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PHOTOPERIOD-INDUCED PHYSIOLOGICAL AND BIOCHEMICAL ADAPTATIONS IN MIGRATORY AND NON-MIGRATORY SONGBIRDS: INSIGHTS FROM BLACKHEADED BUNTING AND INDIAN WEAVER BIRD STUDIES

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ABSTRACT

This study delves into the physiological and biochemical adaptations triggered by photoperiod changes in migratory and non-migratory songbirds, focusing on the blackheaded bunting and the Indian weaver bird. Through a detailed examination, the research reveals how long-day photoperiods induce significant physiological changes in the blackheaded bunting, preparing it for migration. These changes include increased body mass, elevated serum triglycerides, and testis enlargement, all of which are crucial for the bird's long-distance journey. Additionally, the study identifies differential expression of key proteins in the buntings' flight muscles, such as fatty acid binding protein (FABP) and myoglobin, which are vital for meeting the high energy demands and ensuring efficient oxygen transport during migration. These findings highlight the critical role of photoperiod as a trigger for these adaptations, which are notably absent in the non-migratory Indian weaver bird, underscoring the species-specific nature of these physiological changes. The research contributes valuable insights into how migratory birds optimize their physiology to overcome the challenges of long-distance travel, which has important implications for understanding the ecological and evolutionary dynamics of migration, as well as for developing conservation strategies in the face of environmental changes.

Keywords: photoperiod, migration, blackheaded bunting, Indian weaver bird, physiological adaptations, serum triglycerides, fatty acid binding protein, myoglobin, long-distance travel, conservation.

1. INTRODUCTION

In response to environmental signals such shifts in photoperiod, migratory birds endure extensive physiologic changes in the months leading up to their journey. This research set out to discover and describe these alterations in the blackheaded bunting (Emberiza melanocephala), a species known to begin its annual northward migration in response to lengthening daytime hours in early spring. They conducted experiments comparing birds exposed to short days (SD) and long days (LD), as well as a non-migratory Indian weaver bird (Ploceus philippinus) as a control species, all of which shared the same overwintering habitat as buntings, to gain insights into the physiological changes associated with migration.

Body mass, testis size, and triglyceride levels were among the first measurements taken from buntings kept under varying light cycles. Different from the long-day (LD) condition, which had 16 hours of light and 8 hours of darkness (16L: 8D), the short-day (SD) condition had 8 hours of light and 16 hours of darkness (8L: 16D). The protein profiles of buntings' flying muscles were also studied in order to detect alterations related to photoperiod.

Body weight, testis size, and serum triglyceride levels were significantly altered in buntings exposed to LD, but not in those subjected to SD. Increased fat deposition and body weight growth, as well as heightened energy reserves indicated by raised triglycerides, were all consistent with adjustments made in preparation for migration. In addition, electrophoresis examination of flying muscle proteins showed significant variation amongst buntings in SD and LD. Weaver birds, in contrast to migratory buntings, showed no signs of photostimulated alterations in most parameters save for the recrudescence (renewal of activity) of their testes under LD.

Three elevated proteins in the flying muscles of buntings under LD were separated and identified by further analysis using two-dimensional gel electrophoresis (2D-GE). Mass spectrometric analysis (MS-MS) and protein database searches confirmed that they were the enzymes fatty acid binding protein (FABP), myoglobin, and creatine kinase (CK). Long-day exposure particularly raised transcript levels of FABP and myoglobin in buntings, although CK levels did not significantly alter, as validated by subsequent semi-quantitative and quantitative PCR testing. This finding showed that the increased FABP expression could be connected to the higher energy needs involved with migration and that the enhanced myoglobin expression might be related to the heightened physical activity experienced throughout the migration process.

The latitudinal migrations of millions of birds between their breeding and overwintering grounds are among the most remarkable natural events, occurring twice annually. In late October, many species of songbirds embark on their long journey southward to their wintering and breeding habitats, and in late spring, they undertake the equally long return northward. During the pre-migration period, birds undergo critical physiological changes that enable them to migrate as efficiently as possible. Photoperiodic signals play a crucial role in the development of migratory characteristics, while internal circadian clocks help birds anticipate the timing of their spring and fall migrations.

Photoperiodic conditions are pivotal in regulating the migratory response; for instance, the fall migration begins when day lengths decrease, and the spring migration coincides with increasing day lengths. Research on various migrating songbirds has shown that exposure to long days triggers the development of spring migration traits in caged birds, such as body fattening, weight gain, and nocturnal migratory restlessness, known as Zugunruhe.

