A seasonally breeding tropical bird lacks absolute photorefractoriness in the wild, despite high photoperiodic sensitivity

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Summary

1. Photoperiod is an important seasonal cue for temperate seasonal breeders. In birds, increasing photoperiod typically stimulates reproductive maturation, while also triggering gonad regression through photorefractoriness at a later date. Curiously, many tropical birds show photoperiodic reproductive responses, even though day-length changes in their natural habitat are minimal.

2. Spotted Antbirds (*Hylophylax n. naevioides*) from a near-equatorial rainforest in Panama are highly sensitive to small photoperiodic changes at the onset of reproduction. We therefore tested whether these tropical birds use photoperiod as the primary signal for seasonal processes, as do most temperate birds, and terminate reproduction through photorefractoriness.

3. Male and female Spotted Antbirds captured during postbreeding moult significantly increased gonad sizes when exposed to a photoperiod of 22 h. Furthermore, males exposed to a naturalistic 1-h increase in photoperiod (to 13 h) also grew their gonads, suggesting that these birds retain photosensitivity at a time of year when most temperate birds are unable to grow their gonads. Hatch-year birds in all groups showed reproductive development indicating their capability to breed. Unlike in many temperate-zone birds, the moult of adult birds was not inhibited by gonad development.

4. The lack of photorefractoriness allows Spotted Antbirds to flexibly adjust the end of breeding to environmental conditions. Our findings support the view that photoperiodic mechanisms are evolutionary plastic and match the ecology of a particular species.

Key-words: Environmental cue, gonad development, luteinizing hormone, photoperiodism, timing of breeding

Introduction

Reproduction is an energetically expensive process (King 1973; Ricklefs 1974; Bronson 1989). In most animals it is therefore limited to parts of the year during which environmental conditions are most conducive to raising offspring (Perrins 1970; Bronson 1985; Daan *et al.* 1988; Di Bitetti & Janson 2000). To regulate reproduction in a seasonal manner, the vast majority of mid- and high-latitude species respond to changes in photoperiod to initiate and, equally importantly, to terminate breeding (Wingfield & Kenagy 1991). In avian species the typical pattern is that increasing day-lengths in spring stimulate the growth and maturation of the reproductive system (photostimulation), and at

the same time also trigger a process that will eventually terminate reproduction via photorefractoriness (Follett 1984; Nicholls, Goldsmith & Dawson 1988; Ball 1993; Cockrem 1995; Sharp 1996; Hahn *et al.* 1997; Dawson *et al.* 2001). Photorefractoriness is a state of reproductive inactivity in response to long photoperiods that were previously stimulatory (Nicholls *et al.* 1988). The photorefractory condition typically involves the regression of the reproductive system and a post-nuptial moult. Functionally, photorefractoriness ensures the cessation of offspring production before environmental conditions deteriorate. It also enables the preparation for and progression through other costly annual processes such as moult and migration (Farner *et al.* 1983; Jacobs & Wingfield 2000). Typically in temperate-zone birds, the acquisition of photosensitivity is facilitated by exposure to an extended period of short day-lengths, as occurs in the winter (Nicholls *et al.* 1988;

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Cockrem 1995; Bentley 1997). Thus, photoperiod plays an important dual role for the precise regulation of both the onset and end of reproduction in seasonally breeding temperate birds (Dawson *et al*. 2001).

Annual changes in photoperiod are large at mid and high latitudes, but they become exceedingly small at low latitudes. Interestingly, many near-equatorial bird species show annual reproductive cycles that often resemble the seasonal breeding strategy of temperate birds (Murton & Westwood 1977; Dittami & Gwinner 1990; Wikelski, Hau & Wingfield 1999a; Stutchbury & Morton 2001; Wikelski *et al*. 2003). However, it still remains unresolved to what extent the mechanisms controlling seasonal reproduction in temperate and tropical birds are similar to or different from each other (Hau 2001). Curiously, many near-equatorial birds possess photoperiodic features that are comparable to those of temperate birds. For example, when exposed to the large changes in day-length that are characteristic of higher latitudes, most near-equatorial bird species respond by initiating reproductive activity (Rollo & Domm 1943; Marshall & Disney 1956; Wolfson & Winchester 1959; Epple *et al*. 1972; Gwinner & Dittami 1985). Furthermore, in captivity under certain photoperiodic conditions, near-equatorial species can also become photorefractory (Lofts 1962; Gwinner & Scheuerlein 1999). Until recently, the ecological relevance of the photoperiodic reproductive responsiveness of near-equatorial birds remained unclear because they usually do not experience such photoperiodic conditions in the wild (but see Gwinner & Scheuerlein 1999). However, we have shown that one seasonally breeding near-equatorial bird, the Spotted Antbird (*Hylophylax n. naevioides*), is able to use the slight natural photoperiodic changes in its natural habitat to initiate reproduction (Hau, Wikelski & Wingfield 1998). Such a high sensitivity suggests that even in near-equatorial birds photoperiod could play a major role as the primary signal for seasonal processes. Because in Spotted Antbirds photoperiod appears to be an important cue for the initiation of reproduction, we tested whether in these birds it plays a similarly dual role as in most temperate birds by terminating reproduction via photorefractoriness in the wild.

