

# Visual and Nutritional Food Cues Fine-Tune Timing of Reproduction in a Neotropical Rainforest Bird

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**ABSTRACT** Food may act as a proximate factor in the regulation of avian seasonal breeding. Food cues could provide particularly important seasonal information to birds living in variable tropical environments, but this has not yet been tested. Spotted antbirds (*Hylophylax n. naevioides*) inhabiting a humid forest in central Panama (9°N) likely use changes in the tropical photoperiod to time reproduction on a long-term, seasonal basis. We predicted that these insectivorous birds also adjust reproduction to short-term cues such as food availability because the onset of the rainy season and the resulting increase in insect abundance varies considerably between years. To test this prediction, prior to their breeding season (when they had half-maximal gonads), we either exposed captive male spotted antbirds to an ad libitum standard diet only or added live crickets to this diet. Males that received live crickets significantly increased gonad sizes within 3 weeks over controls on the standard diet. Moreover, in six additional experiments cricket availability always increased song rate, usually within a few days. The stimulatory effect of live crickets on song activity may function independent of nutritional aspects: Freshly killed crickets, providing similar nutritional content as live crickets, did not stimulate the birds' song activity. However, song activity increased to intermediate levels when live crickets were shown under a clear plastic wrap, i.e., when birds could see but not eat crickets. We hypothesize that the opportunity to see and handle live insects stimulates song and reproductive activity in these birds. Our data indicate for the first time that a tropical rainforest bird can use food cues to evaluate the suitability of local environmental conditions for breeding. *J. Exp. Zool.* 286:494–504, 2000. © 2000 Wiley-Liss, Inc.

Many tropical habitats are seasonal environments and most tropical bird species studied to date show well-defined breeding seasons (see, e.g., Moreau, '50; Skutch, '50; Voous, '50; Snow and Snow, '64; Fogden, '72; Murton and Westwood, '77; Brown and Britton, '80; Bell, '82; Stiles, '80; Chandola-Saklani et al., '90; Dittami and Gwinner, '90). The majority of birds live at tropical latitudes, but we have only a vague idea of how they adjust reproduction to seasonal events in their environment (Levin and Wingfield, '92; Hau et al., '99). The climatic patterns to which birds are exposed at near-equatorial latitudes (here defined as those within 15° from the equator), can be quite different from those in temperate areas: some factors such as photoperiod and temperature may vary only slightly, whereas seasonal changes in other factors such as rainfall may be dramatic (Grant, '86; Leigh et al., '96). In addition, the year-to-year variability in the timing and duration of seasonal

events (e.g., rainy seasons) is often pronounced (Grant and Boag, '80; Windsor, '90). How do tropical birds that live in such environments time reproductive activities such that they concur with favorable seasonal environmental conditions?

In principle, two ways are conceivable: (i) Birds keep their gonads in a near-functional state throughout the year and "opportunisticly" react to short-term changes in environmental conditions such as food availability or rainfall (e.g., Immelmann, '71, '73; Serventy, '71). Although some tropi-

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cal birds may employ this strategy, several recent studies have shown that most other tropical birds do not breed opportunistically (e.g., Chakravorty et al., '85; Chandola-Saklani et al., '90; Dittami and Gwinner, '90; Hau et al., '98; Wikelski et al., '99, 2000). Alternatively (ii) tropical birds could adopt a seasonal breeding strategy and show regular seasonal changes in gonad sizes. Like many of their temperate zone conspecifics, they could use long-term cues such as photoperiodic changes to initiate gonad growth prior to the breeding season and additionally exploit short-term stimuli such as food availability to fine-tune the rate of gonadal development to environmental conditions (e.g., Murton and Westwood, '77; Wingfield et al., '92; Ball '93; Cockrem '95).

Recently, we obtained evidence that near-equatorial spotted antbirds (*Hylophylax n. naevioides*) from the rainforest in Panama (9°N) undergo regular seasonal changes in gonad development (Wikelski et al., 2000). Furthermore, experimental results indicated that these birds can perceive very small changes in photoperiod, thus supporting the attractive possibility that they use these to initiate gonad growth (Hau et al., '98). These findings suggested that spotted antbirds are seasonal breeders that use photoperiod as a long-term cue to regulate reproductive activity. If these birds were seasonal breeders similar to many temperate birds, we would predict that they also access short-term cues to precisely adjust reproduction with environmental conditions. Spotted antbirds are insectivorous birds which breed during the rainy season (Willis, '72; Sieving, '92; Hau et al., '98, '99; Wikelski et al., 2000), when insect abundance is high (Wolda, '78, '96; Levings and Windsor, '82; Smythe, '96). In central Panama, the onset of the rainy season can vary by up to 6 weeks between years (e.g., Windsor, '90). Thus, it may be an advantage for spotted antbirds to adjust reproductive readiness to local environmental conditions: birds that grow their gonads too early may suffer a cost, while birds that grow their gonads too slowly may miss the first opportunity to breed after a long dry season. Indeed, our field observations indicated that the rate of gonad growth in these insectivorous rainforest birds is related to the amount of rainfall (Wikelski et al., 2000), which in turn correlates with abundance of insects (Wolda, '78, '96; Levings and Windsor, '82; Smythe, '96).

