entered as a cofactor and repeated sampling of individuals was controlled by

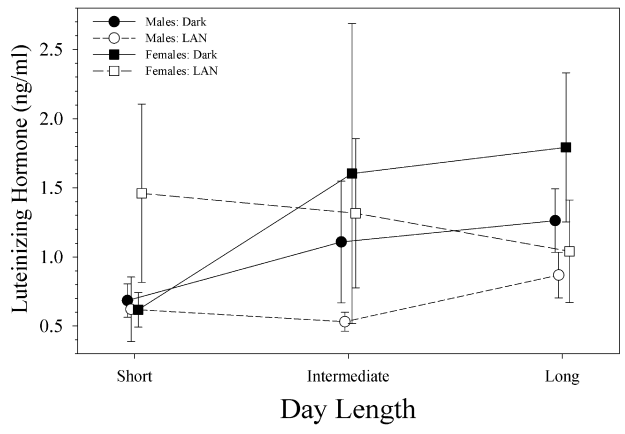

Figure 1. Plasma levels of luteinizing hormone of males (circles) and females (squares) under both dark nights (filled symbols) and while exposed to LAN (open symbols). Day lengths are as follows: short, 9 and 9.5 hr; intermediate, 11.33, 12, and 13 hr; and long, 14.5 hr. Data are presented as means ± SE and are slightly offset to minimize overlap of symbols and error bars.

including individual identity as a random variable in each model. Interaction terms among the three fixed factors were also included in all models. Stepwise removal of nonsignificant terms followed by rerunning models was employed in some instances to further explore observed trends. Post-hoc significance tests used Bonferroni corrections for multiple comparisons.

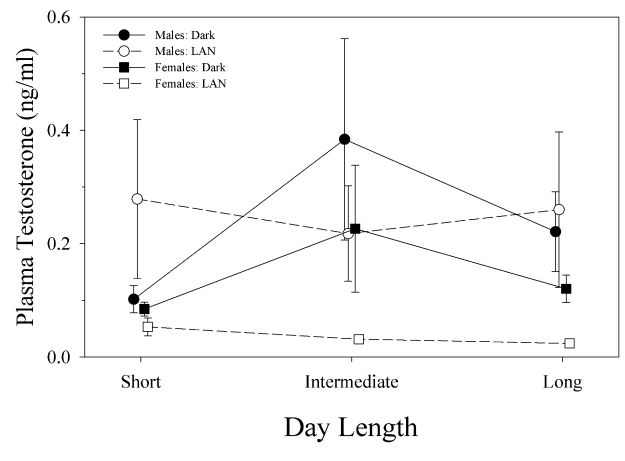

Figure 2. Plasma levels of testosterone of males (circles) and females (squares) under both dark nights (filled symbols) and while exposed to LAN (open symbols). Day lengths are as follows: short, 9 and 9.5 hr; intermediate, 11.33, 12, and 13 hr; and long, 14.5 hr. Data are presented as means ± SE and are slightly offset to minimize overlap of symbols and error bars.

Because of possible relationships between MEL levels and those of the reproductive hormones examined, we used Pearson's correlations among the four hormones. However, given that sampling dates for MEL and the other hormones were often not precisely synchronized, the MEL data were compared with the sex hormone data from the closest dates (i.e., MEL date 31 with 22, MEL date 96 with 85, MEL date 2 with 8, and MEL date 33 with 28; see Table 1). We first examined correlations among the hormones with all samples included irrespective of sex or treatment and subsequently split the data, first by sex and then by night light and examined the suite of correlations (see Table 2).

All analyses were conducted with PASW ver. 18. Data presented below are estimated marginal means ± SE of the mean. Data presented graphically are of means ± SE of the mean.