A critical physiological adaptation in migratory birds is the ability to replenish energy stores during long flights. This adaptation is often tracked through the storage of chemical energy reserves, including carbohydrates, lipids, and proteins. During the premigratory stage, birds store glucose as glycogen and fat as triglycerides (Jenni and Eiermann, 1999; McWilliams et al., 2004). Unlike carbohydrates and lipids, proteins are not stored in the body but are instead broken down directly from muscle and digestive tissues (Bauchinger and Biebach, 2001). Studies have shown that protein breakdown also leads to increased uric acid levels (Landys et al., 2005). Energy is first derived from carbohydrates, followed by the slow mobilization of lipids and proteins after about one to four hours of flight (Schwilch et al., 1996; Gerson and Guglielmo, 2013).

In conclusion, the phenomenon of bird migration involves extraordinary feats of endurance and adaptability. Migratory birds undertake these journeys out of necessity, relying on complex physiological changes to travel from their breeding grounds to their overwintering sites. Photoperiodic signals play a critical role in timing these migrations, with the onset of spring and fall migrations triggered by changes in day length. These migrations are characterized by specific responses to photoperiodic conditions, including fattening, weight gain, and nocturnal migratory restlessness.

Energy storage in the form of carbohydrates, lipids, and proteins is a vital adaptation for successful migration. Proteins are broken down from muscles and digestive organs to support flight and tissue repair, while carbohydrates and fats are stored in preparation for the journey. These metabolic adjustments are essential to meet the high energy demands of long-distance migration.

Metabolic enzymes such as creatine kinase, citrate synthase, and carnitine-o-palmitoyltransferase play crucial roles during migration, further contributing to the complexity of the physiological changes that occur. These enzymes are vital for energy production and replenishment, enabling birds to sustain prolonged flights.

Research on migratory birds enhances our understanding of the remarkable traits that allow these birds to traverse vast distances. By exploring the intricate relationship between photoperiodic signals, physiological changes, and behavioral responses, scientists gain a deeper appreciation of the challenges and triumphs faced by migratory birds.

Bird migration is not only a fascinating natural event but also has significant ecological and evolutionary implications. The precise timing of migrations is crucial for the birds' survival and reproductive success. Migratory birds have a limited window of opportunity to reach their breeding and wintering grounds before adverse weather conditions threaten their survival.

Understanding the physiology and behavior of migratory birds provides valuable insights into the impacts of environmental changes, such as climate shifts and habitat loss. The timing and success of migrations may be influenced by alterations in photoperiodic signals, food availability, and disruptions to stopover sites. As the world undergoes significant environmental changes, understanding how migratory birds respond and adapt to these challenges is critical for conservation efforts.

Fatty acids (FAs) acquired from food and stored in adipose tissues are the primary source of energy for migratory birds. One of the first produced proteins for fatty acid absorption in the liver is the plasma membrane fatty acid binding protein (FABPpm), which aids in the process of cellular fatty acid uptake. In addition to facilitating the absorption and consumption of fatty acids, guiding intracellular metabolic pathways, and shielding cellular structures from fatty acid damage, fatty acid binding proteins (FABPs) have a variety of other activities.

There is an increase in the expression of proteins involved in fatty acid absorption and transport in the pectoral muscles of migrating birds, such as the white-throated sparrow and the bar-headed goose, during the migratory state. There is also evidence that the amounts of myoglobin, a compact heme protein present in muscle cells, rise in migrating bird populations. Myoglobin is an oxygen-binding protein that serves to store oxygen and facilitate its delivery throughout muscle tissues.

Muscular system modifications, especially in flying muscles, are highly indicative of the physiological effects of migration. Because of the demanding nature of migration, the flying muscles are often the subject of research into the physiology of this process.

The purpose of this research was to examine the physiologic adjustments made by latitudinal migratory songbirds prior to their long-distance journey. The blackheaded bunting (Emberiza melanocephala) was the main topic of discussion since it is a long-distance migrant songbird that travels from its breeding sites in western Asia and eastern Europe to its overwintering grounds in India throughout the night. The migration route is around 7,000 kilometers long and crosses a latitudinal gradient.

The purpose of this research was to compare the effects of long and short day lengths on the body mass and serum triglyceride levels of buntings. In addition, the research found that buntings subjected to lengthy days had differing protein expression levels in their flying muscles compared to controls subjected to short days. The Indian weaver bird (Ploceus philippinus), a non-migratory songbird that shares the same overwintering habitat as buntings but does not display the same migratory-related changes, was subjected to the same protocol to confirm that these modifications were linked to migration rather than gonadal maturation.