Hence, the question emerged: can spotted antbirds adjust gonad growth rates to local environmental conditions such as food supply? To the best

of our knowledge, there is no experimental evidence to evaluate such a prediction, although correlations between food abundance and reproductive activity in tropical birds clearly exist (e.g., Boag and Grant, '84; Chakravorty et al., '85; Narasimhacharya et al., '88; Sailaja et al., '88; Chandola-Saklani et al., '90; Young, '94; Komdeur, '96). This question is important in most, if not all, tropical bird species because food abundance also appears to be an important ultimate factor for the timing of reproduction (e.g., Skutch, '50; Ward, '69; Fogden, '72; Snow, '76; Sinclair, '78; Worthington, '82; Boag and Grant, '84; Grant, '86; Poulin, et al., '92; Young, '94; Komdeur '96).

Here we test experimentally whether food can accelerate or inhibit gonad growth rates of captive male spotted antbirds. In our first experiment we improved both food abundance and quality for prebreeding birds that had half-maximal gonad sizes, and measured several parameters indicative of the birds' reproductive state. First, we used luteinizing hormone (LH) as an indicator of the endocrine reproductive system. LH is secreted from the pituitary gland and indicates a stimulation of gonad growth as it usually parallels the secretion of follicle-stimulating hormone (e.g., Follett, '84; Wingfield and Farner, '93). Second, as a more direct measure of reproductive readiness, we monitored gonadal development via unilateral laparotomy (Wingfield and Farner, '76; Hau et al., '98; Wikelski et al., 2000). Third, we observed whether improved food affected behavioral parameters, especially song rate. In an attempt to characterize the mechanism by which food stimulates gonadal development, in a second experiment we separated nutritional from visual food cues. This was done by providing crickets either alive or freshly dead or by only showing crickets to hungry birds. Our results demonstrate for the first time that a tropical bird species can adjust reproductive activity to food availability.

## METHODS

### *Food-stimulation experiment*

#### **Housing and environmental conditions**

Between May 1 and 6, 1997, we caught 14 male and two female spotted antbirds in Soberania National Park (9°N 79°W), Republic of Panama. Birds were housed in individual cages and initially kept together in a room at the Gamboa field station of the Smithsonian Tropical Research Institute (STRI) in Panama. Birds had access to natural daylight and photoperiod. Birds were of-

ferred fresh-made egg-food similar to the recipe of Gwinner et al. ('87), live mealworms (*Tenebrio larvae*), and water ad libitum. In brief, the egg-food contained minced hard-boiled eggs, ground egg shells, raw extra-lean minced beef, cooked yogurt, carrot puree for babies, biscuit crumbs, dried insects ('Bag-o-bugs', West Coast Seed and Supply, Gladstone, OR), and vitamins (Vionate®). Food and water were renewed twice a day; cage floors were cleaned every day. At least every third day a small water bath was put on the cage floor. On May 7, all birds were transferred to another room in Gamboa, where they were separated into two groups with seven males each. Cages were placed into two environmental chambers (Perceival Scientific, Boone, IA) which had their doors propped open during daytime. Photoperiod was kept constant at 12.75 hr by closing the doors of the environmental chambers at set times in the evening and opening them in the morning, in order to prevent any photostimulation by the increasing day length from outside. During daytime, birds had access to the natural daylight, supplemented by artificial light (fluorescent light bulbs, Phillips 40 W, 1 m long, regulated by an automatic timer). Visual contact between birds was prevented by covering the cages with paper on both sides and the back. Birds in each group were matched for date of capture to minimize any effect of time spent in captivity. Apart from that constraint, birds were assigned randomly to the two groups. In each group, one female was placed in the middle of the chamber since previous experiments have indicated that song activity of males was higher when females were present in the groups (own unpublished data).

### Experimental manipulation

On May 9, food availability for one group of birds (further on referred to as control group) was controlled such that the birds maintained their weights. Control birds received egg-food ad libitum, but the amount of mealworms was limited to 10–20 per day (exact amounts were adjusted for needs of individual birds). Birds of the other group (food-stimulated group) also received egg-food ad libitum, mealworms (20–30 per day), and in addition received 5–20 live juvenile crickets (*Acheta domestica*, about 1.5 cm) per day.