RESULTS

There was no evidence of an effect of LAN ($F = 0.72$, $P = 0.40$), photoperiod ($F = 0.95$, $P = 0.39$), or elapsed time ($F = 0.11$, $P = 0.74$) on LH Levels, and none of the interactions were significant ($P \geq 0.26$ in all cases). However, LH levels between the sexes nominally differed ($F = 3.75$, $P = 0.054$; Fig. 1). Rerunning the model with the nonsignificant factors removed in a stepwise fashion revealed that females exhibited higher overall LH levels than males ($F = 5.19$, $P = 0.024$; 1.32 ± 0.17 and 0.77 ± 0.17 ng/mL, respectively). The sex difference led us to reanalyze the LH data separately by sex. In males, exposure to LAN significantly depressed overall LH levels ($F = 6.09$, $P = 0.016$; 1.06 ± 0.13 and 0.64 ± 0.094 ng/mL). Similarly, LH levels differed among photoperiods ($F = 4.01$, $P = 0.022$) with levels during long days (1.13 ± 0.13 ng/mL) significantly higher than those from short days (0.62 ± 0.12 ng/mL; $P = 0.018$), though there were no differences for the other pair-wise comparisons ($P \geq 0.41$). We found no effect of elapsed time ($F = 2.05$, $P = 0.16$) and the interaction between photoperiod and LAN was nonsignificant ($F = 0.80$, $P = 0.45$). In females, LH levels did not change in association with LAN ($F = 0.013$, $P = 0.91$), photoperiod ($F = 0.25$, $P = 0.78$), or elapsed time ($F < 0.0001$, $P = 0.99$), and the interaction term was also nonsignificant ($F = 0.91$, $P = 0.41$).

LAN did not influence levels of T in males ($F = 0.16$, $P = 0.69$; Fig. 2) nor did T levels differ among photoperiods ($F = 0.87$, $P = 0.42$), although T levels tended to decrease with elapsed time ($F = 3.46$, $P = 0.066$: a plot of T on elapsed time [not shown] revealed the direction of the relationship). The interaction term was not statistically significant ($F = 0.89$, $P = 0.41$). In females, overall T levels were significantly depressed under LAN ($F = 4.32$, $P = 0.040$; 0.038 ± 0.037 and 0.14 ± 0.032 ng/mL; Fig. 2), though there was no effect of photoperiod ($F = 0.77$, $P = 0.47$) or elapsed time ($F = 0.034$, $P = 0.86$). The interaction term was also nonsignificant ($F = 1.087$, $P = 0.34$).

In females, overall levels of E_2 were over fourfold lower in birds exposed to LAN (0.12 ± 0.03 and 0.027 ± 0.04 ng/mL; Fig. 3), but

Table 2. Correlation matrices of the relationships among the four hormones assessed collapsed across photoperiods.

	LH			T			E_2		
	r	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*
All									
MEL	0.051	0.59	112	0.062	0.51	116	−0.16	0.095	116
LH	–	–	–	0.06	0.42	183	0.082	0.27	183
T	–	–	–	–	–	–	0.59	**<0.0001**	227
Males									
MEL	0.18	0.17	57	0.12	0.38	58	−0.22	0.09	58
LH	–	–	–	0.29	**0.005**	93	0.005	0.96	93
T	–	–	–	–	–	–	0.51	**<0.0001**	112
Females									
MEL	0.029	0.84	55	−0.20	0.14	58	−0.12	0.37	58
LH	–	–	–	0.11	0.29	90	0.087	0.42	90
T	–	–	–	–	–	–	0.94	**<0.0001**	115
Dark									
MEL	0.17	0.36	31	−0.04	0.82	35	−0.11	0.52	35
LH	–	–	–	0.21	0.066	75	0.10	0.38	75
T	–	–	–	–	–	–	0.80	**<0.0001**	119
LAN									
MEL	0.084	0.46	81	0.078	0.49	81	−0.06	0.57	81
LH	–	–	–	0.022	0.82	108	0.037	0.70	108
T	–	–	–	–	–	–	−0.010	0.92	108
♂ Dark									
MEL	0.42	0.12	15	−0.14	0.60	16	−0.08	0.78	16
LH	–	–	–	0.44	**0.007**	37	−0.28	0.10	37
T	–	–	–	–	–	–	0.79	**<0.0001**	56
♂ LAN									
MEL	0.23	0.15	42	0.11	0.48	42	−0.08	0.62	42
LH	–	–	–	0.34	**0.010**	56	0.26	**0.050**	56
T	–	–	–	–	–	–	0.12	0.39	56
♀ Dark									
MEL	0.10	0.70	16	0.14	0.58	19	−0.15	0.53	19
LH	–	–	–	0.27	0.11	38	0.27	0.11	38
T	–	–	–	–	–	–	0.96	**<0.0001**	63
♀ LAN									
MEL	0.059	0.72	39	−0.34	**0.035**	39	−0.06	0.70	39
LH	–	–	–	−0.07	0.62	52	−0.003	0.99	52
T	–	–	–	–	–	–	0.14	0.33	52

