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Food cues and gonadal development in neotropical spotted antbirds (*Hylophylax naevioides*)

Received: 23 March 2005 / Revised: 25 July 2005 / Accepted: 27 July 2005 / Published online: 7 September 2005
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Abstract Most temperate-zone birds live in environments with a regular seasonality, and primarily use the long-term changes in photoperiod as a cue to initiate gonadal development in anticipation of the breeding season. Short-term cues such as food and temperature are later used to fine-tune the rate of gonadal development to local conditions. Many tropical habitats are seasonal, but the timing of the seasons (e.g., rainy season) can vary considerably between years. We hypothesize that to time breeding in environments with seasonal variability, tropical birds respond to both long-term and short-term environmental cues to initiate gonadal growth. We tested the effectiveness of photoperiod and food cues for the initiation of gonad growth in captive male spotted antbirds (*Hylophylax n. naevioides*) from Panama. A 'control' group was maintained on the short natural photoperiod of 12 h light and 12 h dark (LD 12:12) and adequate food. A 'food-stimulated' group was also held on LD 12:12 but received an increase in food quantity and quality. A 'photo + food-stimulated' group experienced an increase in daylength by 1 h (LD 13:11, the maximal photoperiod in Panama) and an increase in food quantity and quality. Within 3 weeks testis sizes of 'food-stimulated' birds increased significantly, suggesting that food cues alone can initiate gonad development. As expected from the previous experiment, testis sizes of 'photo + food-stimulated' birds, but not 'control' birds,

also increased. We suggest that the capability to respond to both food and photoperiodic cues allows animals to flexibly adjust reproductive activity to variable environmental conditions each year. Future work should elucidate whether food provides nutritional or non-nutritional cues, and the neurophysiological mechanisms by which food stimulates reproductive activity.

Keywords Food cues · Environmental seasonality · Reproduction · Tropical bird · Photoperiod

Introduction

Seasonally variable environments necessitate a precise timing of reproduction in many animals, including birds. To maximize reproductive success, most vertebrate species track cues from the environment that provide information about hospitable conditions for successful reproduction. Birds from temperate habitats have traditionally served as model systems to study the use and integration of environmental cues to time breeding efforts [47]. In temperate latitudes photoperiod changes in a regular manner that is highly predictive of changes in environmental conditions such as ambient temperature and food abundance. This allows birds to use photoperiod as a long-term cue ('initial predictive' cue, [47]) to physiologically prepare for the coming breeding season by initiating gonadal development and other physiological processes in advance. The majority of temperate birds prioritizes the use of environmental cues in a hierarchical fashion and use photoperiod as the primary cue to initiate the onset of reproductive activity [47]. Short-term cues such as food availability or quality, temperature, water availability and social interactions are subsequently used as secondary cues to fine-tune reproductive activities to local environmental conditions [1, 8, 27, 45–47]. These secondary (or 'supplementary', [47]) cues provide more accurate information about short-term changes in environmental conditions.

Communicated by F. Bairlein

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Most temperate birds live in regular seasonal environments. In contrast, many tropical birds are exposed to habitats that undergo seasonal fluctuations in environmental conditions, but with less precision from year-to-year than in temperate zone environments. How do tropical birds from seasonally variable environments integrate environmental cues to successfully time reproduction? Here we test the idea that short-term cues are used by animals living in seasonally variable environments to regulate reproductive activity [48]. In contrast to photoperiod, short-term cues such as food provide more immediate information about local environmental conditions. A responsiveness to short-term cues therefore can increase the precision in the timing of reproduction and thus improve reproductive success.

