

## Artificial photoperiod induced migratory changes in the redheaded bunting

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### Abstract

Photosensitive redheaded buntings were kept at short daylength (SD, 8L/16D) for 7 days. These birds were then exposed to long daylength (LD, 13L/11D) to induce migratory restlessness i.e. zugunruhe. The experiment was continued till termination of zugunruhe in each bird. The actogram shows that birds held at short days show activity only during light period (pre-migratory stage). Upon exposure to long days birds start showing nighttime activity (zugunruhe). Nighttime activity indicates that birds have attained migratory stage in simulated photoperiodic conditions. Birds stop their nighttime activity after showing zugunruhe for many days. The total activity count is highest during migratory stage of buntings. We can say that with increase in day length the buntings show night time restlessness i.e. zugunruhe and this is the mark of onset of migration. Because of their night time activity, the total activity count is high during migration. Buntings store highest amount of body fat in sub-cutaneous adipose tissue during migratory period. This adipose-stored fat helps the bird to fuel their migratory flight. Increase in bodily fuel stores prior to long-distance flights is an important metabolic feature of migratory birds.

Key words: Migration, physiology, flight, metabolism, fuel

### Introduction

Migration is a complex process and composed of successive phases. First, the genetic, molecular and biochemical mechanisms underlying migration are initiated followed by cycles of fueling and flight. During fueling migratory birds undergo hyperphagia followed by fattening that includes lipogenesis in liver, storage in adipose tissue and deposition in flight muscle for the oxidative functions supporting long-distance flight (Jenni-Eiermann and Jenni, 1992; Jenni and Schaub, 2003; McFarlan et al., 2009; Ramenofsky et al., 2011). Other organs also may change in size and activity during preparation of migratory flight. Coordination of internal (e.g., fuel levels) and external (e.g., environmental) cues and behavioral and metabolic changes help to fuel long-distance flight in migration (Guglielmo, 2010; Jenni-Eiermann et al., 2011). The fuel types required for migratory flight comprises maximum of lipids and a minimum of protein. Migrants stop at stopover sites to rest and refuel, after they complete a long flight. Consecutive cycles of fueling and flight thus continue until the destination is reached.

Captive birds also show migratory fat deposition, which is a key component of preparation for migration. This is promoted by hyperphagia and a higher digestibility (Bairlein, 2002). Along with increase in food intake and change in body mass reproductive system also develops and there are changes in energy expenditure, which is related to migratory locomotor activity (Dixit et al, 2014). Migratory hyperphagia and fat deposition are photoperiodically regulated. The photoperiodic control of hyperphagia and fattening shares much in common with that of seasonal reproduction where photoperiod provides initial predictive information (Wingfield, 2004).

Following the fueling stage of migration birds depart and their departure is driven by both endogenous and exogenous information. Exogenous information i.e. environmental cues (e.g., barometric pressure, rain, wind, temperature etc.) is combined with information

concerning intrinsic migratory state (e.g., motivation, physiological state, etc.) to affect departure decisions in many migrants (Jenni and Schaub, 2003; Newton, 2008). Departure occurs soon after the birds have accumulated sufficient energy stores for the flight and with favourable environmental or social conditions (Dingle, 1996; Berthold, 2001; Jenni and Schaub, 2003; Newton, 2008).

Termination of migratory behavior upon arrival at a breeding or over-wintering site probably involves mechanisms of habitat imprinting, recognition and resource evaluation (Gwinner and Czeschlik, 1978; Hahn et al., 1995; Ketterson and Nolan, 1990). It is unknown whether plasma metabolites contribute to the termination of migration, although they may play a role in the transition between the migratory and reproductive life cycle stages. When birds terminate migration and begin to breed there is a transitional phase where birds express characteristics from both migratory and breeding stages alternatively or simultaneously (Ramenofsky and Wingfield, 2006; Jc, 2008). Long distance migrants show highly flexible Behavior and physiology during this transition. Long distance migrants base the timing of migration on highly predictable cues, such as photoperiod, and so arrive at the breeding sites with little predictive power of when breeding will begin or what conditions they might face. Migratory birds mainly depend on stored lipid to derive energy required for their flight.

