

## Food deprivation during photosensitive and photorefractory life-history stages affects the reproductive cycle in the migratory Red-headed Bunting (*Emberiza bruniceps*)

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### SUMMARY

In a seasonally breeding bird species, food deprivation affects reproduction. A key question is whether food shortage at any time in the year will affect reproduction even though the food supply subsequently becomes adequate. A prediction would be that a food supply that is of shorter duration than that optimally required during a life-history stage will have consequential effects on the succeeding life-history stages. Two experiments investigated this in gonadally regressed migratory Red-headed Buntings (*Emberiza bruniceps*) at two life-history stages: photosensitive and photorefractory. Experiment 1 employed two groups of photosensitive birds, and experiment 2 employed two groups of photorefractory birds. In both the experiments, birds were exposed for 8 weeks to a neutral day length at dim light intensity (12 h light: 12 h darkness, 12L:12D; L $\approx$ 5 lux, D=0 lux) with restricted feeding regimes (6 h food present: 18 h food absent, P:A 6:18 or P:A 12:12) and subsequently maintained for another 13 weeks at a highly stimulatory day length (16L:8D; L=400 lux; D=0 lux) with food *ad libitum*. We report that the pretreatment with restricted food cycles influenced the subsequent photoperiodic induction of reproductive (testis growth and molt) but not of metabolic (body fattening and mass gain) functions. The testicular response cycle under 16L:8D had a significantly lower amplitude in birds pretreated with P:A 6:18 than with P:A 12:12. Similarly, the recovery of photosensitivity was slower in photorefractory birds pretreated with P:A 6:18 than with P:A 12:12 food cycles. Overall, our findings show for the first time in a seasonally breeding vertebrate species that food deprivation during non-breeding periods of the annual cycle can affect reproductive functions later in the year.

Key words: feeding, molt, Red-headed Bunting, refractoriness, restricted feeding, reproduction.

### INTRODUCTION

Day length regulates seasonal responses in many vertebrates, including several songbird species. In a long-day-breeding migratory songbird, the increasing day lengths of spring and summer induce events associated with the spring migration and reproduction and time the post-reproductive regression and photorefractoriness (Jain and Kumar, 1995; Kumar, 1997; Dawson et al., 2001; Deviche and Small, 2001; Dawson and Sharp, 2007; Hahn and MacDougall-Shackleton, 2008). The cycle of growth–regression–refractoriness can be reproduced under laboratory conditions by exposing birds to long days. In several photoperiodic species that exhibit absolute photorefractoriness, the refractory individuals show reinitiating of photoperiodic responses under long days only after they have been pre-exposed for at least eight weeks to short days (e.g. 8 h light: 16 h darkness, 8L:16D) (Farner et al., 1983; Kumar and Tewary, 1984; Nicholls et al., 1988).

The daily light period is not the only environmental signal with a regulatory influence on seasonal cycles. Food availability also has significant effects in several species. Food cycles (periodic access to food) synchronize endogenous circadian clocks and affect photoperiodic induction of the seasonal response in several species [see Kumar et al. (Kumar et al., 2001) and references therein]. Food availability at stopover sites and fat reserves can affect the time program in a migratory species, as revealed by studies on a trans-Saharan migrant, the Spotted Flycatcher (*Muscicapa striata*) (Biebach, 1985). An experimental study of Gwinner and colleagues

(Gwinner et al., 1985) on migratory Garden Warblers (*Sylvia borin*) that employed a no-feeding and feeding regimen, simulating a poor feeding condition and an oasis with ample food resources, respectively, and measured the effects on body mass and migratory restlessness (zugunruhe) also suggests that food can be an important factor in timing the seasonal migration. Food availability has also been shown to influence the timing of reproduction in neotropical rainforest Spotted Antbirds (*Hylophylax naevioides*) (Hau et al., 2000) and African Stonechats (*Saxicola torquata axillaries*) (Scheuerlein and Gwinner, 2002). Both the duration and timing of the food availability can affect the photoperiodic induction of body fattening and testicular growth in the Black-headed Bunting (*Emberiza melanocephala*) (Kumar et al., 2001) and House Sparrow (*Passer domesticus*) (Bhardwaj and Anushi, 2004). A very recent study shows that food as a cue can have dominance over light in regulation of testicular growth in Zebra Finches (*Taeniopygia guttata guttata*) (Perfito et al., 2008).