Some studies have shown that temperate species such as red crossbills (*Loxia curvirostra*), piñon jays (*Gymnorhinus cyanocephalus*) and canaries (*Serinus canaria*) living in seasonally variable environments rely strongly on secondary cues as important environmental factors to regulate gonadal development [11, 21, 22, 24]. However, only in a few opportunistically breeding species do secondary cues equal (or even exceed) the relevance of photoperiod in triggering gonadal development prior to the breeding season (e.g., [4, 11, 14, 22, 40]). In tropical birds, it has long been thought that responsiveness to short-term cues is high, not the least because photoperiodic changes are minimal in proximity of the equator. Indeed there exists correlative evidence that gonad development is tied to nutritional conditions in tropical birds (e.g., [17, 41]). Furthermore, elegant experiments in wild tropical birds showed that food supplementation can advance laying date more strongly than in most temperate birds [35] and that translocation to a habitat with constant high food supply results in a prolongation of the breeding season [19]. However, these experiments examined how short-term cues affect the end result of reproductive activation—egg laying—and not whether they can trigger the initiation of gonad growth. This initial switch from a reproductively inactive to an active state is a critical step in the timing of breeding since gonads typically need a few weeks to grow from a regressed state to fully active sizes [26].

Here we test the ability of a tropical bird living in a seasonally variable environment to use food cues independently of photoperiod as a cue to initiate gonadal development. The neotropical spotted antbird (*Hylophylax n. naevioides*) from Panama lives in an environment with a distinct wet and dry season which can vary in onset by 6–8 weeks between years [44]. These purely insectivorous birds breed only during the rainy season [42, 43], when insect abundance increases [20]. Spotted antbirds are known to be responsive to small changes in photoperiod to initiate gonad growth [15], as well as to changes in food abundance and quality to later modulate the rate of gonadal development [16]. Since their reproductive season varies considerably from year to year, we experimentally investigated whether these birds are able to use secondary cues independently of photo-

period. If they could respond to cues such as food availability and quality independently of photoperiodic changes, it would allow them to be highly flexible in their timing of reproduction and able to fine-tune it with environmental variability. Ultimately this would allow individuals to initiate reproduction sooner than photoperiodic stimuli would dictate, possibly leading to increased reproductive success.

Methods

Animal collection

Birds were captured in the non-breeding season when gonads are regressed [42]. Birds were caught between December 19, 2000 and January 19, 2001 in various areas of the humid lowland forest in Soberania National Park, Republic of Panama (9°N, 79°W). Birds were released back onto their home territories at the end of the study.

Animal housing

Birds were kept in individual cages with partitions between cages for visual isolation to prevent aggressive interactions, and housed in two separate experimental rooms at the Smithsonian Tropical Research Institute (STRI), Gamboa Field Station. The rooms were made lighttight to prevent outside light from coming in. Initially all groups were maintained on a schedule of 12 h light and 12 h dark (12L:12D), which is similar to the light schedule in their natural habitat at this time of the year [15]. Previous studies showed that the presence of female spotted antbirds enhances singing in males [15], thus a female was housed with each group in order to provide some social stimulation.

Animal care

Birds were fed a mixture of nutrient-complete soft egg food similar to that of Gwinner [9], live mealworms (*Tenebrio larvae*) and water ad libitum. Food and water were renewed every day and water baths were placed in the bottom of each cage once or twice per week. Cages and perches were thoroughly cleaned once or twice a week. Food containers were cleaned every day and water containers were cleaned at least every other day.

Birds were allowed to acclimate to captive conditions for at least 1 week (and up to approximately 6 weeks depending upon the capture date) before the initiation of the experiment on January 27, 2001. On that day, birds were randomly divided into groups, while controlling for date of capture to avoid any effect of time spent in captivity on experimental results. Birds were assigned to one of the three groups: (1) a control group, kept under 12L:12D and a diet consisting of egg food and live

mealworms; (2) a food-stimulated group exposed to 12L:12D and a diet consisting of egg food, increased numbers of live mealworms plus live crickets (*Acheta domestica*); and (3) a photoperiod and food-stimulated (photo + food) group exposed to an increase in photoperiod by 1 h resulting in a 13L:11D schedule and a diet consisting of egg food, increased numbers of live mealworms and live crickets. All crickets were juveniles and did not sing.