One method of testing for photorefractoriness is to expose birds that have regressed their gonads after the breeding season and are undergoing postbreeding moult to increases in photoperiod that are stimulatory at the start of the breeding season. During this seasonal stage, photorefractory species lack a reproductive response to previously stimulatory photic cues (Nicholls *et al*. 1988). For the present experiment, we caught wild male and female Spotted Antbirds during postbreeding moult and kept a control group on short days (12 h of light) typical for their natural habitat. We exposed a second group to artificially long days of 22 h, and a third group to the longest day-length they can experience in their natural habitat (13 h). Our data show that even during moult the reproductive system

of Spotted Antbirds remains responsive to increases in photoperiod, suggesting that these neotropical birds do not become absolutely photorefractory in the wild.

Materials and methods

ANIMAL HOUSING

Between 30 September and 21 October 2002, 29 male and 12 female Spotted Antbirds were caught in Soberania National Park in the Republic of Panamá (9°N, 79°W). Birds were immediately housed in individual cages ($40 \times 35 \times 40$ cm^3) in one of three indoor rooms at the Gamboa Schoolhouse field station of the Smithsonian Tropical Research Institute. Rooms were visually and acoustically isolated from one another, and each room housed birds of both sexes. Birds in individual cages were visually isolated from each other. Water and food were provided *ad libitum*. Food consisted of live crickets (*Acheta domestica*), live mealworms (*Tenebrio* spp.), and a freshly prepared egg-food mix (as in Hau *et al*. 1998; Hau, Wikelski & Wingfield 2000). Water was changed once a day and food was renewed twice daily. All animal procedures were approved by the Princeton University Institutional Animal Care and Use Committee and met all applicable state and federal guidelines.

PHOTOPERIODIC MANIPULATION

After capture, birds were exposed to a photoperiod of 12 h of light (which approximates the natural photoperiod at this time in Panama; see Wikelski, Hau & Wingfield 2000) for a period of 5–14 days to acclimate birds to captivity. A combination of fluorescent and halogen light sources illuminated the rooms. Ambient temperature in the bird rooms was around 28 °C, which is a temperature experienced by wild birds regularly during the day (Wikelski *et al*. 2000). Birds were assigned to experimental groups at random as they were captured. Birds in the control group remained on a 12 h photoperiod ('12-h group': seven adult and one hatch-year males, one adult and two hatch-year females). A second group of birds was exposed to a long photoperiod of 22 h ('22-h group': seven adult and three hatch-year males, three adult females). A third group was transferred to a naturalistic increase in photoperiod by 1 h, resulting in 13 h of light ('13-h group': eight adult and four hatch-year males, three adult and three hatch-year females). Some birds in each group died of unknown causes, resulting in slightly decreased final sample sizes in each group (see Fig. 1).

PHYSIOLOGICAL AND MORPHOMETRIC MEASUREMENTS

Gonad sizes of all birds were measured before, after 3 weeks (between 20 and 25 days) and after 5 weeks (between 30 and 38 days) of the start of the experiment.
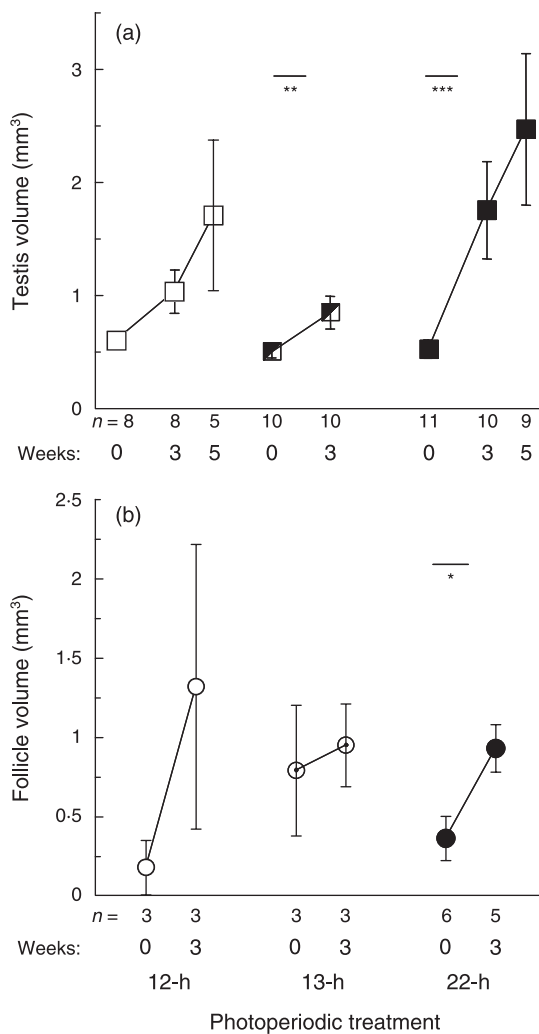
Fig. 1. (a) Testis and (b) follicle volumes (mm³; mean ± 1 SEM) of Spotted Antbirds before, and after 3 and 5 weeks of exposure to a photoperiod of 12 h (open symbols), 13 h (hatched symbols) or 22 h of light (filled symbols). For 13-h group measurements for the 5-week time point are not available. Sample sizes and weeks of treatment are given below *x*-axis. Significant differences between before and after 3 weeks of photoperiodic treatment were determined by *post-hoc* tests (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, for males: Bonferroni-adjusted $\alpha = 0.016$).