Most studies have assessed the effects of restricted food availability on gonadal growth and maturation. A key question that has not been answered yet is whether food shortage or starvation during a life-history stage will have consequential effects on succeeding life-history stages in a seasonally breeding species even though the food supply has subsequently become adequate. We attempted to answer this in the present study on a Palaearctic–Indian migratory species, the Red-headed Bunting (*Emberiza bruniceps*). This latitudinal migrant flies south (or south-east) to its wintering

grounds in the autumn (fall migration) and returns north (or north-west) to its breeding grounds in the spring (vernal migration). Thus, during the year, it experiences varying environmental conditions, including changes in food conditions or food abundance. As food availability affects reproduction and associated events (see above), the prediction would be that a food supply shorter in duration than optimally required during a life-history stage would have effects on subsequent life-history stages. This is consistent with the suggested carry-over effects when events in one life-history stage affect the events in another life-history stage (see Norris, 2005; Norris and Taylor, 2006; Norris et al., 2004; Taylor and Norris, 2007). However, there are few, if any, in the way of experimental mechanistic data on carry-over effects in birds. Therefore, a laboratory study testing carry-over effects in a migratory species can provide potentially novel results with broad implications.

A migratory bird such as the Red-headed Bunting goes through several life-history stages, viz a preparatory and progressive phase, spring migration, recrudescence (breeding), post-reproductive regression and refractoriness, and autumnal migration. These closely coupled stages are temporally spaced in the annual cycle in order to avoid a physiological conflict (Kumar et al., 2006). Most migratory songbirds investigated thus far are photoperiodic, and hence the two physiologically distinct life-history stages of the annual cycle that they also exhibit under laboratory conditions are the photosensitive stage (extends from preparatory to reproductive phase) and photorefractory stage (begins post reproduction and lasts until the following preparatory phase). Therefore, we aimed to ask whether food deprivation for a period during either of these two stages would affect the subsequent response cycle under long days when the food supply has again become adequate.

## MATERIALS AND METHODS

This study was performed on males of the migratory Red-headed Bunting (*Emberiza bruniceps* Brandt), an emberizid finch that breeds in west Asia and east Europe and overwinters in India (Ali and Ripley, 1974). Buntings are still photosensitive when they leave their wintering grounds in early April (spring migration) and are photorefractory when they leave their breeding grounds in July/August (autumn migration). When held in outdoor aviaries providing natural day length (NDL) at Lucknow, India (26 deg.55' N; 80 deg.59' E), they clearly exhibit the photosensitive and photorefractory life-history stages (Trivedi, 2005). A previous study of Rani and colleagues demonstrated the role of photoperiod on seasonal cycles of this species (Rani et al., 2005). Briefly, photoperiods of  $\geq 12$  h per day induce growth and regression cycles in body fattening and testes in a period of 9–12 weeks.

We performed two identical experiments, each with two groups of birds ( $N=6-8$  each). Experiment 1 employed two groups of photosensitive birds (groups 1 and 2) that were captured from the overwintering flock in late February and early March 2007 and maintained subsequently on short days (8L:16D). At this time, the birds did not have fat and the testes were small. Buntings do not show initiation of body fattening and testis recrudescence at least until April. Experiment 2 employed two groups of photorefractory birds (groups 3 and 4) that were captured from the overwintering flock in early March 2006 and maintained subsequently on long days (16L:8D). Thus, these birds had undergone growth and regression cycles in a period of approximately one year. Because post-stimulation buntings exhibit absolute photorefractoriness, birds will not respond to long days until they have experienced at least eight weeks of short days.