The yearly migration of millions of birds between breeding and overwintering habitats is a fascinating example of avian behavior. Many species of songbirds migrate thousands of kilometers twice yearly, first in the late fall to their wintering and breeding grounds, and then again in the late spring to their summer grounds. These migrations are propelled by both genetic and environmental stimuli, and the animals' bodies must undergo profound modifications in order to endure the grueling travels.

Photoperiod seems to be a critical regulator of the development of migratory features, even though birds have their own intrinsic timing systems like endogenous clocks. Changes in day duration are linked to seasonal migrations like those that occur in the fall and spring. For instance, the timing of the fall migration correlates with shorter days, whereas the timing of the spring migration corresponds to longer days. A series of physiological reactions is set in motion by these alterations, getting the birds ready for their migrations.

Energy reserves, including carbohydrates, lipids, and proteins, are built up as part of these alterations in physiology to meet the needs of migration. Glycogen and triglycerides are the forms in which carbohydrates and lipids are stored for use as an immediate source of energy. During these long trips, the body immediately catabolizes proteins stored in the muscles and digestive organs to replenish energy stores. These power sources are essential for meeting the rigorous power needs of long-haul flights.

Fatty acid binding proteins (FABPs) are an important class of proteins involved in cellular energy metabolism. Fatty acids are a significant source of energy for migrating birds, and FABPs help in their absorption, transport, and usage. During migration, these proteins make sure that fatty acids are efficiently used to fuel the flying muscles.

Myoglobin, which is found in high concentrations in muscle cells and is responsible for the storage and distribution of oxygen throughout the body's tissues, is another crucial protein. Myoglobin's greater affinity for oxygen than hemoglobin allows it to gather and distribute oxygen to working muscles more effectively during strenuous exercise or flight.

During migration, when birds must traverse great distances, the ability to fly is crucial. This is why we zeroed attention on how these muscles adapt physiologically in the run-up to departure. The research looked at how long-day vs short-day exposure affected the migratory blackheaded bunting's body mass, serum triglyceride levels, and protein expression in its flight muscles. The goal was to identify the molecular processes that underpin the migratory bird's high energy demands and specialized physiology.

A non-migratory songbird, the Indian weaver bird, which shares the same overwintering habitat as the migratory buntings but does not exhibit the same migratory-related changes was analyzed in a similar fashion to confirm the link between these changes and migration rather than other factors like gonadal maturation.

In conclusion, the research hoped to provide light on the function of proteins like FABPs and myoglobin in energy metabolism and on how migratory birds' bodies adapt in anticipation of migration. Researchers may learn more about how migratory birds overcome the difficulties of lengthy flights and make the most efficient use of their energy by delving into these systems.

The annual migration of millions of birds between their breeding and overwintering grounds is a fascinating and intricate habit. Many species of songbirds perform a massive yearly migration, travelling thousands of miles south in the late fall and thousands of miles north in the late spring. Birds go through complex physiological changes in order to accomplish lengthy voyages, adjusting their behavior and metabolism to suit the demands of long flights.

Despite the presence of endogenous clocks and other internal systems for timing, it is clear that photoperiod plays a significant role in initiating and controlling migratory behaviors and physiological changes in birds. Changes in day duration are mirrored in the ebb and flow of migration, with fall migration coinciding with shorter days and spring migration with longer ones. The bird's physiological responses are fine-tuned in response to the interplay between photoperiod and migratory signals.

The storage of nutrients including carbohydrates, lipids (fats), and proteins for later use is an important part of these alterations in physiology. These fuel reserves are critical for meeting the energetic requirements of migration. In order to provide energy for flight, the body stores carbohydrates as glycogen and lipids as triglycerides. However, proteins help keep muscle tissue healthy and provide fuel for the body.

In particular, fatty acid binding proteins (FABPs) stand out among the proteins involved in these activities. Migration requires a great deal of energy, and fatty acids are transported and used with the help of FABPs. These proteins facilitate the bird's ability to execute long-distance flights by ensuring that fatty acids are effectively taken up and channeled towards energy generation in the muscles.

Myoglobin is a vital protein because it stores and transports oxygen across muscle cells. Myoglobin has a stronger affinity for oxygen than hemoglobin and is thus better at scavenging it from the circulation and transporting it to muscle cells, where it is needed most during high-demand activities like running or flying.