### Measurements

Song activity of each group was counted each day for 30 min, in the early morning before birds were fed and cages were cleaned (usually around 6:00

a.m.). For this, either an observer sat behind a shield inside the experimental room and counted number of songs per group simultaneously, or a Dictaphone was placed in front of each chamber, which was analyzed later. The song of spotted antbirds is a characteristic "peety weety" song about 4 sec long. Four notes per second are usually uttered, amounting to a total of 10–15 phrases at an average frequency of about 3 kHz (for sonogram and detailed description of song, see Willis, '72).

Birds were weighed to the nearest 0.2 g in the early morning before being fed. Initially, birds were weighed daily, but to reduce handling stress birds were later on weighed only every other day. Gonad sizes of all males were determined to the nearest 0.1 mm via unilateral laparotomy under Isoflurane (Enflurane, Abbott) anesthesia (see also Hau et al., '98). The first (baseline) measurement was taken between the second and fourth day after capture. At the same time, a small (40 µl) blood sample was taken for LH analysis (see also Wingfield and Farner, 1976). Nine days after the start of the food manipulation in the two groups, another blood sample for LH analysis was taken (on May 19). On May 30, a final laparotomy and blood sampling were done and the experiment was terminated. The person performing the laparotomies was not aware of the group to which a bird belonged.

### Radioimmunoassay

Blood was kept cool until centrifugation. Plasma was separated and frozen at –20°C until analysis. A 3% solution of β-propiolactone was added according to USDA regulations. β-Propiolactone is an antiviral substance that does not interfere with the assay (Wingfield and Lewis, '93). Samples were transported to Seattle on dry ice. LH was measured using the postprecipitation, double-antibody radioimmunoassay for avian LH developed by Follett et al. ('72) and Sharp et al. ('87), with micromodifications. Samples were randomly distributed in two assays. Detection limits ranged between 0.08 and 0.26 ng/ml. Intra-assay variations were 12.5% and 4.8%, and inter-assay variation 23.4%.

### Cricket experiments

#### Song activity with and without crickets

In a previous experiment, we had noticed a conspicuous decrease in song activity on days when no crickets could be given to the birds (see also Hau et al., '98; this happened accidentally during a delayed shipment of crickets to Panama). Note that crickets (*Gryllidae* sp.) are common in the

forest understory in Panama (Levings and Windsor, '82), and spotted antbirds forage on them (Poulin and Lefebvre, '96). Since this effect was so pronounced and immediate in captivity, we experimentally investigated it in more detail.

We temporarily omitted crickets from the diet of different groups of captive birds. These experiments were done at different times of the year (February, March, and November 1996, April and May 1997) and involved different photoperiodic conditions (ranging from 12 to 13 hr of light per day). The number of males per group varied between 3 and 8. All birds previously had access to crickets and were used to them as food. Each group served as its own control and was first deprived of crickets for 2–3 days and then received crickets again.

### *Song activity and nutritional cues*

On May 23, during the previous “food abundance experiment” (see above), we recorded song activity of each individual bird as a baseline for the current experiment, by taking the cage of each bird outside the chamber and putting it opposite to a cage with a female. This robustly induced song and enabled us to assess song activity of individual males. For each male, such a trial was conducted and his songs counted by a hidden observer for 30 min.

The dietary treatment of the two experimental groups from the food abundance experiment was reversed on May 30. Now birds from the former control group received standard food plus live crickets whereas the former food-stimulated group received the standard diet only (which always included live mealworms). On June 5 and 6, individual song activity was recorded as described above. After this crossover treatment of the two groups, birds from both groups received the same food. On June 6, 7, and 8, all birds were given dead (deep-frozen and freshly thawed) crickets, which the birds readily ate. On June 9, we placed a plastic tray sealed with clear plastic wrap containing live crickets on each cage floor. Thus, the birds could see the crickets but had no access to them. On June 10, all birds were fed live crickets again. On the last day of each cricket treatment, song activity of each individual was recorded as described above.

For statistical analysis, data from the two groups were lumped for each “cricket” treatment. This was justified because during this experiment, birds responded similarly to the different cricket treatments regardless of the group they previously

belonged to. Also, body masses of all birds became very similar after the control group was given the diet enriched with crickets (repeated measures ANOVA,  $F_{(1,12)} = 0.00$ ,  $P > 0.9$ ), indicating that physiological states of both group were comparable.

### *Data analysis*

All statistical analyses were conducted using SPSS for Windows (Version 6.0) and SPSS Advanced Statistics (Version 7.5, SPSS Inc., Chicago, IL). We used a general linear model repeated measures (abbreviated as rANOVA in the text) to analyze the data, unless stated otherwise in the text. Where necessary, raw data were ln-transformed.