The experiments began on 16 May 2007. At this time, all the birds did not have body fat, and the testes were small. The birds were housed in cages (size=45 $\times$ 31 $\times$ 41 cm,  $N=3$  or 4 per cage) and placed within the photoperiodic boxes that provided identical lighting and husbandry conditions. The experimental design was as follows. Birds were exposed initially for eight weeks to neutral day lengths (equinox photoperiod) at dim light intensity (12 h light: 12 h dark, 12L:12D; L= $\sim$ 5 lux, D=0 lux) that was sufficiently long for adequate feeding [buntings normally eat only during the daytime (P.B., S.R. and V.K., unpublished observations)]. However, a 12L photoperiod at this light intensity will be non-inductive (P.B., S.R. and V.K., unpublished observations). This meant that the 12L photoperiod would not cause photoperiodic induction in photosensitive birds (experiment 1) and might initiate the recovery of the photosensitivity in photorefractory birds (experiment 2). While under the 12L:12D regime, one group in each experiment (group 1 or 3) received food for 6 h (ZT 0–6; zeitgeber time 0=light on) and the other group (group 2 or 4) received food for 12 h (ZT 0–12). Thus, along with the L:D cycle, birds were presented with one of the two food cycles defined by the presence (P) and absence (A) of food as follows: P:A 6:18 (6 h food present: 18 h food absent) and P:A 12:12. At the end of the 12L:12D exposure, birds were subjected for another 13 weeks to a highly stimulatory long day length (16L:8D; L=400 lux, D=0 lux) and the food restriction was ceased – that is, the birds were provided food *ad libitum*.

Observations were made on body fattening, changes in body mass, molt score and testis size at the beginning and the end of the experiment, and at intervals of 1–4 weeks, as appropriate, during the experiment. While the changes in body mass were considered to reflect the accumulation of fat (about three-quarters) and protein (about one-quarter) (Klaassen et al., 1997), the size of the testis indicated the summation of gonadotropic stimulation over a period of time (Lofts, 1975). The molt was considered to reflect the specific phase of the seasonal cycle (Newton, 1966). Body mass was measured using a top-pan balance to an accuracy of 0.1 g. Body fattening (fat deposition in furcular, scapular and abdominal areas) was assessed using a subjective criterion with a score index of 0–5, as per the scheme of the fat-score outlined by Malik and colleagues (Malik et al., 2004). Briefly, this score index runs as follows: 0=no subcutaneous fat, 1=light fat deposits overlying the musculature, with the vasculature clearly visible, 2=heavier fat deposits overlying musculature, with the vasculature still visible, 3=fat deposits overlie entire region, 4=area filled with whitish, bulging fat deposits, and 5=copious fat deposits all over. Similarly, the molt was studied by the scores of feathers of primary flight (wing primaries) and body feathers, as per Trivedi and colleagues (Trivedi et al., 2006). We scored primaries in a score range of 0–5, whereby: 0=worn or old feather, 1=missing feather (just dropped), 2=from a new feather papilla emerging up to attainment of one-third growth, 3=new feather that has attained two-thirds growth, 4=new feather grown, but growth is still incomplete, 5=new feather that is fully grown. Thus, each primary could have a minimum score of 0 and maximum of 5. Because there are nine primaries on each wing, the maximum score for one wing could be up to 45 ( $9 \times 5=45$ ), and, for each bird, the score could therefore total up to 90 ( $2 \times 45=90$ ). For recording the body molt, we divided the whole body of the bird into 12 different regions: 1=head, 2=neck, 3=shoulder, 4=back, 5=pelvic, 6=caudal, 7=throat, 8=chest, 9=abdomen, 10=flank, 11=shank and 12=sub-caudal. Any region could have a score of either 0 (no molt, fully grown or old feathers) or 1 (molt: no feathers or new feathers emerging), and hence body molt score could total in the range of 0–12. The testicular response was assessed by laparotomy under