Matrices represent, from top to bottom: (1) All—all individuals irrespective of sex or light treatment; (2) Males—all males collapsed across light treatments; (3) Females—all females collapsed across light treatments; (4) Dark—all individuals (males and females combined) exposed to a non-illuminated dark phase, (5) LAN—all individuals (males and females combined) exposed to low levels of light during the dark phase of the daily photic cycle, (6) ♂♂ Dark—males exposed to a non-illuminated dark phase, (7) ♂♂ LAN—males exposed to low levels of nocturnal light, (8) ♀♀ Dark—females exposed to a non-illuminated dark phase, and (9) ♀♀ LAN—females exposed to low levels of nocturnal light. Bold-face font highlights significant *P*-values.

this difference only approached statistical significance ($F = 3.52$, $P = 0.063$). E_2 levels did not differ across photoperiods ($F = 2.12$, $P = 0.13$) or with elapsed time ($F = 0.005$, $P = 0.94$), nor was the photoperiod × LAN interaction significant ($F = 1.32$, $P = 0.27$). Our observation of lower E_2 levels in association with LAN prompted us to rerun the statistical model without elapsed time, which revealed a marginally significant effect of LAN ($F = 3.81$, $P = 0.054$). As before, there was no effect of photoperiod ($F = 2.26$,

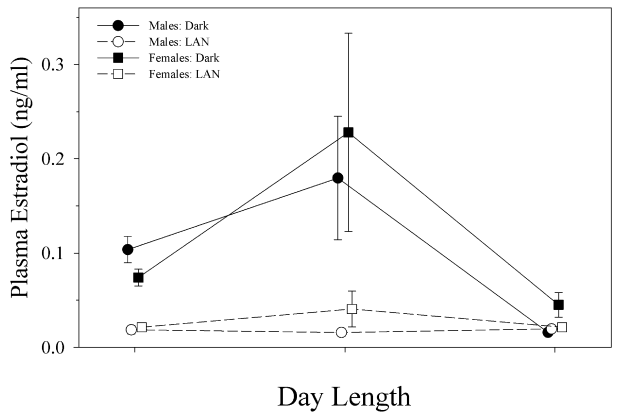

Figure 3. Plasma levels of estradiol of males (circles) and females (squares) under both dark nights (filled symbols) and while exposed to LAN (open symbols). Day lengths are as follows: short, 9 and 9.5 hr; intermediate, 11.33, 12, and 13 hr; and long, 14.5 hr. Data are presented as means ± SE and are slightly offset to minimize overlap of symbols and error bars.

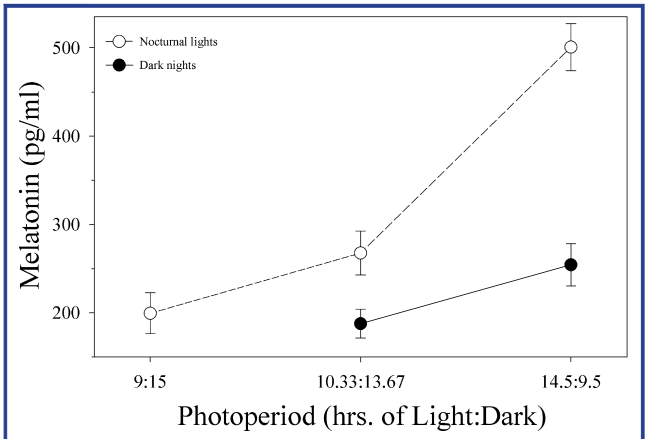

Figure 4. Plasma levels of melatonin in jays exposed to dark nights (closed circles) and LAN (open circles). Data are collapsed across sex as there were no statistical effects of sex. Data are presented as means ± SE.