At the same time a small blood sample (100–150 µl) was taken for later analysis of plasma concentrations of luteinizing hormone. Body mass and furcular fat stores were also determined, but since neither measure differed between treatment groups the data are not reported here. After taking morphometric measurements, each bird was examined for moult. The moult of the nine primaries was also scored (0 = old, 1 = missing, 2 = pin, 3 = half grown, 4 = three-quarters grown, 5 = complete new feather, maximum moult score = 45; after Newton 1966). Birds were aged as juveniles (hatch-year) or adults (after hatch-year) based on skull ossification (Miller 1946; any incompletely ossified birds were considered hatch-year birds). At the end of the experiment birds were released on their original territories.

Length and width of the left testis for males and diameter of the largest follicle for females were measured to the nearest 0·1 mm with modified digital callipers by unilateral laparotomy under a light isoflurane anaesthesia (for details on these standard procedures see Hau *et al*. 1998; Wikelski *et al*. 2000). The person determining gonad sizes was blind with respect to the treatment of the birds. Testis volume was calculated using the formula $V = 4/3\pi a^2 b$, with *a* being half of the width and *b* half the length of the testis. The volume of the largest follicle was calculated using the formula $V = 4/3\pi r^3$, with *r* being the diameter of the follicle.

Blood samples were kept cool until centrifugation. Plasma was separated and frozen at −20 °C until analysis. Samples were transported to the US on dry ice under permission of Panamanian and US authorities. Plasma concentrations of luteinizing hormone (LH) were determined in a single radioimmunoassay following Follett, Scanes & Cunningham (1972) and Sharp, Dunn & Talbot (1987), with minor modifications. The detection limit of the assay was 0·039 ng ml⁻¹. The intra-assay coefficient of variation was under 4·9%.

DATA ANALYSIS

Data are presented as mean ± 1 SEM throughout. Hormone concentrations below the lower detection limit were set at detection limit for statistical analysis. Parametric tests (repeated-measures ANOVAs and paired *t*-tests) were used on raw or log-transformed data. Because of the recent discussion about the usefulness of Bonferroni corrections (e.g. Moran 2003), Bonferroni-adjusted α values are reported along with the original *P*-values of *post-hoc* tests. Moult data were analysed only for adult males.

Results

REPRODUCTIVE MEASURES

Males

Only the 12-h and 22-h groups experienced 5 weeks of photoperiodic treatment. However, testis sizes and plasma LH concentrations in both groups did not change from 3 to 5 weeks of photoperiodic treatment (paired-sample *t*-tests on log-transformed data, each treatment group separately, all $P > 0.18$; Fig. 1a, Table 1). We therefore used data only from before and after 3 weeks of photoperiodic treatment for further statistical analyses.

After 3 weeks of photoperiodic treatment, there was an overall increase in testis size (repeated-measures ANOVA on log-transformed data; effect of 'time': $F_{1,25} = 35.97$, $P < 0.0005$, Fig. 1a), but groups differed in their extent of testis growth (interaction of 'time' × 'group': $F_{2,25} = 3.75$, $P = 0.038$; but no effect of 'group' alone: $P > 0.16$). Both the 13-h and 22-h groups showed highly significant increased in testis sizes, while the

Table 1. Plasma LH concentrations (ng ml^{-1}; mean ± 1 SEM) of male and female Spotted Antbirds before and after 3 weeks of exposure to a photoperiod of 12 h, 13 h or 22 h. Data for 5-week treatments are not reported because not all groups experienced this treatment duration and, in general, LH concentrations did not differ between 3 and 5 weeks. Sample sizes are given in brackets

	Before	After 3 weeks
Males		
12-h	2·79 ± 0·68 (8)	1·85 ± 0·44 (8)
13-h	1·42 ± 0·38 (10)	2·76 ± 0·51 (10)
22-h	2·73 ± 0·68 (11)	2·76 ± 0·42 (9)
Females		
12-h	2·96 ± 0·69 (3)	1·42 ± 0·78 (2)
13-h	2·32 ± 0·51 (3)	3·69 ± 0·7*** (3)
22-h	4·88 ± 1·5 (6)	2·06 ± 0·56* (4)

Significant differences as determined by paired-sample *t*-tests: *$P < 0.05$, ***$P < 0.005$. (For exact *P*-values and Bonferroni-adjusted α values see text.)

12-h group showed a trend for an increase (*post-hoc* paired-samples *t*-tests; Bonferroni-adjusted α = 0·016; control group: $t = -2.51$, $P = 0.04$; 13-h group: $t = -3.3$, $P = 0.009$; 22-h group: $t = -4.6$, $P = 0.001$; Fig. 1a).

In contrast to testis growth, there were no overall effects of treatment on plasma LH concentrations in males (Table 1, effects of 'time' or 'group': repeated-measures ANOVA, both $P > 0.5$), but there was a significant interaction between 'time' × 'group' ($F_{2,24} = 3.64$, $P = 0.042$). Males in the 12-h group seemed to decrease LH levels while males in the 13-h group appeared to increase LH levels. However, *post-hoc* tests were not significant.