The flying muscles of the migratory blackheaded bunting were examined to gain insight into the physiological changes that take place in the species during the premigration period. The purpose of this research was to determine whether and how long-day and short-day exposure affected body mass, blood triglyceride levels, and protein expression in the flying muscles of birds. This study aimed to learn how birds deal with the energy drain and physical obstacles of migrating at the molecular level.

The research expanded their investigation to a non-migratory songbird species, the Indian weaver bird, to show that these alterations were truly connected to migration and not impacted by other variables, such as gonadal maturation. Through this comparison, scientists were able to deduce that the blackheaded bunting's physiological changes coincide with its migratory patterns.

The purpose of the research was, in a nutshell, to illuminate the complex physiological adjustments that migrating birds make before setting off on their long journey. The purpose of the research was to shed light on how these

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birds maximize their energy metabolism and physical capacities to go on their remarkable long-distance excursions by examining the involvement of proteins such FABPs and myoglobin.

2. MATERIALS AND METHODS

The research involved two distinct cohorts of adult male avian species, specifically Passerina (buntings) and Ploceus (weaverbirds). These birds were procured directly from their natural habitats to ensure the ecological validity of the study. Following their initial capture, the birds underwent a seven-day acclimatization period in a controlled open-air aviary. This acclimation allowed the birds to adapt to their new surroundings and conditions before being transferred to an indoor aviary. In this controlled environment, the birds were exposed to non-stimulatory short days, with a photoperiod of 8 hours of light and 16 hours of darkness, for 1.5 weeks. This specific light-dark cycle was deliberately chosen to establish a consistent and standardized baseline for the birds' physiological and behavioral responses before the start of the experimental phase.

During the experiment, light intensity remained stable, with no significant fluctuations, and the temperature was meticulously maintained at approximately 24°C to ensure a controlled environment for the study. At the beginning of the experiment, all avian specimens were in a state of reproductive inactivity, evidenced by the absence of significant adipose tissue accumulation and the relatively small size of their testes.

The study strictly adhered to ethical guidelines for animal treatment as prescribed by the Institutional Animal Ethics Committee (IAEC) at the University of Lucknow, where the experiment was conducted.

The experimental design included two groups for each species: one group was exposed to prolonged illumination (16 hours of light and 8 hours of darkness), while the other group was kept under conditions of abbreviated daylight exposure (8 hours of light and 16 hours of darkness). Due to the natural variability in the rate of testicular induction among avian species when exposed to extended daylight, the experimental period lasted three weeks for buntings and eleven weeks for weaverbirds. The extended duration for weaverbirds was necessary due to their characteristically slower response to prolonged daylight, which required a longer exposure period to induce testicular maturation.

Various measurements and assessments were conducted during the experiment:

- **Body Mass and Testis Measurements:** Body mass of the birds was recorded using a top pan balance, and the dimensions of the left testis were measured through laparotomy under local anesthesia. Testis volume was calculated using specific formulas, allowing categorization of the birds into different reproductive states.

- **Feeding:** Birds were provided with cereal grain-based diets and a supplementary food mixture. Food and water were provided ad libitum.

- **Blood Collection and Serum Harvesting:** At the end of the experiment, birds were sacrificed, and blood was collected for serum analysis. Serum was separated from the blood and stored for later assays.