$P = 0.11$) on E_2 levels, nor was there a photoperiod–LAN interaction ($F = 1.44$, $P = 0.24$). When the model was rerun without the interaction term, LAN exhibited a statistically significant effect on E_2 levels ($F = 5.43$, $P = 0.022$), and the effect of photoperiod approached statistical significance ($F = 2.50$, $P = 0.087$; Fig. 3). In males, E_2 levels were nearly sevenfold lower under LAN than when the scotophase was dark ($F = 15.43$, $P = 0.0002$; 0.015 ± 0.016 and 0.10 ± 0.015 ng/mL; Fig. 3). E_2 levels also differed among photoperiods ($F = 3.39$, $P = 0.037$) with levels during the intermediate photoperiod being significantly greater than during the long period ($P = 0.037$); none of the other pair-wise comparisons approached statistical significance ($P \geq 0.42$). Elapsed time had no effect upon E_2 levels of males ($F = 0.85$, $P = 0.36$), although the photoperiod–LAN interaction was statistically significant ($F = 4.47$, $P = 0.014$).

MEL levels were significantly greater under LAN than when the dark phase was not lighted ($F = 36.00$, $P < 0.0001$; with overall MEL levels of 320.40 ± 13.50 and 223.41 ± 21.86 pg/mL, respectively; Fig. 4). Similarly, MEL levels varied greatly among photoperiods ($F = 29.80$, $P < 0.0001$). Long day (14.5L:9.5D) MEL levels (374.35 ± 19.46 pg/mL) were significantly greater that those from birds exposed to 9L:15D, 9.5L:14.5D, and 10.33L:13.67D ($P \leq 0.006$; 199.49 ± 24.12, 193.65 ± 29.39, and 266.19 ± 24.17 pg/mL, respectively); however, MEL levels did not differ among the other pair-wise comparisons ($P \geq 0.34$). MEL levels did not differ between the sexes ($F = 0.009$, $P = 0.93$) and there was no effect of elapsed time ($F = 0.016$, $P = 0.90$). None of the among factor interaction terms (i.e., sex × LAN, se × photoperiod, and LAN × photoperiod) were significant ($P \geq 0.55$).

The among-hormone correlations are presented in Table 2. One of the most robust relationships are the tight correlations ($r \geq 0.79$) between T and E_2 levels under dark nights and the absence of this relationship ($r \leq 0.14$) with LAN.

DISCUSSION

Our data offer no support for the hypothesis that exposure to dim light during the dark phase of the daily cycle is stimulatory to the HPG axis. Contrarily, our data suggest the opposite. Although the initial examination of LH levels noted no main effect of LAN, the significant main effect of sex led us to further analyze the data separately by sex. This analysis found that males exhibited a marked depression of LH levels under LAN. Additionally, inspection of the graphically presented LH data (see Fig. 1), suggests a degree of disruption due to exposure to LAN. Whereas LH levels in both males and females increase with day length during dark nights, under LAN this pattern is no longer evident. Similarly, in both males and females birds exposed to LAN had levels of E_2 that were markedly lower, seven- and fourfold lower, respectively, than birds under dark nights. Whereas T levels of males did not differ between LAN and dark nights, levels of T in females were significantly depressed under LAN. In general, consideration of the figures that depict sex hormone profiles across photoperiods suggests that LAN interfered with the patterns of the endocrine responses to increased day length (Figs. 1–3). In all cases, the largely "flat" profiles of jays exposed to LAN suggest that the treatment interfered with their responses to changes in photoperiod.

Further support in our study for LAN having disruptive effects upon the HPG axis comes from the among-hormone correlations presented in Table 2. Among the presented correlations, the most

robust and ubiquitous one is the strong positive relationship between T and E_2 that is observed in virtually all of the matrices. It is only when correlations are examined by exposure to, or the absence of, LAN that a pattern is revealed. Clearly, in the three comparisons of jays that were not exposed to LAN (i.e., noted as "dark" in Table 2), these correlations were highly significant ($P < 0.0001$), whereas in jays that experienced LAN, the correlation between T and E_2 was not observed. Given that T is the precursor to E_2 at both the Leydig cells of the testes and granulosa cells of the ovary, it is not unreasonable to speculate that the observed lack of correlation reflects disruption of this conversion. Because the T to E_2 conversion is mediated by the enzyme, aromatase, it would be interesting to determine the impact of LAN upon aromatase levels and function.