Young (hatch-year) males were present in each group ($n = 1$ in 12-h group, $n = 3$ in 13-h group, $n = 3$ in 22-h group). These sample sizes precluded a statistical analysis of the effect of photoperiod on testis growth of young *vs* adults separately for each experiment group. However, all hatch-year males increased testis sizes in the 3 weeks of photoperiodic treatment. Gonads of hatch-year males were smaller than those of adults at the start of the experiment (one-way ANOVA, groups lumped, $n = 22$ adults, $n = 7$ hatch-year, $F_{1,27} = 6.52$, $P = 0.017$), but not after 3 or 5 weeks (after 3 weeks: $n = 22$ adults, $n = 6$ hatch-year, $P > 0.3$; after 5 weeks: $n = 11$ adults, $n = 3$ hatch-year, $P > 0.4$). Because of the overall low sample size and high interindividual variability, we did not analyse LH concentrations of hatch-year males.

Females

There were no changes in follicle sizes in females in the 12-h and the 13-h groups (because of the small sample size we used paired-sample *t*-tests on log-transformed data, $P > 0.2$ in both analyses), but females of the 22-h group did significantly grow their gonads ($t = -4.02$, $P = 0.016$, Fig. 1b; females did not yolk up follicles). A total of five hatch-year females were included in the experiments ($n = 2$ in 12-h group, $n = 3$ in 22-h group). All hatch-year females, except for one bird in the 12-h group, grew their gonads.

Repeated measures for LH were obtained for only two females in the 12-h group and therefore this group could not be included in the statistical analysis. There was a significant increase in LH concentrations in the 13-h group (paired-sample *t*-test, $t = -16.5$, $P = 0.004$), while the 22-h group showed a significant decrease in LH over the 3 weeks of photoperiodic treatment ($t = 3.5$, $P = 0.039$; Table 1).

MOULT

Males

At the start of the experiment adult males were in various stages of primary wing moult and most of them also showed heavy body moult at their belly, sides, back, head and tail. All birds that actively moulted primaries before the start of the experiment continued to lose new feathers during the first 3 weeks of the experiment, and there were no indications of interrupted moult in any bird. There was no correlation between the degree of wing moult (as determined by the number of newly lost or growing feathers) of individuals and their changes in gonad size from before to after 3 weeks of photostimulation (groups lumped, Pearson's ρ = 0·49, $P > 0.8$). The rate of wing moult (as calculated by the change in the cumulative moult score for primaries; Newton 1966) from before to 3 weeks after the start of the experiment did not differ between experimental groups (one-way ANOVA, $P > 0.17$, Table 2). Within this experimental period, birds on average advanced their primary feather moult by 7·2 ± 1·16 score points, or about 1·5 new feathers.

Females

Adult females were in a similar moult stage to adult males. All adult females showed active body and wing

Table 2. Moult of primary wing feathers in adult males from each experimental group before and after 3 weeks of photoperiodic treatment (mean ± 1 SEM). %: percentage of males in active moult in each group. Score: cumulative moult score for primaries. Moult rate: change in cumulative moult score from before to after 3 weeks of photoperiodic treatment

	Before %	Score	After 3 weeks %	Score	Moult rate
12-h ($n = 7$)	85·7	20·9 ± 5·1	88·4	26·1 ± 3·9	5·3 ± 5·1
13-h ($n = 7$)	85·7	25·3 ± 4·0	71·9	31·6 ± 3·6	6·3 ± 1·7
22-h ($n = 8$)	62·5	31·6 ± 4·3	50	36·4 ± 3·6	4·9 ± 1·6

moult in each group before the start of photoperiodic treatment and most of them continued to do so 3 weeks after the start of the photoperiodic treatment. As in males, there were no indications of moult being interrupted.

Discussion

Our data show that seasonally breeding, highly photo-sensitive Spotted Antbirds from the near-equatorial tropics do not become absolutely photorefractory in the wild. Spotted Antbirds thus differ from many temperate zone birds in the control of gonad regression at the end of the reproductive season. The ability to respond to a changing photoperiod provides an important annual template for the timing of reproduction, which in many avian species causes reproduction to begin and end on the same date every year (Wingfield *et al.* 1992; Hahn *et al.* 1997). We suggest that Spotted Antbirds possess a high degree of plasticity of the photoperiodic template, which allows reproduction to continue as long as environmental conditions allow, even under short photoperiods.

LACK OF ABSOLUTE PHOTOREFRACTORINESS IN ADULT AND HATCH-YEAR SPOTTED ANTBIRDS

We conducted this experiment on birds that had fully regressed gonads after the breeding season and that were in the midst of postbreeding moult. We chose this seasonal stage because it is the period when temperate (Farner *et al.* 1983; Nicholls *et al.* 1988; Dawson *et al.* 2001) and equatorial bird species (Gwinner & Scheuerlein 1999) studied so far show photorefractoriness and are reproductively unresponsive to long photoperiods. Two types of photorefractoriness have been distinguished: relative and absolute (Nicholls *et al.* 1988; Cockrem 1995; Bentley 1997; Hahn *et al.* 1997). Absolutely photorefractory birds collapse their gonads during long days and cannot re-grow them in response to photostimulation until they have regained photosensitivity, which in many species requires exposure to short day-length. Relatively refractory species need to experience a decrease in photoperiod to regress their gonads, but can re-initiate rapid gonad development when presented with long photoperiods during gonad regression (Robinson & Follett 1982). The significant increase in gonad sizes in birds exposed to 22 h of light (22-h group; Fig. 1) clearly demonstrates that Spotted Antbirds do not become absolutely photorefractory when tested at the end of breeding. Our experimental design does not allow us to exclude the possibility that Spotted Antbirds become relatively photorefractory. Despite this, the demonstration that Spotted Antbirds are not absolutely photorefractory in the wild and therefore lack a rigid termination of breeding is functionally the most critical aspect of their seasonality (see also Hahn *et al.* 1997).