In this study we have investigated some physiological changes of the migratory redheaded buntings to get a view of their physiological response to different migratory stages under artificial photoperiodic conditions.

## Materials and methods

### *Model species*

The present work was carried out on male redheaded bunting, *Emberiza bruniceps*, a small migratory passerine bird in the family Emberizidae. It has a slender and small body size of about 17-18 cm in length. This bird exhibits sexual dimorphism. The breeding male has bright yellow underparts, green upperparts and a brownish-red face and breast. The wings and tail are dark brown in colour and tail coverts are brown. It has a conical bill and a forked tail. The female and juvenile have paler underparts, a grey-brown back and greyish head. This avian species is a long-distance migrant with distinct breeding and wintering grounds. It breeds in central Asia- Afghanistan, Iran, Kazakhstan, Kyrgyzstan, Mongolia; Russian Federation (European Russia, Central Asian Russia), Tajikistan, Turkmenistan, Uzbekistan. This bird winters in India and Bangladesh. It covers a distance of about 7,000 km during the migratory journey.

Adult male redheaded buntings were captured from overwintering flocks in India and were acclimatized to captive conditions by keeping them in an outdoor aviary for 7 days where they experienced natural variation of photoperiod, temperature and humidity. These birds were then brought indoors and exposed to short days (8L/16D) for two months to terminate photorefractoriness, if they had any in nature, and to make them photosensitive. These photosensitive birds were used for the present study. Buntings (n=5) were kept singly in activity cages for the recording and analyses of the activity data (actogram) by using the Chronobiology Kit (Release Version 1c, © 1998–2004) software programme of the Stanford Software Systems (Stanford, USA). The activity cages carrying birds were exposed to short daylength (SD, 8L/16D) for 7 days. The intensity of light during light period was  $250 \pm 20$  lux and that of dark period was 0.1 lux throughout the experiment. Birds were provided with ad libitum food and water. These birds were then exposed to long daylength (LD, 13L/11D) to induce migratory restlessness i.e. zugunruhe. The experiment was continued till termination of

zugunruhe in each bird. Observations on food intake, body mass and fat score were recorded after 7<sup>th</sup> day of LD (pre-migratory), 7<sup>th</sup> day of appearance of zugunruhe (migratory) and 7<sup>th</sup> day of termination of zugunruhe (post-migratory).

#### *Body mass*

Birds were individually weighed on a top pan digital balance to the nearest accuracy of 1.1 g. For this, the bird was placed in a cotton bag, the weight of which was tared to zero. Then the bird with the cotton bag was weighed.

#### *Fat score*

Fat deposition was scored from the furcular, scapular, and abdominal areas in an index of 0-5 as described by Boswell, 1991 and outlined below:

- 0- No subcutaneous fat
- 1- Light deposits underlying musculature and vasculature easily seen
- 2- Heavier deposits, underlying musculature and vasculature still visible
- 3- Fat deposits overlies entire region
- 4- Area filled with whitish, bulging fat deposits
- 5- Copious deposits overflowing onto outlying areas

#### *Food intake*

Fixed amounts of food in the food cups were provided to birds housed in activity cages. The difference between the initial and final weight of food gave the amount of food consumed by a bird. The food intake was measured according to the method described by Jain and Kumar (1995).

### **Statistical analysis**

All data are presented as mean  $\pm$  S.E.M. They were analyzed using one-way ANOVA followed by Newman-Keul's Multiple range 't' test or Bonferroni post-hoc mean comparison test, if ANOVA indicated a significance of difference. All statistical analyses were done using GraphPad Prism software (version 6.0, San Diego, CA, USA). The significant was taken at the 95% confident level.

