

The effect of duration and time of food availability on the photoperiodic response in the male house sparrow, *Passer domesticus*

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Abstract – We investigated the effects of food availability on the seasonal testicular growth in the photoperiodic house sparrow (*Passer domesticus*). Two experiments were performed, each lasting 4 weeks. In experiment 1, sparrows were exposed to natural (NDL; group 1), short (8L:16D; group 2) and long (16L:8D; groups 3–5) day lengths with access to food ad libitum (groups 1–3) or for 10 h (zeitgeber time (zt) 0–10, group 4; zt 0 is the time of light onset) or for 8 h (zt 8–16, group 5). Testes recrudesced under long, but not short and natural, day lengths, and the recrudescence under long days was influenced by the duration of food availability. In experiment 2, the sparrows were exposed to short (8L:16D, group 1) and long (16L:8D, groups 2–5) day lengths with access to food ad libitum (for groups 1 and 2) or for 6 h (for groups 3–5) at different times during the 16 h light period (group 3- zt 0–6, group 4- zt 5–11, group 5- zt 10–16). As the expected, the testes recrudesced only under long lengths, but the photoinduction was variable among the 4 groups. The testes grew to full size in groups 2 and 3 that received food either ad libitum or for 6 h at zt 0–6, but to sub-maximal size in the groups that received 6 h food either at zt 5–11 (group 4) or at zt 10–16 (group 5). Altogether, these results support the idea that the photoperiodic regulation of reproduction in a seasonally breeding species is influenced both by the duration and the time of food availability.

sparrow / photoperiod / timing / food / reproduction

1. INTRODUCTION

Day length regulates reproduction in many birds [1–4]. In the majority of bird species, which have been investigated, long day lengths are photostimulatory, short day lengths are ineffective, and a period of post-reproductive refractoriness develops in birds that were photostimulated for a longer period of time [3, 5–8]. An endogenous photoperiodic clock, which is circadian in nature, mediates the inductive effects of day length [3, 4, 9].

The clock appears to have a non-photoinducible phase, beginning at the time of light onset, and a photoinducible phase, beginning approximately after 11–12 h of the day in most species (see [10]). Light at the beginning of the day entrains the photoperiodic clock, and when the light period is long enough to extend into the photoinducible phase, photoperiodic induction occurs [1, 4, 11]. The results from several species of birds are consistent with this model of photoperiodic induction called the external coincidence model [6, 12–15].

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The photoperiodic clock is also phasically sensitive to the intensity and spectrum of light of the environment (for review see [16]).

However, additional factors, called the supplementary factors (e.g. rain fall, food availability, temperature cycles), of the environment could also be critical in the fine-tuning of the actual onset of breeding [17]. The role of these supplementary factors has not been adequately addressed although strong indications emanate from some studies that food can be an important supplementary factor. In fact, in recent years, the role of food in the regulation of the circadian activity rhythms has been clearly recognized [2]. The fact that relatively little attention has been paid to the idea that food may be important in photoperiodic regulation of seasonal reproduction could perhaps have stemmed from a few early studies that were negative. For example, Dawson [18] found no effect of food restriction on the photoinduction of testicular development in the European starling (*Sturnus vulgaris*). But, Hahn [19] showed that a close integration between light and food cues was critical in the timing of the testicular cycle of the red-cross bill, *Loxia curvirosta*. A very clear effect of food availability has now been shown in a recent study on the migratory blackheaded bunting (*Emberiza melanocephala*). Kumar et al. [20] showed that both the duration and the time of food availability modulated the photoperiodic induction of body fattening and subsequent weight gain, and testicular growth in the migratory blackheaded buntings. Because the blackheaded bunting is a migratory species, with breeding grounds located at $\sim 40^\circ$ N [21], a study on resident species will be important in determining whether the role of food availability in the photoperiodic induction can be generalized. Therefore, we investigated the role of food availability in the photoperiodic induction of testicular growth of house sparrows (*Passer domesticus*) for the following reasons. Firstly, this species is cosmopolitan in distribution, and the role of the photoperiod

in the regulation of testis growth is known for the temperate population of sparrows [22, 23]. Secondly, food cycles (periodic access to food) can synchronize the house sparrow's circadian activity rhythms [24] suggesting a role of food as a timing cue. In this study, we specifically determined whether a reduction in the duration and/or change in the timing of food supply will influence the photoperiodic induction of testis growth of house sparrows subjected to stimulatory long day lengths.