Males experiencing a 1-h increase in photoperiod (13-h group; Fig. 1a) showed a highly significant increase in testis size, suggesting that even slight changes in photoperiod remain stimulatory. Females in the same group lacked a significant reproductive response (Fig. 1b), which is probably related to the small number of females tested. However, we cannot rule out the possibility that females cannot respond to a small increase in photoperiod at this time of year and that the two sexes differ in reproductive control mechanisms (Ball 1993; Astheimer & Buttemer 1999; Bentley *et al.* 2003). Interestingly, males in the 12-h (control) group also tended to grow their testes, albeit at a slower rate than males in the 13-h group (Fig. 1a). An attractive explanation related to the ecology of this species (see discussion at end) could be that males remain responsive to both photoperiodic and non-photic environmental cues at this time of year (see also Ball 1993; Hahn *et al.* 1997). Food cues can stimulate the rate of gonad growth in Spotted Antbirds prior to the breeding season (Hau *et al.* 2000), and social cues such as song might be stimulatory in captive birds housed closely together. Alternatively, Spotted Antbirds might possess endogenous annual rhythms like other temperate and tropical species (Gwinner 1986), and under benign conditions in captivity spontaneously re-initiate slow gonadal development irrespective of day-length (in the wild, Spotted Antbirds maintain regressed gonads at this time of year; Wikelski *et al.* 2000).

Males in the 22-h group showed the highest rate of testis growth of all groups, but despite such a large increase in photoperiod did not achieve the large gonad sizes of males exposed to a 1-h increase in photoperiod prior to the breeding season males reached testis sizes of about 2·5 mm^3 within 5 weeks of photostimulation, compared to about 17 mm^3 within 4 weeks of photostimulation in Hau *et al.* (1998). The size at which the testes probably produce active sperm in this species is 5 mm^3; Wikelski *et al.* (2000). This discrepancy could result from a seasonally variable response of male Spotted Antbirds to photoperiod, which might be related to internal (e.g. annual rhythms, body reserves, moult, initial gonad sizes) or external factors (e.g. actions of other environmental stimuli).

Plasma LH concentrations in birds from the different groups differed in the initial sample and changes in LH did not parallel the reproductive development of birds in the different groups very well (Table 1). In previous experiments LH has not been a reliable marker for reproductive activation in Spotted Antbirds either (Hau *et al.* 1998; Hau *et al.* 2000), perhaps because of a suppression of hormone levels in captivity or overall low concentrations in plasma LH in these birds even in the wild (Wikelski *et al.* 2000). LH also failed to parallel gonad maturation in studies of other temperate and tropical species, perhaps as a consequence of negative feedback from gonadal steroids (Sailaja *et al.* 1988; Silverin & Viebke 1994; Wingfield *et al.* 1997; Bentley *et al.* 2000; Perfito *et al.* 2004).

All hatch-year birds increased gonad sizes in the present experiment. The variation observed among hatch-year birds in the degree of gonad growth might be related to their hatch date (i.e. their age at the start of the experiment), or to photoperiodic treatment. The attainment of reproductive maturation in the first year of life in Spotted Antbirds is similar to opportunistic breeders (Vaugien 1953 in Lofts & Murton 1968; Marshall & Serventy 1958; Gibbs, Grant & Weiland 1984; Follett 1991). In complete contrast, the young of most seasonally breeding temperate birds hatch in a photorefractory state and are unable to breed in the year in which they were born (Farner *et al.* 1983; Follett 1991).

GONAD GROWTH DURING MOULT

In seasonal breeders, reproduction and moult are often considered to be mutually exclusive life-cycle stages (Payne 1972; Murton & Westwood 1977; Wingfield & Farner 1980; Dawson 2002), presumably because both are energetically costly. It is therefore remarkable that in the present experiment moult rates of males were similar among treatment groups and that none of the birds interrupted moult. Wild Spotted Antbirds have not unequivocally been shown to exhibit moult–breeding overlap (M. Hau & M. Wikelski, unpublished data), but the present finding suggests that in these birds these two life-cycle stages are not mutually exclusive (see also Hahn *et al.* 1992).

In the present experiment, moult and reproductive maturation might have been energetically compatible because of unlimited abundance of high-quality food in captivity and the fact that gonads had just started to grow and were still small. Alternatively, slow moult rates in tropical birds (Miller 1961; Fogden 1972; Gwinner, Dittami & Gwinner 1983) might render an overlap of moult and breeding generally more feasible. Indeed, the duration of the primary wing moult in our captive population (roughly 120 days, or 17 weeks) falls within the slow moult rates reported for other tropical birds rather than the fast moult rates of temperate birds. Finally, the reproductive hormone testosterone appears to be one mediator of the incompatibility of reproduction and moult in temperate birds (Schleussner, Dittami & Gwinner 1985; Hahn *et al.* 1992; see also summary in Payne 1972). Spotted Antbirds generally have low circulating concentrations of testosterone (Wikelski, Hau & Wingfield 1999b; Wikelski *et al.* 2000) and this hormone might therefore not have inhibited moult.

ENVIRONMENTAL CONSTRAINT OR EVOLUTIONARY ADAPTATION?