## RESULTS

### *Food-stimulation experiment*

As a result of the different food treatments, body masses of birds in the two groups diverged (Fig. 1a, comparison of the body mass at day of capture against last day of the experiment, between-subjects effect,  $F_{(1,12)} = 15.7$ ,  $P = 0.002$ ; within-subject effect,  $F_{(1,12)} = 14.8$ ,  $P = 0.002$ ; interaction of treatment and time:  $F_{(1,12)} = 10.7$ ,  $P = 0.007$ ). Control birds maintained their weight, whereas food-stimulated birds increased their body mass by more than 20% (post-hoc paired-sample  $t$  test, control group,  $t = 0.5$ ,  $P > 0.6$ ; food-stimulated group,  $t = 4.3$ ,  $P = 0.005$ ; since we performed the  $t$  test twice on the same data set, we only considered  $P < 0.025$  a significant difference).

Song activity in the morning was initially very low in both groups (around 10 songs/30 min, Fig. 1b). About 1 week after the start of the food manipulation, song activity in the control group decreased and birds did not sing for a couple of days, whereas song activity began to increase in the food-stimulated group. At the end of the experiment food-stimulated birds sang about 30 songs/30 min. (Fig. 1b), which was seven times more than song activity of the control group (about 4 songs/30 min). A conspicuous peak in song activity occurred in both groups on May 27, coinciding with a strong morning rainfall at the end of the dry season (see Fig. 1b).

Initially, birds had half-maximal gonad sizes (for comparison see, e.g., Hau et al. '98, Wikelski et al., 2000). After 3 weeks, gonad sizes between the control and the food-stimulated group were significantly different (Fig. 1c, between-subjects effects,  $F_{(1,12)} = 13.56$ ,  $P = 0.003$ ; within-subjects effect,  $F_{(1,12)} = 0.001$ ,  $P = 0.977$ ; interaction of treatment and time,  $F_{(1,12)} = 14.8$ ,  $P = 0.002$ ). Food-

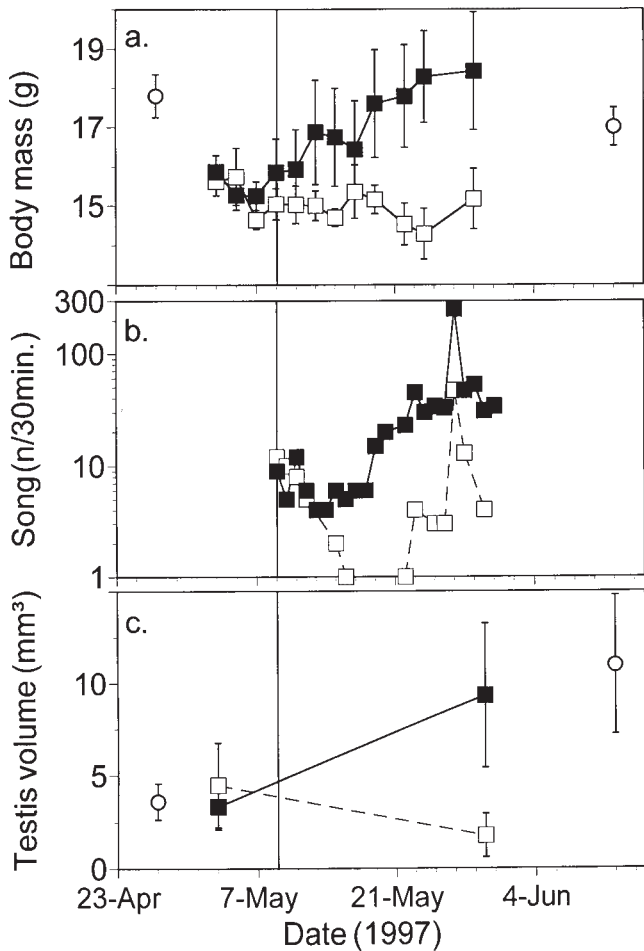


Fig. 1. Changes in (a) body mass (g), (b) group song (number of songs in each group per 30 min in the early morning), and (c) testis volumes ( $\text{mm}^3$ ) of male spotted antbirds in the first experiment. Values are mean  $\pm$  95% CI (except for group song). Open symbols, dashed line: control group. Closed symbols, solid line: food-stimulated group. Solid vertical line: start of food manipulation. Please note the logarithmic scale for group song. Highest song activity occurred on a morning with a strong rainfall, at the end of the dry season. Open circles in top and bottom panel: body masses and gonad sizes of free-living spotted antbirds caught during a parallel field study (data from Wikelski et al., 2000).

stimulated birds significantly increased gonad sizes, while control birds showed no change in gonad sizes (post-hoc paired sample  $t$  test, food-stimulated group,  $t = -3.7$ ,  $P = 0.01$ ; control group,  $t = 2.2$ ,  $P > 0.07$ ; since we performed the  $t$  test twice on the same data set we only considered  $P < 0.025$  a significant difference).