local anesthesia (Kumar et al., 2001). Briefly, 2% xylocaine (Astra-IDL, Bangalore, India) was injected subcutaneously and 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 (TV) was calculated from the formula  $4/3\pi ab^2$ , where  $a$  and  $b$  denote half the length of the long and short axes, respectively. We also acquired a subjective grading of the testis size, as per Kumar and colleagues (Kumar et al., 2002), to explain the testicular response:  $TV=0.33$  to  $<2.35$  mm<sup>3</sup> = no response; 2.35 to  $<9.82$  mm<sup>3</sup> = initiation of response;  $TV=9.82$  to  $<18.86$  mm<sup>3</sup> = small response; 18.86 to  $<41.9$  mm<sup>3</sup> = moderate response; 41.9 mm<sup>3</sup> and above = full response.

The data are presented as means  $\pm$  s.e.m. We used two-way ANOVA to analyze simultaneously the effects of two factors over the period of the experiment (e.g. food condition and the duration of the experiment) followed by *post hoc* Bonferroni test. The effects of treatment were also analyzed using one-way analysis of variance with repeated measures (one-way RM ANOVA) followed by the *post hoc* Newman-Keuls test if ANOVA indicated a significance of the difference. Two groups at one time-point were compared using the student's *t*-test. Significance was taken to be:  $P<0.05$ .

## RESULTS

The results from experiment 1 are shown in Fig. 1. The lack of body fattening and testicular recrudescence during the first eight weeks of the experiment (Fig. 1A–C) confirmed that the 12 h photoperiod was perceived as the non-inductive day length. Regardless of the imposed restricted feeding regimes during the 12L experiment (group 1, P:A 6:18; group 2, P:A 12:12), all birds exhibited a

photoperiodic response when subsequently subjected to long days – 16L:8D (Fig. 1). Birds underwent significant gain or loss in fat deposition and body mass in the period between week 8 and week 21 of the experiment when they were held under 16L:8D (fat score: group 1 –  $F_{4,20}=10.71$ ,  $P<0.0001$ ; group 2 –  $F_{4,24}=17.71$ ,  $P<0.0001$ ; body mass: group 1 –  $F_{13,65}=11.77$ ,  $P<0.0001$ ; group 2 –  $F_{13,78}=16.39$ ,  $P<0.0001$ ; one-way RM ANOVA; Fig. 1A,B). Two-way ANOVA did not reveal a significant difference in fat deposition between groups 1 and 2 ( $F_{1,55}=3.222$ ,  $P=0.0782$ ; cf. Fig. 1A) and body mass ( $F_{1,154}=2.695$ ,  $P=0.1027$ ; cf. Fig. 1B). Similarly, the testes also underwent a recrudescence–regression cycle under long days (group 1:  $F_{4,20}=13.11$ ,  $P<0.0001$ ; group 2:  $F_{4,24}=41.05$ ,  $P<0.0001$ ; one-way RM ANOVA; Fig. 1C), but the response cycle was significantly different between the two groups (food condition  $F_{1,55}=19.65$ ,  $P<0.0001$ ; duration of the exposure  $F_{4,55}=31.42$ ,  $P<0.0001$ ; food condition  $\leftrightarrow$  duration of the exposure  $F_{4,55}=2.86$ ,  $P=0.0319$ ; two-way ANOVA; Fig. 1). The rate of induction was slower and the overall amplitude of the testicular response cycle was significantly attenuated in group 1 (P:A 6:18) than in the group 2 (P:A 12:12) ( $P<0.05$ , Bonferroni *post hoc* test). By the end of the first four weeks of long days, six of seven birds showed full responses and one a moderate response in the P:A 12:12 group. At this time, of six birds from P:A 6:18, only two had moderate and four had small responses, respectively. At the end of eight weeks of long days, all birds from P:A 12:12 had shown a full response, but, from the P:A 6:18 group, only four showed a full response and one had a small response and another had a moderate response. The body molt (shedding of feathers) began after six weeks of long days (group 1 –  $F_{13,65}=54.49$ ,  $P<0.0001$ ; group 2 –  $F_{13,78}=66.50$ ,  $P<0.0001$ ; Fig. 1D), and the response was similar in both the groups