Early research had argued that equatorial birds might not become photorefractory in the wild because the critical day-length to induce this state might not be exceeded (Murton & Westwood 1977). This suggestion was based on the fact that in most photoperiodic bird species, the threshold for the induction of photorefractoriness is higher than that for reproductive maturation (Murton & Westwood 1977; Nicholls *et al.* 1988; Cockrem 1995). However, there now exists evidence that the critical day-length for the induction of photorefractoriness is below 13 h (which is the maximal day-length in Panama; Wikelski *et al.* 2000) in temperate bird species (Nicholls *et al.* 1988). Furthermore, it has been shown that even equatorial birds can become photosensitive when exposed to their natural photoperiod (e.g. Gwinner & Scheuerlein 1999). Since Spotted Antbirds have an almost exclusively tropical phylogenetic history (Ridgely & Tudor 1994), they are likely to have evolved photoperiodic thresholds that are functionally relevant in their environment, like many other avian species (Lofts, Murton & Westwood 1966; Lofts, Murton & Westwood 1967; Lofts & Murton 1968; Silverin, Massa & Stokkan 1993; Hahn *et al.* 2004; MacDougall-Shackleton, Pereyra & Hahn, in press).

The lack of absolute photorefractoriness matches the ecology of Spotted Antbirds, in particular their long breeding season that extends past the summer solstice (Wikelski *et al.* 2000). Absolute photorefractoriness is typically found in species with a short breeding season that need to terminate reproductive processes while day-lengths are very long, while species with a long breeding season typically are relatively or not photorefractory (Farner *et al.* 1983; Nicholls *et al.* 1988; Hahn *et al.* 1997; Dawson *et al.* 2001). It also fits the seasonal variability of the environment in Panama, where the beginning and end of the wet season can vary between years by up to 11 weeks (Windsor 1990). In such a climate it appears beneficial for Spotted Antbirds to keep the end of the reproductive period flexible and extend it in years in which favourable conditions persist (see also Hahn *et al.* 1997). The environmental and physiological processes that actually terminate breeding and lead to a regression of the reproductive system in these birds remain to be investigated, but factors such a decrease in day-length, resource availability or body condition may contribute to it.

References

Astheimer, L.B. & Buttemer, W.A. (1999) Gonadal and hormonal patterns in the annual cycle of an Australian honeyeater. *Proceedings 22 International Ornithological Congress Durban* (eds N. Adams & R. Slotow), pp. 1768–1783. BirdLife South Africa, Johannesburg.

Ball, G.F. (1993) The neural integration of environmental information by seasonally breeding birds. *American Zoologist* **33**, 185–199.

Bentley, G.E. (1997) Thyroxine and photorefractoriness in starlings. *Poultry and Avian Biology Reviews* **8**, 123–139.

Bentley, G.E., Spar, B.D., MacDougall-Shackleton, S.A., Hahn, T.P. & Ball, G.F. (2000) Photoperiodic regulation of the reproductive axis in male zebra finches, *Taeniopygia guttata*. *General and Comparative Endocrinology* **117**, 449–455.

Bentley, G.E., Audage, N.C., Hanspal, E.K., Ball, G.F. & Hahn, T.P. (2003) Photoperiodic response of the hypothalamo-pituitary-gonad axis in male and female canaries, *Serinus canaria*. *Journal of Experimental Zoology* **296A**, 143–151.

Bronson, F.H. (1985) Mammalian reproduction: an ecological perspective. *Biology of Reproduction* **32**, 1–26.

Bronson, F.H. (1989) *Mammalian Reproductive Biology*. University of Chicago Press, Chicago, IL.

Cockrem, J.F. (1995) Timing of seasonal breeding in birds, with particular reference to New Zealand birds. *Reproduction, Fertility and Development* **7**, 1–19.

Daan, S., Dijkstra, C., Drent, R. & Meijer, T. (1988) Food supply and the annual timing of avian reproduction. *Acta XIX Congressua Internationalia Ornithologicua* (ed. H. Ouellet), pp. 392–407. University of Ottawa Press, Ottawa, ON.

Dawson, A. (2002) Photoperiodic control of the annual cycle in birds and comparison with mammals. *Ardea* **90**, 355–367.

Dawson, A., King, V.M., Bentley, G.E. & Ball, G.F. (2001) Photoperiodic control of seasonality in birds. *Journal of Biological Rhythms* **16**, 365–380.

Di Bitetti, M.S. & Janson, C.H. (2000) When will the stork arrive? Patterns of birth seasonality in neotropical primates. *American Journal of Primatology* **50**, 109–130.

Dittami, J.P. & Gwinner, E. (1990) Endocrine correlates of seasonal reproduction and territorial behaviour in some tropical passerines. *Endocrinology of Birds: Molecular to Behavioural* (ed. M. Wada), pp. 225–233. Japanese Science Society Press/Springer-Verlag, Tokyo/Berlin.

Epple, A., Orians, G.H., Farner, D.S. & Lewis, R.A. (1972) The photoperiodic testicular response of a tropical finch, *Zonotrichia capensis costaricensis*. *Condor* **74**, 1–4.

Farner, D.S., Donham, R.S., Matt, K.S., Mattocks, P.W. Jr, Moore, M.C. & Wingfield, J.C. (1983) The nature of photorefractoriness. *Avian Endocrinology: Environmental and Ecological Perspectives* (eds S. Mikami, K. Homma & M. Wada), pp. 149–166. Japanese Science Society/Springer-Verlag, Tokyo/Berlin.