Initial gonad sizes of both groups of captive birds were similar to gonad sizes of free-living males (one-way ANOVA,  $F_{(2,26)} = 0.318$ ,  $P = 0.7$ , see Fig. 1c). At the end of the food-stimulation experiment gonad sizes of captive birds had diverged from those

of free-living birds (one-way ANOVA,  $F_{(2,21)} = 18.18$ ,  $P > 0.001$ ). Food-stimulated birds achieved similar (maximal) gonad sizes as free-living birds (post-hoc Dunnett's T3,  $P > 0.9$ ), whereas control birds retained significantly smaller gonads than free-living conspecifics ( $P = 0.002$ ).

There were no effects of the different food treatments on plasma LH titers. Mean ( $\pm$ SE) LH levels of control and food-stimulated birds were (control/food-stimulated birds, in ng/ml) as follows: on May 3–8, ( $0.6 \pm 0.18$ )/( $0.62 \pm 0.14$ ); on May 19, ( $1.4 \pm 0.45$ )/( $0.91 \pm 0.12$ ); on May 30, ( $1.8 \pm 1.03$ )/( $0.63 \pm 0.12$ ). LH levels of both groups were indistinguishable ( $F_{(1,12)} = 2.48$ ,  $P > 0.1$ ) and did not change during the experiment ( $F_{(2,24)} = 2.26$ ,  $P > 0.1$ ).

To analyze which parameter contributed most to the increase in song activity, we performed a multiple regression analysis, using the songs of the individual birds recorded on May 23 (middle of the food abundance experiment) as dependent variable, and their final gonad sizes and body masses as independent variables (Fig. 2). The linear regression model was significant ( $r^2 = 0.635$ ,  $F_{(2,11)} = 9.58$ ,  $P = 0.004$ ): gonad size contributed significantly to individual song activity ( $P = 0.015$ , see Fig. 2), but not body mass ( $P > 0.5$ ).

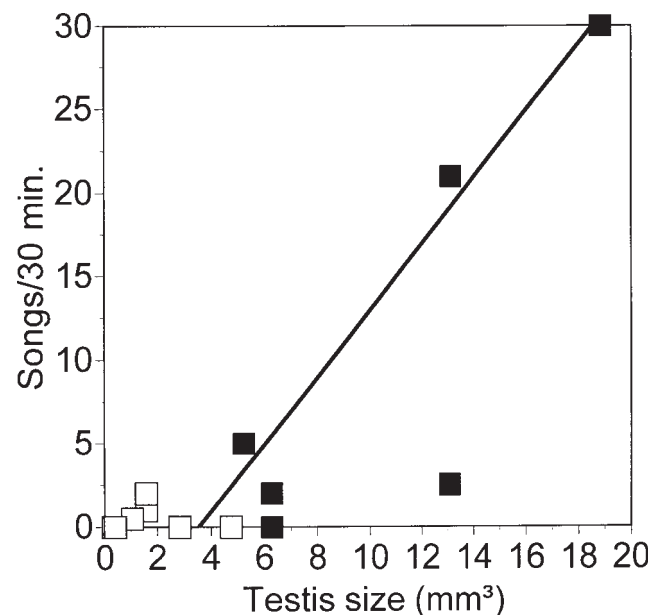


Fig. 2. Correlation between song activity of individual male spotted antbirds (number of songs/30 min) and their final testis size. Song data were obtained on May 23, 1997, during the food abundance experiment. Open symbols: control group. Closed symbols: food group. Both groups were included in the linear regression line. For further explanation and statistics, see text.

### Cricket effects on song

#### Song activity with and without crickets

We divided number of songs per group by the number of males present in each of the six experiments and used these values in further analyses. Spotted antbirds with access to crickets always sang the most songs per 30 min (Fig. 3;  $F_{(1,5)} = 12.55$ ,  $P = 0.017$ ). As a consequence of both time of year when birds were caught and photoperiodic conditions to which they were exposed during the different experiments, male spotted antbirds had different gonad sizes (mean gonad sizes ranging between 0.3 and 18 mm<sup>3</sup>). Mean gonad size influenced the birds' response to cricket availability (gonad size versus the increase in song activity between days with and without cricket, Pearson's correlation coefficient,  $r^2 = 0.74$ ,  $P = 0.027$ , see Fig. 3). However, there was no correlation between the percentage of increase in song due to cricket availability and gonad size (Spearman's  $\rho = -0.029$ ,  $P > 0.9$ , compare with Fig. 3).