2. MATERIALS AND METHODS

Adult male house sparrows (*Passer domesticus*) were procured locally ($\sim 29^\circ$ N) in November 2002. They were then acclimatized to laboratory conditions for about 2 weeks. The experiments began on the 1st of December 2002.

2.1. Experiment 1

This experiment examined the effects of the *duration* of food supply on testicular growth using 5 groups (groups 1–5; $n = 8$) of birds. Group 1 was kept under natural day lengths (NDL) at 29° N, group 2 was exposed to a short day length (8 h of light: 16 h of darkness, 8L:16D), and the remaining 3 groups (groups 3–5) were exposed to a long day length (16L:8D). Whereas the birds of groups 1–3 were fed ad libitum (these birds had unrestricted access to food all 24 h, 24 h), the birds of group 4 were fed for 10 h during light period (zt 0–10; zeitgeber times 0 refers to the time of the beginning of the lights-on period), and of group 5 for only 8 h. The duration of food availability of group 5 was equal to that of birds under short day lengths (8L:16D since sparrows feed primarily during light hours – our unpublished observations), but the timing of food availability was different; it was available in the second half of the 16 h light period (zt 8–16) so that the beginning of access to the food and light

periods did not coincide so that we could discern the relative effects of two zeitgebers (food and LD cycles).

2.2. Experiment 2

This experiment determined the effects of the *timing* of food availability on the photoperiodic induction of testicular growth. Again, we had 5 groups (groups 1–5; $n = 8$) of birds in the experiment. Group 1 was exposed to 8L:16D, and the remaining 4 groups (groups 2–5) were exposed to 16L:8D. The birds in groups 1 and 2 were fed ad libitum. The birds in the groups 3–5 were fed for only 6 h, but at different times. Group 3 had food from zt 0–6, group 4 from zt 5–11, and group 5 from zt 10–16.

The birds caged in groups of 4 wire-mesh cages (size – 45 × 30 × 30 cm) were placed in the photoperiodic box (size – 75 × 70 × 60 cm); two cages were thus in one box. The birds under NDL were also housed similarly and kept in a room that received unrestricted environmental light and air from large windows. Food was a mixture of seeds of *Pennisetum* and *Setaria*. Sparrows survive well and remain healthy for a long period of time on this food (our unpublished observations). Water was available all the time to all groups. Artificial light was provided by 14 watt fluorescent tubes (CFL) at an intensity of ~ 500 lux at the perch level, and the timing of light and dark was regulated by an automatic Mueller electronic timer.

The observations of body mass and testicular size were recorded at the beginning and at the end of the 4-week experiment. Body mass was recorded using a top pan balance to an accuracy of 0.1 g. The testicular growth was recorded by laparotomy performed under local anaesthesia as per procedure described by Kumar et al. [20]. Briefly, a small incision was made between the last two ribs on the left flank, and the left testis was located within the abdominal cavity with the help of a spatula. The dimensions of the left testis were

recorded and testis volume was calculated from $\frac{4}{3} \pi ab^2$, where a and b denote half of the long and short axis, respectively.

Data are presented as mean ± S.E.M. We have excluded the data on the body mass from the results and figures since there was no significant effect on body mass in any of the treatments. Mean values from the same group obtained before and after the treatment were compared by the student paired *t*-test. We used one way analysis of variance (ANOVA) when we compared the effects of different groups. The data from a few birds that died in one of the groups (see figure legends) during the course of the study were completely excluded.

3. RESULTS

3.1. Experiment 1. The effects of the duration of food availability

Figure 1 shows the results. The testes were enlarged in all groups exposed to long days, but not in those exposed to short days and NDL. However, the magnitude of testis growth differed among different groups under long days, and this difference clearly reflected on the duration of food availability. The birds that were fed ad libitum (group 3) had fully enlarged testes, but the birds that were fed for only 10 (group 4) and 8 (group 5) h had significantly smaller testes ($F = 11.32$, $P = 0.0006$; One-way ANOVA). The testes of the birds in group 4 were, however, larger than those of the birds in group 5 (cf. Fig 1).