### **Results**

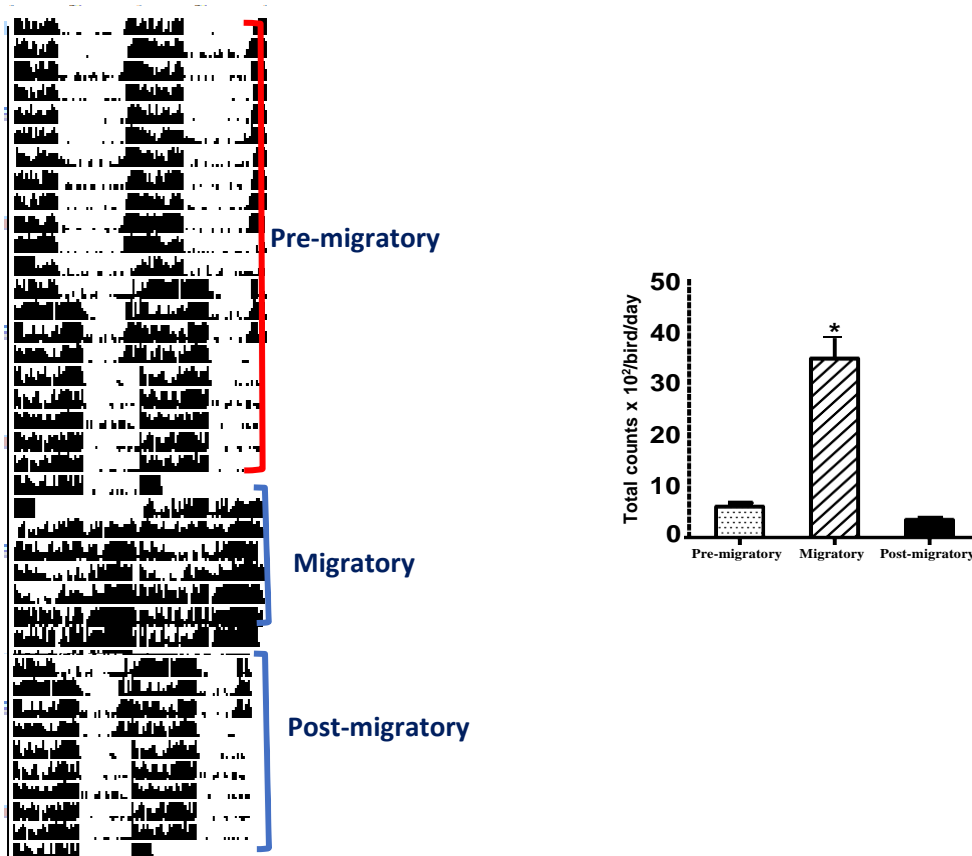
#### *Onset of zugunruhe*

The actogram (Fig. 1a) showed that birds held at short days showed activity only during light period (pre-migratory stage). Upon exposure to long days birds started showing nighttime activity (zugunruhe). Nighttime activity indicated that birds attained migratory stage in simulated photoperiodic conditions. Birds stopped their nighttime activity after showing zugunruhe for many days. The total activity count was highest during migratory stage of buntings (Fig. 1 b).

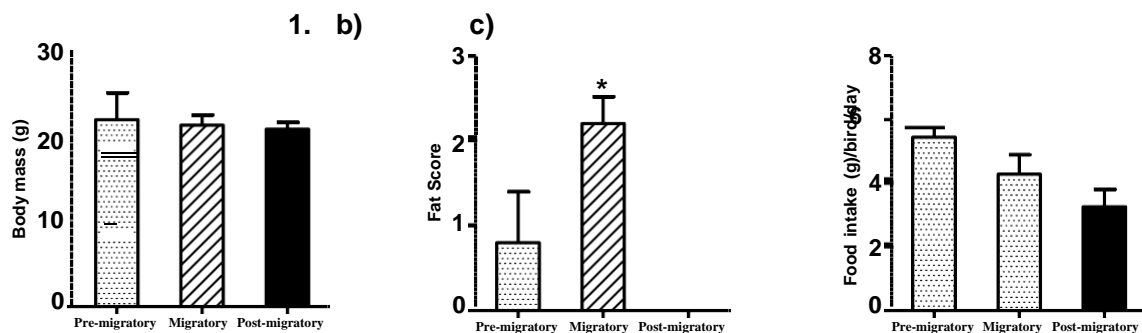
#### *Body mass, fat score and food intake*