Fogden, M.P.L. (1972) The seasonality and population dynamics of equatorial forest birds in Sarawak. *Ibis* **114**, 307–343.

Follett, B.K. (1984) Birds. *Marshall's Physiology of Reproduction* (ed. G.E. Lamming), Vol. 1, pp. 283–350. Churchill Livingston, Edinburgh.

Follett, B.K. (1991) The physiology of puberty in seasonally breeding birds. *Follicle Stimulating Hormone: Regulation of Secretion and Molecular Mechanisms of Action* (eds M. Hunzicker-Dunn & N.B. Schwartz), pp. 54–65. Springer Verlag, New York.

Follett, B.K., Scanes, C.G. & Cunningham, F.J. (1972) A radioimmunoassay for avian luteinizing hormone. *Journal of Endocrinology* **52**, 359–378.

Gibbs, H.L., Grant, P.R. & Weiland, J. (1984) Breeding of Darwin's Finches at an usually early age in an El Nino year. *Auk* **101**, 872–874.

Gwinner, E. (1986) *Circannual Rhythms*. Springer-Verlag, Berlin Heidelberg.

Gwinner, E. & Dittami, J. (1985) Photoperiodic responses in temperate zone and equatorial stonechats: a contribution to the problem of photoperiodism in tropical organisms. *The Endocrine System and the Environment* (eds B.K. Follett, S. Ishii & A. Chandola), pp. 279–294. Japanese Science Society/Springer-Verlag, Tokyo/Berlin.

Gwinner, E. & Scheuerlein, A. (1999) Photoperiodic responsiveness of equatorial and temperate-zone stonechats. *Condor* **101**, 347–359.

Gwinner, E., Dittami, J. & Gwinner, H. (1983) Postjuvenile molt in East-African and Central European stonechats (*Saxicola torquata axillaris*, *Saxicola torquata rubicula*) and its modification by photoperiod. *Oecologia* **60**, 66–70.

Hahn, T.P., Swingle, J., Wingfield, J.C. & Ramenofsky, M. (1992) Adjustments of the prebasic molt schedule in birds. *Ornis Scandinavica* **23**, 314–321.

Hahn, T.P., Boswell, T., Wingfield, J.C. & Ball, G.F. (1997) Temporal flexibility in avian reproduction. *Current Ornithology* (eds V. Nolan, E.D. Ketterson Jr & C.F. Thompson), Vol. 14, pp. 39–80. Plenum Press, New York, London.

Hahn, T.P., Pereyra, M.E., Sharbaugh, S.M. & Bentley, G.E. (2004) Physiological responses to photoperiod in three cardueline finch species. *General and Comparative Endocrinology* **137**, 99–108.

Hau, M. (2001) Timing of breeding in variable environments: tropical birds as model systems. *Hormones and Behavior* **40**, 281–290.

Hau, M., Wikelski, M. & Wingfield, J.C. (1998) A neotropical forest bird can measure the slight changes in tropical photoperiod. *Proceedings of the Royal Society of London B* **265**, 89–95.

Hau, M., Wikelski, M. & Wingfield, J.C. (2000) Visual and nutritional food cues fine-tune timing of reproduction in a neotropical rainforest bird. *Journal of Experimental Zoology* **286**, 494–504.

Jacobs, J.D. & Wingfield, J.C. (2000) Endocrine control of life-cycle stages: a constraint on response to the environment? *Condor* **102**, 35–51.

King, J.R. (1973) Energetics of reproduction in birds. *Breeding Biology of Birds* (ed. D.S. Farner), pp. 78–120. National Academy of Science, Washington, DC.

Lofts, B. (1962) Photoperiod and the refractory period of reproduction in an equatorial bird, *Quelea quelea*. *Ibis* **104**, 407–414.

Lofts, B. & Murton, R.K. (1968) Photoperiodic and physiological adaptations regulating avian breeding cycles and their ecological significance. *Journal of Zoology, London* **155**, 327–394.

Lofts, B., Murton, R.K. & Westwood, N.J. (1966) Gonadal cycles and the evolution of breeding seasons in British Columbidae. *Journal of Zoology, London* **150**, 249–272.

Lofts, B., Murton, R.K. & Westwood, N.J. (1967) Interspecific differences in photosensitivity between three closely related species of pigeons. *Journal of Zoology, London* **151**, 17–25.

MacDougall-Shackleton, S.A., Pereyra, M.E. & Hahn, T.P. (in press) GnRH, photorefractoriness, and breeding schedules of cardueline finches. *Functional Avian Endocrinology* (eds A. Dawson & P.J. Sharp). Narosa Publishing House, New Delhi.

Marshall, A.J. & Disney, H.J.D.S. (1956) Photostimulation of an equatorial bird (*Quelea quelea* Linnaeus). *Nature* **177**, 143–144.

Marshall, A.J. & Serventy, D.L. (1958) The internal rhythm of reproduction in xerophilous birds under conditions of illumination and darkness. *Journal of Experimental Biology* **35**, 666–670.

Miller, A.H. (1946) A method for determining the age of live passerine birds. *Bird Banding* **17**, 33–35.

Miller, A.H. (1961) Molt cycles in equatorial andean sparrows. *Condor* **63**, 143–161.

Moran, M.D. (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**, 403–405.