3.2. Experiment 2. The effects of the time of food availability

As expected, the testes were photostimulated in all three groups under long day length, but remained regressed in birds under short day length (Fig. 2). However, the magnitude of testis response was different among different photostimulated groups ($F = 6.615$, $P = 0.0028$; one way

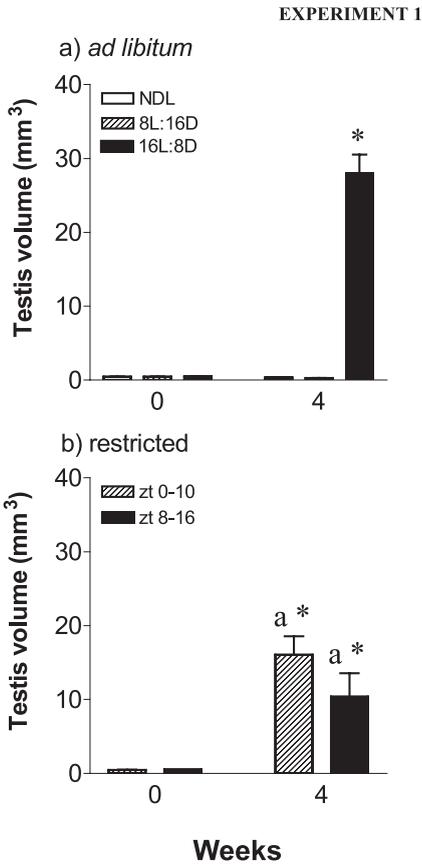


Figure 1: Results from experiment 1 (the values are means \pm S.E.M.). Groups of house sparrows ($n = 8$ per group) were exposed to ND (group 1), short day lengths (8L:16D, group 2) and long day lengths (16L:8D, groups 3–5) for 4 weeks and received food ad libitum (groups 1–3) or for 10 h (zeitgeber time (zt) 0–10, group 4; zt 0 is light onset) or 8 h (zt 10–16, group 5). * Indicates a significant difference ($P < 0.05$) from day 0 values (student t -test); ^a indicates a significant difference compared to the long day ad libitum group at the end of the experiment. Three birds of group 4 died, and data from them is excluded.

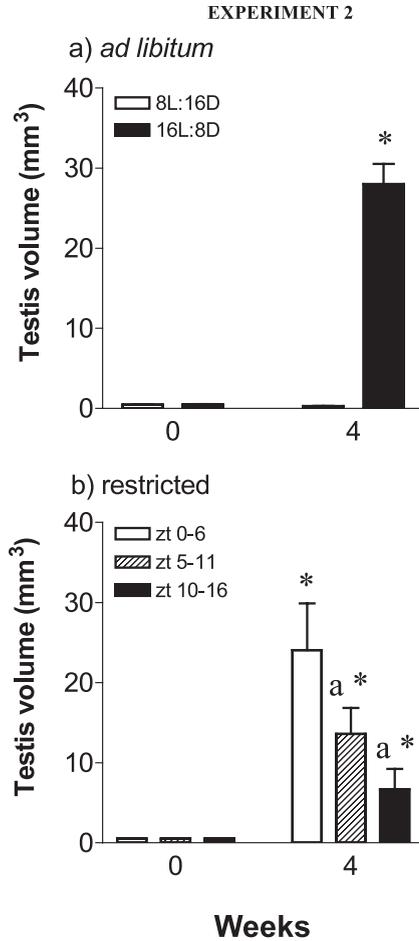


Figure 2: Results from experiment 2 (the values are means \pm S.E.M.). Groups of house sparrows ($n = 8$ per group) were exposed to short day lengths (8L:16D, group 1) and long day lengths (16L:8D, groups 2–5) for 4 weeks and received food ad libitum (groups 1 and 2) or for 6 h (groups 3–5; group 3- zt 0–6, group 4- zt 6–11, group 5- zt 10–16). * Indicates a significant difference ($P < 0.05$) from day 0 values (student t -test); ^a indicates a significant difference compared to the long day ad libitum group at the end of the experiment.

ANOVA). The testes grew to full size in birds (group 2) that received food ad libitum. Although the testes were slightly smaller in group 3 birds, which had 6 h food in the morning, the mean testis vol-

ume was not statistically different from that of the birds in group 2. But, testis recrudescence was compromised to almost 50% in the birds (group 4) that received

food in the middle of the day (zt 5–11), and to almost 75% in birds that received food at the end of the day (zt 10–16).

4. DISCUSSION

Clearly, photoperiodic induction of testis growth in house sparrows at this latitude is influenced by food availability. Both the duration and the time of food availability has a direct effect on the magnitude of testis growth. For example, testis growth was attenuated, compared to those birds that received food *ad libitum*, by about 40% in birds receiving food for 10 h and about 70% in birds receiving food for 8 h (experiment 1, Fig. 1). This suggests that a shorter duration of food availability (e.g. of 8–10 h) is insufficient to maintain the costs of physiological events like gametogenesis induced by long day lengths. However, the results from experiment 2 do not entirely support this argument, in which birds with food access for 6 h in the morning had testis recrudescence not significantly different than birds that were given food *ad libitum*. But, if the time of food was varied from the morning to noon (6 h in the middle of the day, zt 5–11) or to the evening (6 h late in the day, zt 10–16), testis growth was attenuated by almost 50 to 75%.