#### Song activity and nutritional cues

Individual birds significantly changed song activity during the different cricket treatments

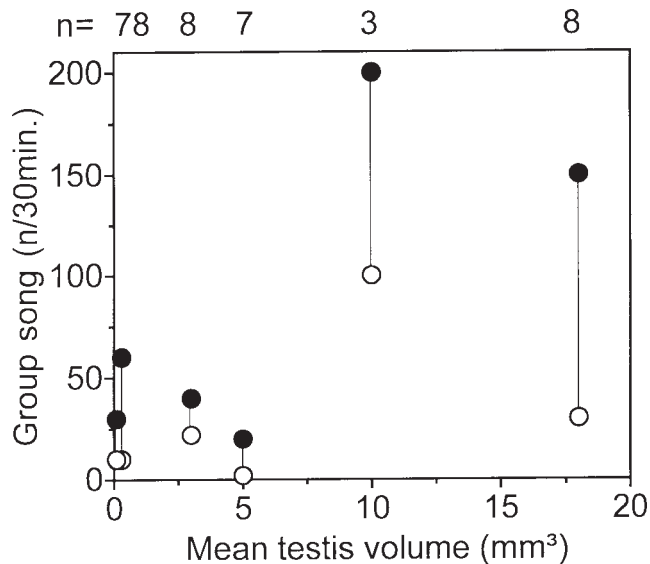


Fig. 3. Effect of cricket availability on songs (per 30 min in the early morning) of groups of spotted antbirds in six different experiments, with different mean testis volumes per group. Open symbols: song activity without crickets. Closed symbols: song activity with crickets. Vertical lines connect repeated measurements of the same group. Numbers above graph indicate number of males in each group. Note that song activity was always higher when live crickets were available, and that general level of song activity was increased at larger gonads.

(Friedman two-way ANOVA on the difference in song activity between subsequent treatments,  $\chi^2 = 12.04$ ,  $n = 14$ , D.F. = 3,  $P = 0.007$ , Fig. 4). We analyzed differences between groups using a post-hoc Wilcoxon matched-pairs signed-ranks test which was corrected for multiple comparisons ( $n = 4$ ) with the Bonferroni method. Significant differences are indicated in Fig. 4. Birds sang most when they had access to live crickets (22 songs/30 min), and showed low song activity when they only received mealworms. Birds retained a very low level of song activity when dead crickets were provided (note that the birds ate as many dead crickets as they ate live crickets). When live crickets were provided in a dish sealed with a clear wrap, birds sang strongly during the first 15 min of observation, but then song activity decreased. Over the 30 min observation time, this resulted in a significant increase in song activity over that of birds receiving dead crickets. Song activity increased again significantly (and to levels statistically indistinguishable from the first live cricket treatment) when the birds had access to live crickets again.

### DISCUSSION

These experiments show that food stimuli enhance reproductive readiness in male spotted antbirds. Birds with access to a rich food source significantly increased gonad sizes over controls

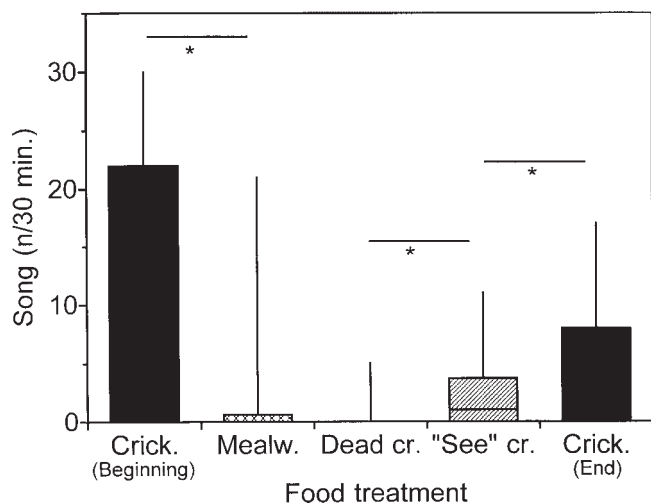


Fig. 4. Effect of cricket availability on song activity (number of songs/30 min) of individual male spotted antbirds ( $n = 14$ ). Bars represent median  $\pm$  quartiles. Horizontal bars with an asterisk indicate that two neighboring bars differ by  $P < 0.0125$  (post-hoc tests, see text). Please note that the live cricket treatments in the beginning and the end of the experiment were not statistically different.