Measurements

Measurements were taken only for male birds. Before dividing birds into different treatment groups, they were laparotomized between January 21 and 24, 2001 to determine pre-treatment testis sizes. Laparotomies were carried out via an incision in the skin and peritoneum at the left side of the bird. The last two ribs were parted and length and width of the left testis was determined [15]. All laparotomies were performed under light Isoflurane (Abbott Laboratories, Enflurane) anesthesia [15]. To determine post-treatment testis sizes laparotomies were conducted on February 15 and 16, 2001. Testis volume was calculated using the formula $V = 4/3\pi a^2b$, with a being half of the width and b being half the length of the testis. Birds were weighed two to four times a week and scored for the presence of molt.

Statistical analysis

Because of the modest sample sizes in the control and photo + food-stimulated groups, we analyzed changes in testis size separately for each group using paired t -tests (SPSS Inc., Chicago). Body mass at four time points (before the start of the experiment until 10 days before the second laparotomy) was analyzed using repeated measures ANOVA. For this analysis, we substituted missing data points in some individuals with the mean of their body masses before and after the missing data point. We then further analyzed which groups changed body masses during the experiment by conducting post-hoc repeated-measures ANOVA for each treatment group separately. As the applicability of Bonferroni-corrections for post-hoc tests is currently being debated [25], we report Bonferroni-adjusted α 's along with the original P values of post-hoc tests. Significance for all tests was set at $\alpha = 0.05$. All data are presented as mean \pm 1SEM.

Results

Paired t -tests showed significant increases in testis sizes in both the food-stimulated ($t = -3.12$, $P = 0.012$) and the photo + food-stimulated groups ($t = -5.8$, $P = 0.004$), but not in the control group ($t = -1.26$, $P > 0.2$; Fig. 1). Treatment groups changed in body mass over time

($F_{3,18} = 10.43$, $P < 0.005$), but they did so in different patterns (interaction of treatment and time: $F_{6,38} = 3.48$, $P < 0.01$, Fig. 2). There was also a significant difference between groups ($F_{2,20} = 4.98$, $P < 0.02$). Analyzing changes in body mass separately for each treatment group (Bonferroni-adjusted $\alpha = 0.016$) showed that neither the control group ($F_{3,18} = 2.74$, $P > 0.15$), nor the photo + food group ($F_{3,18} = 2.24$, $P > 0.25$) varied over time, but that food-stimulated birds significantly increased body mass over the course of the experiment ($F_{3,18} = 14.01$, $P < 0.005$, Fig. 2)

Discussion

Our data suggest that male spotted antbirds can initiate testis development in response to food cues alone, independent of photoperiodic stimulation. As expected, photo + food-stimulated birds also increased testis sizes, confirming that a small increase in photoperiod in the presence of a high abundance of high-quality food stimulates the reproductive system of these birds [15]. Although testis sizes of food-stimulated males increased over the course of 3 weeks, they remained relatively small (averaging about 1.5 mm^3 , Fig. 1; testes probably become spermatogenic at around 5 mm^3 in this species, [42]). Photo + food-stimulated birds showed a slightly bigger increase in testis sizes than food-stimulated birds, suggesting that either the photoperiodic stimulation is more effective than the food stimulation, or that the two factors are acting additively. There might also be a temporal difference in the perception of photoperiodic and food cues, leading to different temporal patterns in gonad growth. Photoperiodic cues can be perceived very