A number of studies have explored the ecological and behavioral impacts of exposure to nocturnal lights upon birds, although relatively few have assessed such effects on reproduction and fewer still have measured reproductive hormones. Miller (2006) noted that "light pollution" led to earlier onset of dawn song in American robins (*Turdus migratorius*) and, given the links between song and sex steroid hormones, this may reflect a stimulatory effect of LAN upon the reproductive axis. Unfortunately, the study did not evaluate any aspect of reproduction. However, a recent study of the congeneric European blackbird (*Turdus merula*) showed LAN exposure advanced both the timing of song onset and seasonal testes growth (Dominoni et al., 2013).

In studies of visual (as opposed to tactile) foragers, waders such as ringed (*Charadrius hiaticula*), Kentish (*C. alexandrinus*), and gray (or black-bellied) plovers (*Pluvialus squatarola*), as well as the "diver" common murre (*Uria aalge*), LAN resulted in increased foraging efficiency (Santos et al., 2010; Regular et al., 2011). A study of five common woodland passerines in Europe found that four (blue tit, *Cyanistes caeruleus*; great tit, blackbird, and robin, *Erithacus rubecula*: with the chaffinch, *Fringilla coelebs*, being the exception) exhibited earlier onset of dawn song in areas with LAN (Kempenaers et al., 2010). These researchers also found that in blue tit territories with LAN, males had greater success in obtaining extra-pair paternity and females laid earlier than conspecifics not exposed to LAN. Conversely, de Molenaar et al. (2006) found that black-tailed godwits (*Limosa limosa*) tended to delay laying slightly when nesting near roadway lights.

Clearly, not all LAN effects are beneficial and in species that are nocturnally active, LAN can cause disorientation and grounding, which can often result in the death of the bird. A long-term study on *Procellariiformes* on Tenerife of the Canary Islands highlights such negative effects (Rodríguez and Rodrguez, 2009). Similarly, collisions with manmade structures are exacerbated with night lighting and are known to kill millions of migrating birds annually (for reviews see Longcore et al., 2008; Arnold and Zink, 2011).

We know of no avian studies that have specifically addressed plasma levels of reproductive hormones in response to dim LAN. However, there is an increasing interest in apparent links among LAN exposure, altered reproductive cycles (accompanied with changes in ovarian and pituitary hormone secretions), and health in humans, primarily in female night shift or alternating shift workers (for reviews see Blask et al., 2011; Wang et al., 2011).

Our melatonin findings are intriguing and paradoxical. Because it has been known for decades that the exposure of animals to LAN invariably results in depressed levels of MEL (Reiter, '91), our expectation was that the exposure to LAN would result in lower plasma levels of MEL. However, it is important to note that the majority of avian-based experimental studies have used LAN levels that were quite bright, equivalent to those used during the light phase of the L:D cycle (e.g., pigeon, *Columba livia*, [Vakkuri et al., '85]; pied flycatcher, *Ficedula hypoleuca* [Schneider et al., '94]; and geese, *Anser anser f. domestica* [Zawilska et al., 2003]). Numerous studies have held animals under continuous dim light to explore the nature and persistence of circadian rhythms in the absence of a photic *zeitgeber* (e.g., exploration of the rhythmicity of perch-hopping and feeding behaviors in European starlings [Ebihara and Gwinner, '92; Kumar et al., 2007]).