The results are presented in the figure 2 (a-c). One-way ANOVA showed no significant differences in body mass of birds ( $F_{2,14}=0.2598$ ,  $P=0.1167$ ). Buntings showed deposition of fats during the migratory stage. Deposition of fat was significantly high during migratory stage

as compared to pre-migratory and post-migratory stages ( $F_{2,14}=6.00$ ,  $P=0.0256$ ). Change in food intake during different migratory stages was not significant ( $F_{2,14}=10.95$ ,  $P=0.051$ ).



**Fig. 1:** (a) Representative actogram and (b) total activity counts of the redheaded buntings under simulated migratory conditions. \* $P<0.05$



**Fig. 2:** (a) Body mass, (b) fat score and (c) food intake of the redheaded buntings under simulated migratory conditions. \* $P<0.05$

**Discussion**

With increase in day length the buntings showed night time restlessness i.e. zugunruhe and this is the mark of onset of migration. Because of their night time activity, the total activity count is high during migration. Birds travel thousands of kilometers between their breeding areas in summer and overwintering sites. Birds show uninterrupted bouts of flight for several days during their migration. Buntings stored highest amount of body fat during migratory period. Migratory birds accumulate large amount of fat stores and they deposit fat as high as 50-60% of their total body mass during migration. Long distance migratory flights are fueled by adipose-stored fats (Landys et al., 2005; Guglielmo, 2018). Increase in bodily fuel stores prior to long-distance flights is an important metabolic feature of migratory birds. These body stores comprise mainly of lipids and proteins (Lindstorm and Piersma, 1993; Piersma et al., 1999). Fats, universal storage forms of energy in living organisms, are derivatives of fatty acids. Major amount of fat is stored in sub-cutaneous adipose tissue (Berthold, 1993; Maillet and weber, 2006). Fat is also stored in flight muscles (pectoralis), liver, mesenteries and connective tissue in the abdominal cavity, sometimes enveloping the intestine (Blem, 1976; Pond, 1978; Maillet and Weber, 2006; Guglielmo, 2018). These stored fatty acids are transported and oxidized at very high rates in the migratory birds to sustain flights for many hours or days. Birds rely more on hepatic and muscular lipids during their short fasting periods (Jenni-Eiermann and Jenni, 1996). Migratory journeys impose tremendous energy demands and birds rely solely on energy stored in the body. Birds obtain energy from lipids stored as triglycerides in their adipose tissue to support the migratory flight. Migratory birds maintain their metabolism using body fat, carbohydrate and protein as fuels. It has been suggested that migratory songbirds may supplement fatty acids with triglycerides in plasma to provide more energy to muscles (Jenni-Eiermann and Jenni, 1992). Protein is stored in large quantity as body structure in living organisms. Proteins from muscles and digestive organs are used during migratory flights (McWilliams and Karasov, 2001). It is suggested that catabolism of body protein also occurs in migratory birds (Mori and George, 1978; Robin et al., 1987). Digestive organs, which have greater protein turnover rates than muscle, catabolize proteins during long flights in migratory birds (Battley et al., 2000; Bauchinger et al., 2005; Bauchinger and McWilliams, 2009). Catabolism of protein leads to the reduction of flight muscle size and loss of body mass over a flight (Jenni and Jenni-Eiermann, 1998; Pennycuick, 1998). This reduction in body mass makes the flight easier for migratory birds.

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