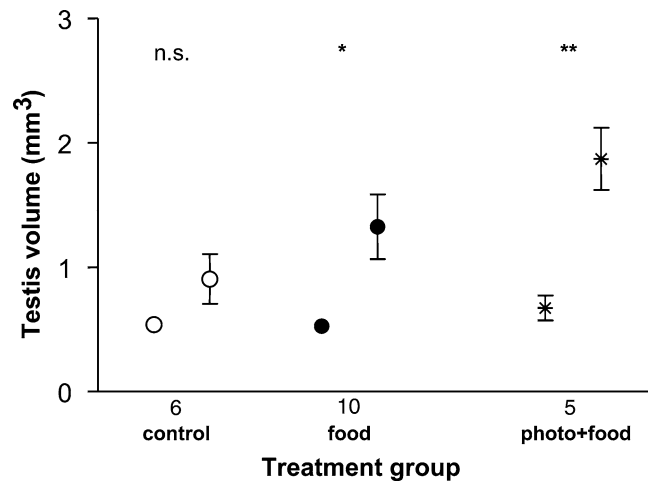


Fig. 1 Testis sizes (mm^3 ; mean \pm SE) of control (open symbols), food-stimulated (filled symbols) and photo + food-stimulated (star symbols) males before and 3 weeks after the start of the experiment. Sample sizes for each group are indicated below the x-axis. The results of post-hoc paired t -tests comparing testis sizes before and at the end of the experiment for each group is indicated by asterisks above each group (* $P = 0.012$, ** $P < 0.005$, n.s. = not significant, for further explanations please see text)

rapidly; in Japanese quail (*Coturnix japonica*) photostimulation can lead to a large increase in LH secretion within 18 h [28]. We are not aware of comparable data for food stimulation in birds, but a similar increase in food quantity and quality as in the present experiment caused an increase in song activity (a measure of reproductive behavior) within 2–3 days in male spotted antbirds [16]. However, even in photo + food-stimulated males average testis sizes reached only 2 mm³ (Fig. 1). The relatively small increases in testis sizes in both groups might be related to the short duration of the experiment and the very small initial testis sizes [2]. Previous experiments that reported larger increases in gonad sizes after a similar photoperiodic (and food) stimulation were conducted for at least a week longer, and birds also had slightly initial larger testis sizes [15].

The present data suggest that spotted antbirds are flexible breeders [13] that do not use photoperiodic and food cues in a strict hierarchical fashion. In that aspect they differ from strict seasonal breeders (most temperate birds) for which photoperiod is the main trigger for the initiation of gonadal development [48]. Spotted antbirds also differ from many temperate birds in their termination of the reproductive season by lacking a period of absolute photorefractoriness [2], which also supports our interpretation that they are flexible seasonal breeders [13].

The increase in body mass over the course of the experiment in the food-stimulated group suggests that food improved the nutritional condition of the birds. Conversely, the lack of a decrease in body mass of control birds confirms that birds in the present study were not food-restricted, thus indeed testing the capacity of food to act as a stimulatory cue rather than as a limiting factor for gonad growth. Why the photo + food-stimulated group did not show a similar increase in body mass as food-stimulated groups remains unresolved at this point, but both food- and photo + food-stimulated groups had similar final body masses (Fig. 2). At present, we cannot resolve which aspect of the food manipulation stimulated the reproductive system of the birds—whether the birds were stimulated by nutritional or non-nutritional cues. The higher body masses of food-stimulated birds suggest that the food presentation might have increased the nutritional state of the birds [36]. Alternatively, food might have acted via non-nutritional pathways. In a previous experiment we have shown that merely seeing live crickets stimulates song activity in male spotted antbirds, indicating that visual cues alone are stimulatory [16]. Along the same lines, the consumption of freshly dead crickets, which should provide a similar nutritional value as live crickets, did not stimulate song activity. However, free availability of crickets led to the strongest stimulation of song in this experiment, suggesting that a combination of visual and handling stimuli might activate reproductive behavior most [16]. Future experimental work controlling for modes of perception of food cues by spotted antbirds can further elucidate this question.