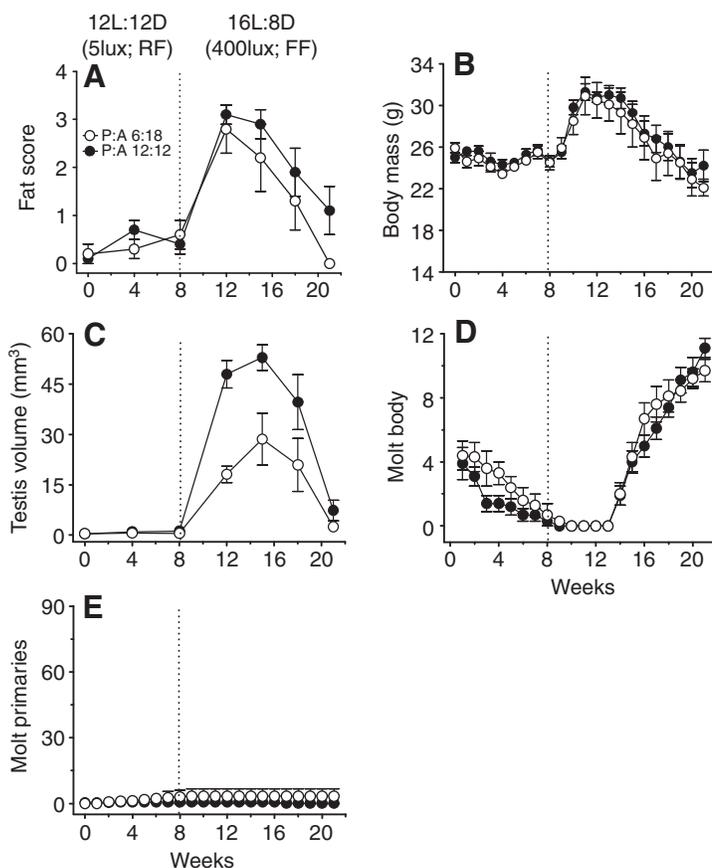


Fig. 1. Effects of prior exposure to restricted feeding regimes on subsequent photoperiodic induction of seasonal responses in photosensitive male Red-headed Bunting (*Emberiza bruniceps*). Birds ( $N=6-8$ ) were first exposed for eight weeks to dim 12 h light: 12 h darkness (12L:12D;  $L\approx 5$  lux,  $D=0$  lux) with restricted feeding (RF) regimes [6 h food present: 18 h food absent, P:A 6:18 (group 1, open circle) or P:A 12:12 (group 2, closed circle)] and then subsequently subjected for another 13 weeks to a highly stimulatory photoperiod (16L:8D;  $L=400$  lux;  $D=0$  lux) with food *ad libitum* (free food, FF). The vertical dashed line indicates the boundary between the 12L:12D and 16L:8D exposures. The response was recorded as changes in fat deposition and body mass, testis growth, body molt (or regeneration of feathers) and wing primary molt at intervals of 1–4 weeks during the 21-week experiment. In all figures, means ( $\pm$ s.e.m.) of the collected data are presented. Clearly, the exposure to restricted feeding regimes had an effect on subsequent induction of reproductive functions (e.g. testis growth and development).

( $F_{1,154}=2.282$ ,  $P=0.1329$ ; two-way ANOVA; cf. Fig. 1D). Wing primaries did not molt during the period of the experiment (Fig. 1E).