In those relatively few studies that have examined the effects of exposure to dim LAN upon MEL, levels have been depressed across taxa, although this can be dependent upon the intensity of the light. For example, lab rats (Sprague–Dawley) exposed to LAN levels of 0.41 lux or less had MEL levels that were equivalent to control animals (i.e., no LAN), whereas those rats exposed to LAN levels of 0.55 lux had levels of MEL that did not differ from animals housed under constant light (Dauchy et al., 2010). Similarly, in humans (*Homo sapiens*), exposure to a white light emitting diode (LED) backlit screen significantly depressed the nocturnal rise in MEL, although the pattern of MEL increase was similar to that of controls (Cajochen et al., 2011). In the only study that we are aware of that has considered this issue in birds, Tarlow et al. (2003) found a significant diel pattern of MEL secretion under a new moon (i.e., dark night) in Nazca bobbies (*Sula granti*) on the Galapagos Islands; however, under illumination from a full moon, the pattern was dampened and there was no pattern of diel variation in MEL levels.

As for why our study produced such anomalous findings with MEL levels not only being higher under LAN but also increasing with photoperiod, we can only speculate. Our finding of increased MEL levels under the photoperiods with the shortest dark phase may be due to our sampling protocol in which samples were collected 3 hr after lights out irrespective of photoperiod. The result would be that under short-days (9L:15D) MEL was measured about 3.5 hr before the expected midnight peak, whereas during long days (14.5L:9.5D) MEL was measured closer (1.5 hr) to the expected midnight peak that has been noted in a variety of taxa (Gwinner et al., '93; Reierth et al., '99; Kumar et al., 2000; Nakahara et al., 2003; Silverin et al., 2009; Moninuzzaman and Maitra, 2012). Hence, one would expect that levels would be lower during short days, which actually was the case.

Another explanation for our finding of elevated MEL under LAN is that this elevation is not really due to LAN but is the result of intrinsic changes in the physiology of the birds over the intervening time between the two sampling periods. At the time of the initial sampling under an unlighted dark period, birds had been in captivity for 1–4 months. A pilot study that coincided with the initial stage of the current study in which samples were collected in January 2005 to examine the effect of disturbance time on plasma corticosterone (CORT) levels found baseline levels (i.e., samples collected within 2 min of entering the room) to be generally high ($n = 4$, 20.8 ± 5.5 ng/mL [$x \pm$ SE]; S.J.S., unpub. data). In contrast, mean baseline CORT levels when assessed 4–5.5 months later were <5 ng/mL (see Fig. 1c in Bridge et al., 2009). The relatively elevated baseline CORT levels suggest that the time in captivity upon initial sampling was insufficient for the birds to have become fully acclimated to captivity. The relationship between glucocorticoid secretion and MEL can be complex. Studies indicating that CORT can sometimes lead to increased MEL, and that exogenous MEL can ameliorate the effects of CORT, have led to the hypothesis that MEL functions as a protector against negative effects of stress (Barriga et al., 2002; Saito et al., 2005; Cuoto-Moraes et al., 2009; Detanico et al., 2009; Singh et al., 2010; Baxi et al., 2012). Perhaps more relevant to the current study is evidence that CORT and MEL can be inversely related (i.e., stress and elevated CORT resulted in depressed MEL levels; Jessop et al., 2002; Nikaido et al., 2010). While speculative, our seemingly paradoxical data might reflect: (1) the elevated CORT levels in individuals tested under dark nights resulted in the observed relatively low MEL levels and (2) the apparently elevated MEL under LAN reflect levels that are more in-line with the species norm. Support for the latter point comes from comparable absolute MEL levels in two passerine species housed under similar conditions (e.g., garden warblers, *Sylvia borin* and European starlings; Gwinner et al., '93; Kumar et al., 2000, respectively).

With regard to the suburban population of Florida scrub-jays that motivated this study, our findings suggest that LAN is not the primary factor that accelerates the onset of reproduction. The consistency with which the suburban birds begin laying eggs (always in February) would seem to be indicative of a response to a very consistent seasonal cue, such as photoperiod. However, food is also a very abundant and reliable resource in the suburban site, given that the jays can exploit garbage, bird feeders, and pet food in addition to naturally occurring food sources (Fleischer et al., 2003). Supplementation of wildland jays consistently results in earlier breeding (Schoech and Bowman, 2001, 2003; Schoech et al., 2008), but never to the extent observed among the suburban birds. Our finding that exposure to LAN interferes with sex-related hormone secretion in western scrub-jays is inconsistent with its playing a key role in early breeding in the suburban population of Florida scrub-jays.

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