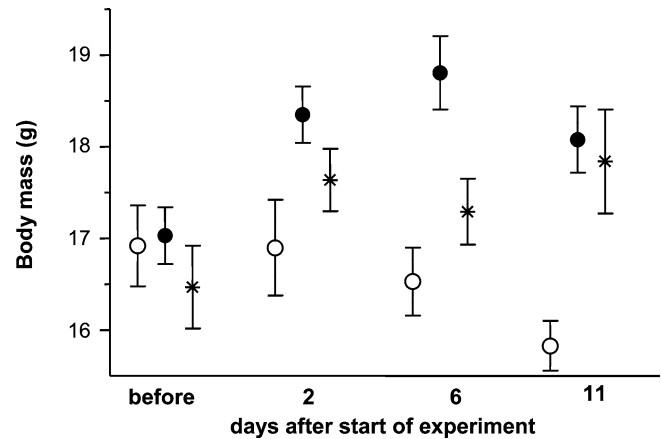


Fig. 2 Body mass (g; mean \pm SE) of control (open symbols, $n=7$), food-stimulated (filled symbols, $n=10$) and photo+food-stimulated (star symbols, $n=6$) males before the start of the experiment, and at various intervals (days) after the start of the experiment. For statistics please see text

Environmental cues are transduced neuronally to the hypothalamo-pituitary–gonadal axis (HPG-axis), where they affect the reproductive system via an endocrine cascade [10]. Upon stimulation, the hypothalamus releases gonadotropin-releasing hormone (GnRH) which then stimulates the pituitary to release luteinizing hormone (LH) and follicular-stimulating hormone (FSH). These two gonadotropins then travel to the gonads to increase gonadal development and maturation. Several forms of GnRH have been identified in the avian brain, chicken GnRH-I, II [5, 37] and recently also lamprey GnRH-III [3]. In temperate birds photoperiod stimulates the release of GnRH-I, which is the main switch for gonadotropin release in these species [6, 23]. Likewise, the gonadal response to photoperiod in spotted antbirds might occur via a stimulation of GnRH-I neurons, or an increase in the number of GnRH-I neurons present [7, 12, 29, 33]. At present it remains unclear how food cues exert their effect on the reproductive axis. Food cues could either be acting on the GnRH-I system, or on other GnRH forms [7, 30, 34]. Data from temperate zone song sparrows (*Melospiza m. melodia*) indeed raise the possibility that short-term cues might not act via the GnRH-I system, since short-term cues affected rate of gonadal development, but not circulating LH concentrations [31]. Instead, food cues might be acting via GnRH-II as suggested by recent findings in female mammals: GnRH-II appears to mediate an animal's energetic status to the reproductive system, stimulating reproductive behavior directly when food availability is limited [18, 39]. Furthermore, we have recently obtained evidence in Zebra finches (*Taeniopygia guttata*) that GnRH-II cell number might be affected by another short-term cue, social factors [32].

The seasonal organization of spotted antbirds may be an evolutionary adaptation to the environment in which they live. The environment in Panama is seasonal in terms of rainfall, but the timing of the rainy seasons may

vary by 6–8 weeks between years [44]. In such an environment it may be advantageous to use both photoperiod and food cues as potent cues for initiating reproductive readiness (see also [48]). By being highly responsive to food cues, spotted antbirds could initiate gonad growth in years in which an increase in food quantity or quality is advanced relative to photoperiodic changes. Spotted antbirds could thus exploit seasons of high food abundance and lengthen their breeding season accordingly without being limited by photoperiod. Similarly, in years in which food availability is delayed, their responsiveness to photoperiod ensures the physiological preparation for reproduction in advance of the breeding season and a fast response to an improvement in food conditions.

In conclusion, our data suggest that a tropical bird from a seasonally variable environment can respond to either photoperiod or food cues to initiate reproduction, and that this flexibility might ultimately increase its reproductive success. While the non-hierarchical processing of these two environmental cues in male spotted antbirds appears different from that of most strict seasonally-breeding temperate birds, further experiments are needed to determine how the mode of perception of environmental cues and their transmission to the HPG axis differs from temperate species. It is interesting to note that like most strict seasonally breeding temperate birds, flexible seasonal breeders such as spotted antbirds, and even opportunistic breeders such as zebra finches and red crossbills, all possess the ability to process and respond to both photoperiodic and food cues [4, 11]. The importance, however, of each of the two cues appears to vary between species using different breeding strategies. Thus, our present findings add to the growing evidence that the seasonal organization of birds is evolutionarily plastic [14, 26, 38]. It would now be interesting to find out how fast the responsiveness to photoperiod versus food cues can evolve in species using different breeding strategies. This knowledge would be important to evaluate the potential for successful reproduction after dispersal into new habitat with different seasonal schedules, as well as possible effects of climate change on the reproduction of populations, which could ultimately determine the fate of some species.