Fig. 2 shows the results from experiment 2. Birds did not show body fattening or testis growth under 12L:12D, as in experiment 1 (Fig. 2A,B). However, there were two noticeable changes. First, birds with access to food for only 6 h (P:A 6:18) showed relatively larger fluctuations in their body mass ( $F_{8,48}=4.399$ ,  $P=0.0005$ ; one-way RM ANOVA; Fig. 2B). Second, unlike in experiment 1, wing primaries of birds in both groups underwent molt (P:A 6:18 –  $F_{7,42}=22.48$ ,  $P<0.0001$ ; P:A 12:12 –  $F_{7,49}=55.70$ ,  $P<0.0001$ ; one-way RM ANOVA; Fig. 2E) and had a significant group difference ( $F_{1,104}=9.533$ ,  $P=0.0026$ ; two-way ANOVA). There was no regeneration of body feathers except in regions marked as 1–4 (see above) in P:A 12:12 birds during the last week of 12L:12D exposure (Fig. 1D).

When transferred to 16L:8D, the birds responded slowly. Between weeks 8 and 21 there was a significant increase in fat stores [group 3 (P:A 6:18):  $F_{4,24}=10.71$ ,  $P<0.0001$ ; group 4 (P:A 12:12):  $F_{4,28}=11.92$ ,  $P<0.0001$ ; one-way RM ANOVA; Fig. 2A] and gain in body mass [group 3 (P:A 6:18):  $F_{13,78}=4.517$ ,  $P<0.0001$ ; group 4 (P:A 12:12):  $F_{13,91}=3.440$ ,  $P=0.0002$ ; one-way RM ANOVA; Fig. 2B]. Similarly, the testes showed small levels of recrudescence, but the response was variable within as well as between groups; the response was more homogenous in the P:A 12:12 group (Fig. 2C). At the end of 4 weeks of long days, of seven birds pre-exposed to the P:A 6:18 condition, only two individuals responded, with one showing initiation and one showing a small response. During the same period in birds pre-exposed to the P:A 12:12 condition, six of eight individuals responded, with four showing initiation of

response, one showing a small response and one a moderate response. Although testis recrudescence was partial, the comparison of means over the experiment revealed that the changes were significant in group 4 (P:A 12:12,  $F_{6,42}=3.146$ ,  $P=0.0123$ ; one-way RM ANOVA) but not in group 3 (P:A 6:18,  $F_{6,36}=1.367$ ,  $P=0.2542$ ; one-way RM ANOVA).

The molt of wing primaries that had already begun during the 12L:12D cycle progressed in both the groups under long days (group 3:  $F_{13,78}=8.976$ ,  $P<0.0001$ ; group 4:  $F_{13,91}=12.08$ ,  $P<0.0001$ ; one-way RM ANOVA; Fig. 2E), but the significance of difference between the groups still persisted ( $F_{1,182}=10.09$ ,  $P=0.0017$ ; two-way ANOVA; Fig. 2E). Regeneration of feathers (body molt) also progressed in both the groups (group 3:  $F_{13,78}=11.93$ ,  $P<0.0001$ ; group 4:  $F_{13,91}=14.02$ ,  $P<0.0001$ ; one-way RM ANOVA; Fig. 2D), but the rate of regeneration was significantly faster in PA 12:12 than in P:A 6:18 ( $F_{1,182}=114.2$ ,  $P=0.0001$ ; two-way ANOVA; Fig. 2D). In birds pre-exposed to P:A 12:12, the regeneration of feathers began with long days, but, in those pre-exposed to P:A 6:18, it did not begin at least until 4 weeks of long days (Fig. 2D).

## DISCUSSION

This is the first study to demonstrate that food deprivation during the non-reproductive phase could have effects on the timing of gonadal growth and regression in the subsequent reproductive phase. Fig. 1C clearly shows that prior exposure to a restricted feeding regime determines the rate and magnitude of the testicular response under the 16L:8D regime in photosensitive birds. In birds pre-exposed to P:A 6:18, the rate and magnitude of the testicular responses under long days were attenuated (experiment 1; Fig. 1C). As birds exposed