(Fig. 1c). Food-stimulated birds also tripled song activity (Fig. 1b). Hence, food cues may serve as a proximate factor for the timing of reproduction in spotted antbirds, presumably by providing information about the suitability of environmental conditions for breeding.

A role of food in the regulation of gonadal development of spotted antbirds was also suggested by our field data. Gonadal development of free-living males was significantly enhanced in the wet spring of 1996 as compared to the dry spring of 1997 (Hau et al., '99; Wikelski et al., 2000). The climatic difference between the two years was reflected in the abundance of leaf litter arthropods at our study area (T.R. Robinson and J.J. Nesbitt, unpublished data). Our present experiment thus appropriately simulated variability in insect abundance that occurs naturally at the beginning of rainy seasons in Panama (Wolda, '78, '96; Levings and Windsor, '82; Smythe, '96): While our control birds maintained gonads in a half-developed state, apparently awaiting better environmental conditions, food-stimulated birds completed maximal gonadal development.

It is unlikely that body mass influenced gonad development directly, or perhaps there exists only a loose relationship between gonad size and body mass in male spotted antbirds. In late April 1997, free-living birds from a comparatively wet habitat had larger body masses (Fig. 1a) but similar gonad sizes as our experimental birds (Fig. 1c), which were caught in a more dry habitat. The observation that relatively lean birds from a dry habitat were able to develop and sustain similar gonad sizes as conspecifics from a more humid area indicates that during our experiment, keeping control birds at their weight specific to their habitat did not impose a physiological stress.

After observing the stimulatory effect of food, we expected that increased gonad growth would have been accompanied by increased LH levels. However, we could not detect changes in plasma LH levels in the present experiment. These results parallel those from a previous study in which photostimulation failed to increase plasma LH in spotted antbirds (Hau et al., '98). Two explanations for this negative result are conceivable. First, LH may have increased rapidly in response to food stimuli, but titers had already decreased again at the time the blood samples were taken (nine days after the change in food conditions). A second possibility is that LH secretion is not regulated according to food cues. This hypothesis is supported by studies failing to find an effect of food restric-

tion on LH levels in two temperate bird species (*Zonotrichia leucophrys*, Wingfield, '80; and *Falco tinnunculus*, Meijer and Schwabl, '89).

Food also exerted immediate effects on behavior since song rate rapidly increased after food improvement. Food cues stimulated song activity of male spotted antbirds in two ways: (1) song rate increased over weeks along with increased gonad size, and (2) song rate increased in the short-term, i.e., separate from changes in gonad size. The first effect (song increase via gonad increase) appeared to be independent of body mass (see Fig. 2 and multiple regression analysis). This could suggest that gonad development directly affects song rate, maybe through the secretion of testosterone (T) from the testes. Experimental manipulation of T has been shown to increase song activity in male spotted antbirds (Hau et al., 2000). The second short-term behavioral effect of food was implied by the immediate increase in song activity when birds were given live crickets (Figs. 3 and 4). The latter response seemed to be gonad-independent since birds increased song activity in response to cricket availability to a similar percentage, irrespective of their gonad sizes (Fig. 3).

Birds only showed a full song response when they could see, handle, and eat crickets. When freshly killed crickets were offered, birds readily ate them but showed no song response. This result contradicts pure nutritional effects as a cause for increased song rate. However, we cannot exclude that some nutritional components in the crickets were broken down during the freezing and thawing process, although we consider this possibility unlikely. The presentation of live crickets under a clear plastic wrap, a purely visual signal, resulted in an intermediary song response. A functional interpretation of a stimulation by moving insects may be that spotted antbirds use live insects either as indicators for increased prey abundance or for the occurrence an army ant swarm (*Formicidae*). The latter would be an indirect indicator for prey availability since spotted antbirds frequently accompany raiding army ants, where they forage on insects that are flushed up by the ants (Willis, '72; own observations). The lack of a full song response to seeing live crickets—relative to the birds' response to eating live crickets—may be explained in two ways: (1) Birds may have been stimulated by the cricket movements, but for a full stimulation of song activity birds would also need to handle the crickets. (2) A full response was not elicited by the visual stimulus alone because the birds were "frustrated" by not being able

to get at the crickets, and thus song activity decreased precociously (perhaps through an increase in adrenocortical hormones, e.g., Romero et al., '95). To distinguish between these possibilities, it will be important to experimentally investigate the physiological and behavioral responses of spotted antbirds to different kinds of live prey, e.g., moving edible versus nonedible insects, and different visual presentations of live insects.