Our results do not suggest the possible mechanism of how does the reduction in food supply influence the photoperiodic induction of testis growth in the house sparrow. However, we can offer some explanations. The simplest explanation is that food deprivation reduces the energy required to meet the physiological costs. Less energy is available to meet the reproductive costs in birds with food-deprivation periods; the longer the period of pre-feeding starvation, the greater is the magnitude of the effect. This is supported by the results of both experiments. In experiment 1, the sparrows with 8 h pre-feeding and no post-feeding deprivation compromised their testicular growth by almost 50%. Similarly, in exper-

iment 2, the sparrows of group 5 with the longest pre-feeding (10 h) and no post-feeding food-deprivation period had attenuated their testicular growth by almost 75%. Group 4 sparrows with a 5 h pre-feeding and post-feeding food-deprivation period showed only a small reduction in testicular growth. Group 3 sparrows, however, with no pre-feeding and the longest post-feeding (10 h) food-deprivation had larger testes. The other explanation can be offered on the basis of results from experiment 2, in which a 6 h food supply induced time-dependent effects. The presence of food at the beginning of the day is clearly favourable for a full testicular response. The time-of-day effects of food on testis growth may suggest that food influences endogenous circadian clock regulating reproduction, as with the circadian locomotor clock of temperate house sparrows [24]. However, these results might also suggest that the effect of food on the photoperiodic clock is not the same as that of light. Testicular growth in sparrows was compromised when food was absent at the beginning of the day. So, the presence of food at the beginning of the day appears to be critical. This was also concluded from the experiments on blackheaded buntings. Kumar et al. [20] have categorically demonstrated the effects of duration and time of food availability on the photoinduction of body fattening and subsequent gain in body mass and testis recrudescence in buntings, and the effects were dependent whether or not food was present at the beginning of the day. The importance of food at the beginning of the day can also explain why European starlings did not show any effect of food restriction [18], since food in all food-restriction regimes was available in the morning. It is puzzling, however, that one of our results did not conform to any of the above explanations. We found that in experiment 1, birds of group 4 which were given food for 10 h from zt 0–10, and had access to food at the beginning of the day, in fact had smaller testes (Fig. 1), but still they were not significantly different from

those receiving the other equivalent food-restriction regimes in experiment 2 (the one which had 6 h food in the morning). The only explanation for this inconsistent response that we have is that 3 of the 8 birds died during the experiment, and so the mean testis volume of this group was for 5 birds against 8 birds in the other groups.

A close integration between light and food cues in the timing of gonads appears to be adaptive, as suggested from studies on the regulation of the annual testicular cycle of an opportunistic breeder, the red-cross bill *Loxia curvirosta* [19]. It is conceivable that there exists an endogenous circadian rhythm of feeding (CRFE), similar to that of the circadian rhythm of photosensitivity (CRPP). CRPP is assumed to mediate photoperiodic induction of gonadal growth in a number of passerine birds [2, 5, 7–9, 25]. In the synchronized state, the onsets of CRFE and CRPP coincide and, hence, feeding occurs at the time when it is most needed to support the physiological events. In ad libitum food supply conditions, the synchrony between light and food cues is never lost. But, in situations such as in experiment 2, the synchrony between the light and food cues is lost and, as a result, the initiation of physiological responses is affected. In the present study as well as those of Kumar et al. [20] on buntings, the photoinduction was compromised when food was present in the evening for 5 or 6 h, although the availability of food later at this time of day did coincide with the photoinducible phase of the photoperiodic clock (see introduction). Could the phase-sensitivity of the photoperiodic clock for food be different than for light? This needs to be investigated.

In conclusion, the present findings support the idea that the photoperiodism in a seasonally breeding species is affected by food availability. Food can have both, direct and temporal (clock) effects. Direct effects are caused because of the non-availability of extra energy from restricted feeding to meet the physiological costs. Temporal effects are induced because of

the asynchrony between light and food cues. A close phase relationship between light and food cues in timing the photoperiod-induced physiological events may clearly have adaptive implications.