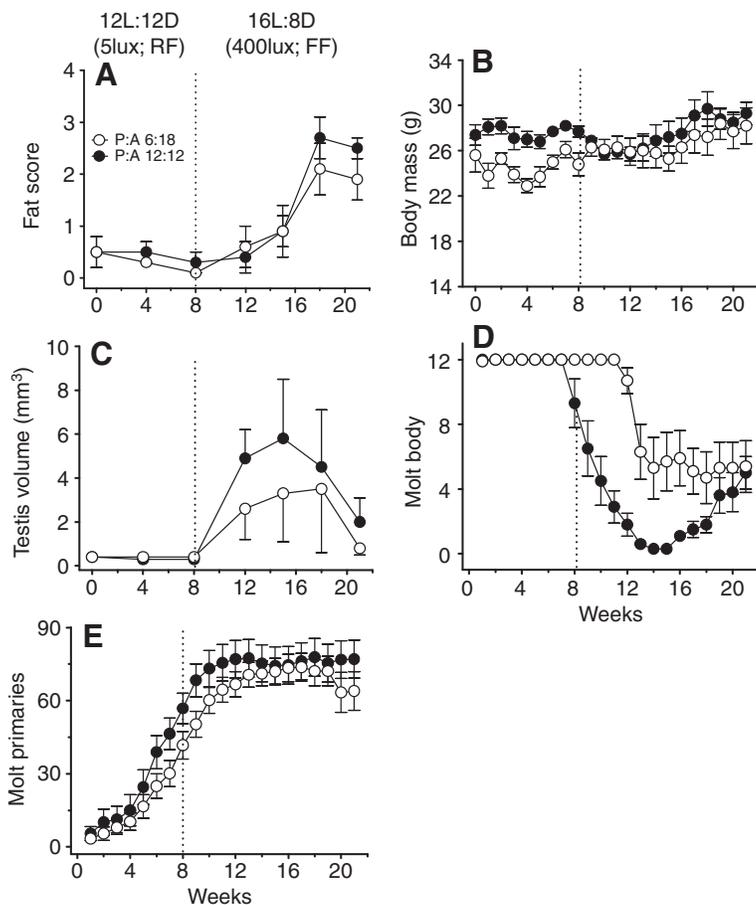


Fig. 2. Effects of prior exposure to restricted feeding regimes on subsequent induction of seasonal responses under long days in photorefractory male Red-headed Bunting (*Emberiza bruniceps*). Birds ( $N=6-8$ ) were first exposed for eight weeks to dim 12 h light: 12 h darkness (12L:12D; L= $\sim$ 5 lux, D=0 lux) with restricted feeding (RF) regimes [6 h food present: 18 h food absent, P:A 6:18 (group 3, open circle) or P:A 12:12 (group 4, closed circle)] and then subsequently subjected for another 13 weeks to a highly stimulatory photoperiod (16L:8D; L=400 lux; D=0 lux) with food *ad libitum* (free food, FF). The vertical dashed line indicates the boundary between the 12L:12D and 16L:8D exposures. All other details are the same as in Fig. 1.

to long days were fed *ad libitum*, the effect on the testicular cycle was clearly the consequence of the restricted feeding regime to which they had been exposed previously. The results from experiment 2 reinforced this conclusion. A 12L photoperiod at the daytime intensity of 5 lux was perceived as a non-stimulatory photoperiod and hence induced the recovery of the photosensitivity in photorefractory birds. The response under long days clearly indicated the recovery of photosensitivity in photorefractory birds. Despite large individual variations, the exposure to restricted feeding regimes appeared to have an effect on the termination of the photorefractoriness under dim 12L:12D conditions. But, as shown by a relatively smaller and variable testicular response on subsequent exposure to long days, the recovery of the photosensitivity in photorefractory birds was still partial. Nevertheless, the degree to which birds had recovered at the end of 12L:12D exposure was clearly related to the food cycles to which they were exposed during the 12L:12D cycle. This was evident from the following observations. When exposed to 16L:8D, six of eight birds of the group pre-exposed to P:A 12:12 (group 2) recrudesced their testes compared with only two of six birds of the group pre-exposed to P:A 6:18 (group 1). Furthermore, birds pre-exposed to P:A 12:12 (group 4) but not to P:A 6:18 (group 3) underwent a significant testicular growth–regression cycle during the subsequent exposure of 13 weeks to the 16L:8D regime (Fig. 2C).