Murton, R.K. & Westwood, N.J. (1977) *Avian Breeding Cycles.* Clarendon Press, Oxford.

Newton, I. (1966) The moult of the bullfinch *Pyrrhula pyrrhula. Ibis* **108**, 41–67.

Nicholls, T.J., Goldsmith, A.R. & Dawson, A. (1988) Photo-refractoriness in birds and comparison with mammals. *Physiological Reviews* **68**, 133–176.

Payne, R.B. (1972) Mechanisms and control of molt. *Avian Biology*, Vol. 2 (eds D.S. Farner & J.R. King), pp. 103–155. Academic Press, New York.

Perfito, N., Tramontin, A.D., Meddle, S.L., Sharp, P., Afik, D., Gee, J., Ishii, S., Kikuchi, M. & Wingfield, J.C. (2004) Reproductive development according to elevation in a seasonally breeding male songbird. *Oecologia* **140**, 201–210.

Perrins, C.M. (1970) The timing of birds' breeding seasons. *Ibis* **112**, 242–255.

Ricklefs, R.E. (1974) Energetics of reproduction in birds. *Avian Energetics* (ed. R.A. Paynter Jr), pp. 152–292. Nuttall Ornithological Club, Cambridge, MA.

Ridgely, R.S. & Tudor, G. (1994) *The Birds of South America*, Vol. II. *The Suboscine Passerines.* University of Texas, Austin, TX.

Robinson, J.E. & Follett, B.K. (1982) Photoperiodism in Japanese quail – the termination of seasonal breeding by photorefractoriness. *Proceedings of the Royal Society of London B* **215**, 95–116.

Rollo, M. & Domm, L.V. (1943) Light requirements of the weaver finch. I. Light period and intensity. *Auk* **60**, 357–367.

Sailaja, R., Kotak, V.C., Sharp, P.J., Schmedemann, R. & Haase, E. (1988) Environmental, dietary, and hormonal factors in the regulation of seasonal breeding in free-living female Indian rose-ringed parakeet (*Psittacula krameri*). *Hormones and Behaviour* **22**, 518–527.

Schleussner, G., Dittami, J.P. & Gwinner, E. (1985) Testosterone implants affect molt in male European starlings, *Sturnus vulgaris. Physiological Zoology* **58**, 597–604.

Sharp, P.J. (1996) Strategies in avian breeding cycles. *Animal Reproduction Science* **42**, 505–513.

Sharp, P.J., Dunn, I.C. & Talbot, R.T. (1987) Sex differences in the responses to chicken LHRH-I and II in the domestic fowl. *Journal of Endocrinology* **115**, 323–331.

Silverin, B. & Viebke, P.A. (1994) Low temperatures affect the photoperiodically induced LH and testicular cycles differently in closely related species of tits (*Parus* spp.). *Hormones and Behavior* **28**, 199–206.

Silverin, B., Massa, R. & Stokkan, K.A. (1993) Photoperiodic adaptations to breeding at different latitudes in great tits. *General and Comparative Endocrinology* **90**, 14–22.

Stutchbury, B.J.M. & Morton, E.S. (2001) *Behavioral Ecology of Tropical Birds.* Academic Press, San Diego, CA.

Wikelski, M., Hau, M. & Wingfield, J.C. (1999a) Seasonal endocrinology of neotropical passerines: A comparative approach. *Proceedings 22nd International Ornithological Congress Durban* (eds N. Adams & R. Slotow), pp. 1224–1241. Bird Life South Africa, Johannesburg.

Wikelski, M., Hau, M. & Wingfield, J.C. (1999b) Social instability increases testosterone year-round in a tropical bird. *Proceedings of the Royal Society of London B* **266**, 1–6.

Wikelski, M., Hau, M. & Wingfield, J.C. (2000) Seasonality of reproduction in a neotropical rain forest bird. *Ecology* **81**, 2458–2472.

Wikelski, M., Hau, M., Robinson, W.D. & Wingfield, J.C. (2003) Reproductive seasonality of seven neotropical passerine species. *Condor* **105**, 683–695.

Windsor, D.M. (1990) *Climate and Moisture Variability in a Tropical Forest: Long-term Records From Barro Colorado Island, Panamá.* Smithsonian Institution Press, Washington, DC.

Wingfield, J.C. & Farner, D.S. (1980) Control of seasonal reproduction in temperate-zone birds. *Progress in Reproductive Biology* **5**, 62–101.

Wingfield, J.C. & Kenagy, G.J. (1991) Natural regulation of reproductive cycles. *Vertebrate Endocrinology: Fundamentals and Biomedical Implications* (eds P.K.T. Pang & M.P. Schreibman), pp. 181–241. Academic Press, New York.

Wingfield, J.C., Hahn, T.P., Levin, R. & Honey, P. (1992) Environmental predictability and control of gonadal cycles in birds. *Journal of Experimental Zoology* **261**, 214–231.

Wingfield, J.C., Hahn, T.P., Wada, M. & Schoech, S. (1997) Effects of day length and temperature on the control of gonadal development, body mass, and fat depots in white-crowned sparrows, *Zonotrichia leucophrys pugetensis. General and Comparative Endocrinology* **107**, 44–62.

Wolfson, A. & Winchester, D.P. (1959) Effect of photoperiod on the gonadal cycle in an equatorial bird, *Quelea quelea. Nature* **184**, 1658–1659.