What is the mechanism behind food stimulation of reproductive activity? We hypothesize that the fast behavioral reaction in song and the slow physiological effect on the gonads may be based on a common, two-step process. The critical stimuli responsible for initiating this process could arise from the food in two ways: (1) Seeing and/or handling crickets provided a non-nutritional stimulus. It is important to note here that mealworms, which were part of the standard diet, usually hid under the dry food and thus were often not visible to the birds. In contrast, crickets were always active and conspicuous. It is so far unclear, if the birds' fast response in song activity to live crickets has been amplified by learning that the crickets are good food. (2) To grow gonads to maximal sizes, birds assessed internal resources. Body fat and especially protein stores have been proposed to be the major factor regulating reproduction in other tropical birds (Ward, '69; Jones and Ward, '76; Fogden and Fogden, '79; but see Meijer and Drent, '99). The concurrent increase in body mass and gonad size of birds in the present experiment (see Fig. 1a) might support such a mechanism. However, it is probable that spotted antbirds are "income breeders" like most other passerines, which do not need to rely on stored energy reserves for breeding (Meijer and Drent, '99). This notion, together with the hypothesized loose connection between body mass and gonad size (see above) could suggest that the increase in body mass of food-stimulated birds is not causally involved in the effect of food abundance on gonad growth. These different mechanisms are entirely testable and will have important implications for the sensory and integrative pathways through which food cues are perceived and processed by the birds.

The current experiment was restricted to males but future experiments should aim at identifying environmental factors that female spotted antbirds use to regulate reproduction. In the field, female spotted antbirds delayed ovarian development during the severe dry season in 1997 to a similar extent as males (Wikelski et al., 2000) sug-

gesting that their reproductive timing mechanism is sensitive to food cues as well. Moreover, male song has been shown to affect the reproductive activity of females in many temperate birds (e.g., Brockway, '65; Lehrman and Friedman, '69; Hinde and Steel, '76; Morton et al., '85). Thus the increased song activity of males could be an additional proximate stimulus for females.

To our knowledge, the present experiment is the first experimental test of food as a regulator of reproductive activity in tropical birds. Similar findings have been obtained on a number of mammalian species (see e.g., Bronson, '87; Wingfield and Kenagy, '91). How do the present results compare to the data available for temperate birds?

Improved food quality stimulated gonad growth in tricolored blackbirds (*Agelaius tricolor*; Payne, 1969), red crossbills (*Loxia curvirostra*; Tordoff and Dawson, '65; Hahn, '95), piñon jays (*Gymnorhinus cyanocephalus*; Ligon, '74, '78), and California quails (*Lophortyx californicus*; Leopold et al., '76). Interestingly, all these species, except for California quail, have been classified as flexible seasonal or opportunistic breeders (Hahn et al., '97). Both flexible seasonal and opportunistic breeders are expected to be more responsive to short-term environmental information than strictly seasonal breeders (Wingfield et al., '92, '93; Hahn et al., '97). Food supplementation also affected laying date in many temperate birds (see reviews by, e.g., Daan et al., '88; Boutin, '90; Svensson, '95; Meijer and Drent, '99). However, most of these studies were conducted when birds already had large gonads, i.e., not during the initial phase of gonad growth like in our experiment. Food reduction, on the other hand, resulted in a slight delay of gonadal development in male European starlings (*Sturnus vulgaris*) but not in females (Meijer, '91). This lack of a strong gonadal effect of food reduction agrees with other studies from captive European starlings (Dawson, '85), bramblings (*Fringilla montifringilla*; Lofts et al., '63), and moorhens (*Gallinula chloropus*; Huxley, '76). Hence, it is still open to discussion whether tropical (especially near-equatorial) birds are indeed more responsive to short-term proximate factors than birds breeding in higher latitudes (e.g., Immelmann, '71, '73; Cockrem, '95). Clearly, more data on tropical birds are needed to make such a comparison informative for further hypotheses.

A likely scenario for the timing of reproduction in spotted antbirds is that early in the year photoperiodic cues (or endogenous factors; Gwinner, '86) initiate gonadal development (Hau et al., '98,

'99). Food stimuli are then important in regulating the rate of gonadal development and determining the actual onset of breeding, as in many temperate birds (e.g., Murton and Westwood, '77; Wingfield, '80; Ball, '93; Wingfield and Farner, '93). A similar situation may apply to other tropical species as well (see, e.g., Dittami and Gwinner, '85; Narasimhacharya et al., '88; Chandola-Saklani et al., '90). However, it is still unclear if food cues can override photoperiodic signals in spotted antbirds, i.e., if birds can respond to food cues also in the absence of photoperiodic stimulation (see also Hahn et al., '97; Hahn, '98; Hau et al., '99). Further experiments will be aimed at determining the hierarchy in which spotted antbirds respond to environmental cues.

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