That the exposure to restricted feeding regimes could have carry-over effects was also supported by data on the molt scores of body and wing primary feathers from experiment 2 (Fig. 2D,E). The regeneration of body feathers (indicating the stage of body molt) and molt of wing primaries in photorefractory birds were significantly faster in birds pre-exposed to P:A 12:12 than to P:A 6:18 (Fig. 2D,E). However, the data on the testicular response did not exactly correspond with those on molt scores of the wing primaries in photorefractory birds (cf. Fig. 2C and Fig. 2E). Wing primaries started molt under 12L:12D when testes were not photostimulated. We discount a suggestion that the birds could have just become photorefractory at the start of the 12L:12D treatment and partly recovered from photorefractoriness by the time of photostimulation under long days for the following reasons. Buntings become absolutely photorefractory under long days (Rani et al., 2005). Because birds had been on long days for approximately a year, they had regressed and were photorefractory before they were employed in experiment 2. We suggest that the testicular and molt cycles were dissociated under the experimental conditions employed.

Long-day-induced cycles of gain and loss in fat deposition and body mass were not significantly different between groups pre-exposed to P:A 6:18 and P:A 12:12 regimes (Fig. 1 and Fig. 2A,B). Thus, the restricted feeding regimes probably had differential effects on the metabolic (e.g. fattening and body mass) and reproductive (e.g. testicular growth and molt) functions. This conforms with the view that the stimulation of lipogenesis and gametogenesis are separate photoperiodic events (Kumar 1988; Kumar et al., 2006). It is likely that the animal prioritizes its energy expenditure when faced with a reduced energy supply condition. In the present experiments, it is probable that our migratory buntings used much of the energy available through restricted feeding regimes for the maintenance of their body mass.

We do not know precisely how a restricted feeding regime exerts a consequential effect on the photoneuroendocrine system. However, we offer a few plausible explanations. One is that food deprivation during the day (i.e. starvation) triggers a cascading effect on the release of neurotransmitters within the hypothalamus. One neurotransmitter that is elevated in response to food deprivation is neuropeptide Y (NPY) (Boswell et al., 1999). It is likely that elevated

hypothalamic NPY inhibits the reproductive axis (Aubert et al., 1998; Raposinho et al., 1999), and this effect is carried over for a longer period under long days even though the food supply has become adequate. A second explanation is that the food and light cues are closely coupled in timing the seasonal cycles (Hahn 1995; Hau et al., 2000; Perfito et al., 2008). The restricted feeding regimes disrupt this coupling and subsequently affect photoperiodic induction of the seasonal cycles. In the Red-headed Bunting, which forages only during light hours presumably owing to photosensory limitations (P.B., S.R. and V.K., unpublished observations), the food and light cues remain relatively well integrated during the P:A 12:12 but not during the P:A 6:18 cycle when food is absent during the second half of the 12 h day. It is conceivable that an induced asynchrony between the food and light cues under P:A 6:18 affects subsequent photostimulation of the testes under long days.

In conclusion, our results strongly support the idea that the food cues exert a significant effect on photoperiodic regulation of seasonal reproduction. Food deprivation can exert direct effects through changes in energy homeostasis and can exert indirect effects through changes in the relationship between food and light cues synchronizing the circadian clock that underlies the timing of gonadal growth and development. The results also indicate that the after-effects of restricted feeding regimes are strong enough to influence the activity of the reproductive axis under long days. In the absence of data on testis histology, which limits our conclusions regarding effects on the reproductive state, the size of testes can be considered to suggest that gametogenesis was affected (Lofts, 1975). To sum up, the current findings suggest for the first time that, in a seasonally breeding vertebrate species, food shortages at any time during the year might potentially affect reproduction.

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