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REFERENCES

- [1] Follett BK. Circadian rhythms and photoperiodic time measurement in birds. *J Reprod Fertil Suppl* 1973, 19: 5–18.
- [2] Gwinner E, Hau M. The pineal gland, circadian rhythms and photoperiodism. In: Sturkie PD (Ed), *Avian Physiology*, 5th Ed, Academic Press, New York, 2000, p 557–568.
- [3] Kumar BS, Kumar V. Seasonal reproduction in subtropical brahminy myna, *Sturnus pagodarum*: Role of photoperiod. *Gen Comp Endocrinol* 1991, 83: 354–365.
- [4] Kumar V, Follett BK. The nature of photoperiodic clock in vertebrates. In: Haldane JBS, *Commemoration Volume*, Proc Zool Soc Calcutta, 1993, p 217–227.
- [5] Follett BK. Birds. In: Lamming GE (Ed), *Marshall's physiology of reproduction*, Churchill Livingstone, Edinburgh, 1984, p 283–290.
- [6] Kumar BS, Kumar V. Photoperiodic control of annual reproductive cycle in subtropical brahminy myna, *Sturnus pagodarum*. *Gen Comp Endocrinol* 1993, 89: 149–160.
- [7] Kumar V. Photoperiodism in higher vertebrates: An adaptive strategy in temporal environment. *Indian J Exp Biol* 1997, 35: 427–437.
- [8] Nicholls TJ, Goldsmith AR, Dawson A. Photorefractoriness in birds and comparison with mammals. *Physiol Rev* 1988, 68: 133–176.
- [9] Dawson A, King VM, Bentley GE, Ball GF. Photoperiodic control of seasonality in birds. *J Biol Rhythms* 2001, 16: 365–380.
- [10] Pittendrigh CS, Minis DH. The entrainment of circadian oscillations by light and their

- role as photoperiodic clocks. *Am Nat* 1964, 98: 261–294.
- [11] Pittendrigh CS. Circadian surfaces and the diversity of possible roles of circadian organization in photoperiodic induction. *Proc Natl Acad Sci USA* 1972, 69: 2734–2737.
- [12] Follett BK, Sharp PJ. Circadian rhythmicity in photoperiodically induced gonadotropin release and gonadal growth in the quail. *Nature* 1969, 223: 968–971.
- [13] Hamner WM. Circadian control of photoperiodism in the house finch demonstrated by interrupted-night experiments. *Nature* 1964, 203: 1400–1401.
- [14] Kumar V, Rani S. Effects of wave length and intensity of light in initiation of body fattening and gonadal growth in a migratory bunting under complete and skeleton photoperiods. *Physiol Behav* 1996, 60: 625–631.
- [15] Rani S, Kumar V. Time course of sensitivity of the photoinducible phase to light in the redheaded bunting, *Emberiza bruniceps*. *Biol Rhythm Res* 1999, 69: 555–562.
- [16] Rani S, Singh S, Kumar V. Light sensitivity of the biological clock. In: Kumar V (Ed), *Biological rhythms*, Narosa Publishing House, New Delhi, 2002, p 232–243.
- [17] Wingfield JC, Farner DS. The reproductive endocrinology of wild species. In: Farner DS, King JR, Parkes KC (Eds), *Avian Biology*, Vol IX, Academic Press, New York, 1993, p 163–327.
- [18] Dawson A. The effect of restricting the daily period of food availability on testicular growth of starlings *Sturnus vulgaris*. *Ibis* 1986, 128: 572–575.
- [19] Hahn TP. Integration of photoperiodic and food cues to time changes in reproductive physiology by an opportunistic breeder, the red cross bill, *Loxia curvirosta* (Aves: Carduelinae). *J Exp Zool* 1995, 272: 213–226.
- [20] Kumar V, Singh S, Misra M, Malik S. Effects of duration and time of food availability on photoperiodic responses in the migratory male blackheaded bunting (*Emberiza melanocephala*). *J Exp Biol* 2001, 204: 2843–2848.
- [21] Ali S, Ripley SD. *Handbook of Birds of India and Pakistan*. Second edition, Vol 10, Oxford University Press, Bombay, London, New York, 1974.
- [22] Binkley S. *The Clockwork Sparrow: Time, Clocks, and Calendars in Biological Organizations*, Englewood Cliffs, NJ: Prentice Hall, 1991.
- [23] Murton RK, Westwood NJ. *Avian Breeding Cycles*. Clarendon Press, Oxford, 1977.
- [24] Hau M, Gwinner E. Food as a circadian zeitgeber for house sparrows: the effect of food access times. *J Biol Rhythms* 1996, 11: 196–207.
- [25] Kumar V, Kumar BS. Entrainment of circadian system under variable photoperiod (T-Photoperiod) alters the critical daylength for photoperiodic induction in blackheaded buntings. *J Exp Zool* 1995, 273: 297–302.