Zusammenfassung

Futterreize und Gonadenentwicklung bei neotropischen Fleckenbrustwaldwächtern (*Hylophylax n. naevioides*)

Die meisten Vögel der gemäßigten Breiten leben in Umwelten mit regelmäßigen saisonalen Veränderungen und nutzen vor allem die zunehmende Hellzeit des Tages im Frühjahr, um die Gonadenentwicklung zu steuern. Futter und Temperatur sind später zusätzliche Umweltreize, um die Gonadenwachstumsrate mit kurzzeitigen Umweltränderungen abzustimmen. Viele tropische

Habitats zeigen ebenfalls saisonale klimatische Veränderungen, jedoch können sich die Zeiten, zu denen bestimmte Jahreszeiten (z.B. Regenzeiten) auftreten, von Jahr zu Jahr merklich verschieben. Um die Brutzeit auch in Habitats mit solcher beträchtlicher saisonaler Variation zeitlich zu steuern, reagieren tropische Vögel in der Regulation ihrer Gonadenentwicklung möglicherweise sowohl auf Langzeit- wie auf Kurzzeit-Umweltfaktoren. Die Wirksamkeit von Tageslängen- und Futterreize auf den Beginn des Gonadenwachstums wurde an gekäfigten männlichen Fleckenbrustwaldwächtern (*Hylophylax n. naevioides*) aus Panama geprüft. Eine Gruppe (Kontrollgruppe) wurde unter der kurzen natürlichen Tageslänge von 12 Stunden Licht und 12 Stunden Dunkel (LD 12:12) und ausreichend Futter gehalten. Eine andere Gruppe wurde ebenfalls unter LD 12:12 gehalten, erhielt aber Futter in erhöhter Menge und Qualität (futterstimulierte Gruppe). Eine weitere Gruppe erfuhr eine Verlängerung in der Tageslänge von einer Stunde (LD 13:11, die längste natürliche Tageslänge in Panama) und zudem die Futterstimulation (tageslänge- und futterstimulierte Gruppe). In der futterstimulierten Gruppe nahm die Hodengröße innerhalb von drei Wochen signifikant zu, was darauf hinweist, dass Futterreize unabhängig von anderen Faktoren das Gonadenwachstum stimulieren können. Wie aus vorhergehenden Experimenten erwartet, zeigten auch die Vögel der tageslänge- und futterstimulierten Gruppe Hodenwachstum, nicht jedoch die Vögel der Kontrollgruppe. Wir nehmen an, dass die Fähigkeit, sowohl auf Futter- wie auch auf Tageslängenveränderungen zu reagieren, den Vögeln erlaubt, ihre Reproduktion flexibel auf die jährlich variablen Umweltbedingungen abzustimmen. Zukünftige Untersuchungen sollten prüfen, ob diese Wirkung von Futter nährwertbezogen ist oder davon unabhängig und über welche neurophysiologischen Mechanismen Futter die Fortpflanzung stimuliert.

Acknowledgements This manuscript is dedicated to the memory of Ebo Gwinner, an outstanding scientist who has been a wonderful friend and mentor to us. Many thanks to J. Touchton, Umberto and Andres for expert field and lab assistance, and Armstrong Cricket Farm, West Monroe, LA, for reliably shipping insects to Panama. The Smithsonian Tropical Research Institute, in particular M. Leone, O. Arosemena, M. Fernandez and R. Urriola provided invaluable logistical support. L. Spinney, L. Martin, M. Wikelski, B. Walker, G. Bentley, N. Perfito and C. Hess provided constructive comments on previous versions of the manuscript. The research reported here was performed under guidelines established by the University of Illinois Institutional Animal Care and Use Committee. This material is based upon work supported by the National Science Foundation under grant no. IBN-0196297 to M.H.

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