3. ANALYSIS

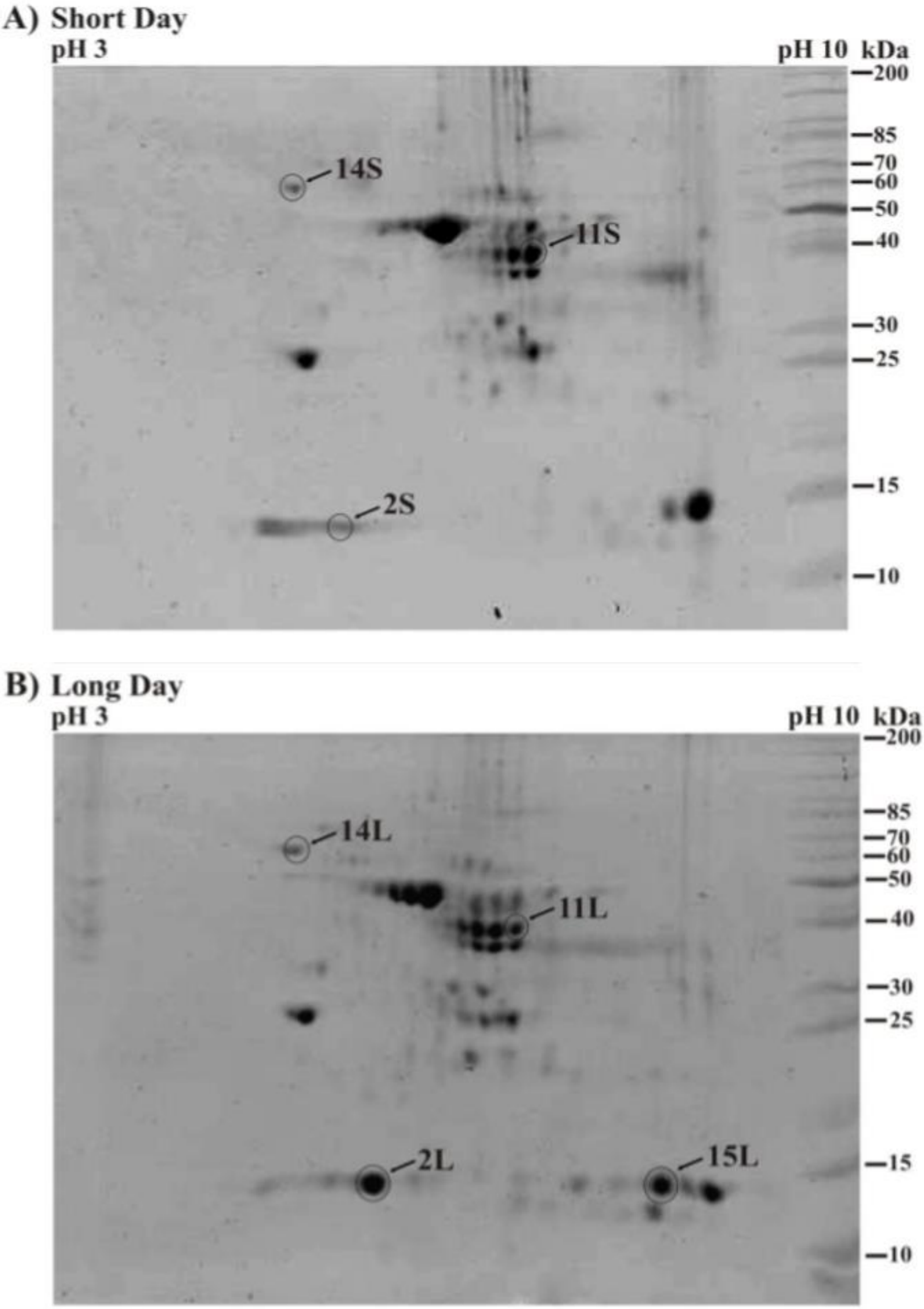

Figure 5: Representative 2D-GE resolution of protein spots in the flight muscle of blackheaded bunting (E. melanocephala) under short days (SD; 8L: 16D) and long days (LD; 16L: 8D). A broad range (10-200 kDa)

unstained protein ladder (cat no. # SM0661; Fermentas Thermo, USA) was used as a reference to estimate the molecular weight of proteins under SD (A) and LD (B) conditions. Differentially expressed protein spots with more than two fold difference between SD and LD groups (n = 4) were circled and numbered as 14S and 11S in SD, and 2L, 11L, 14Land 15Lin the LD condition. Note that spot 15Lis present in LD only .

The utilization of Two-Dimensional Gel Electrophoresis (2D-GE) was employed to carry out a more comprehensive and in-depth analysis of the proteins that exhibited differential expression. This technique, which is widely recognized and employed in the field of proteomics, facilitates the separation of proteins based on their distinctive isoelectric point and molecular weight properties. By capitalizing on the principles of electrophoresis, this method enables the effective separation and visualization of proteins, thereby enabling researchers to gain valuable insights into the intricate and complex nature of protein expression patterns. Through the implementation of 2D-GE, the identification and characterization of differentially expressed proteins can be accomplished with a higher degree of precision and accuracy, thereby contributing to a more comprehensive understanding of the underlying molecular mechanisms and biological processes that govern protein regulation and function. The protein spots that were deemed of interest were carefully excised from the sample and subsequently subjected to identification through the utilization of mass spectrometry, specifically tandem mass spectrometry (MS-MS) techniques.

The primary objective of this research endeavor was to comprehensively investigate and elucidate the intricate physiological alterations that occur in avian species during the migratory state. To achieve this, the study meticulously examined and analyzed a multitude of crucial parameters, including variations in body mass, serum metabolites, and protein expression within the flight muscles of two distinct avian species: the migratory blackheaded bunting and the non-migratory Indian weaver bird. By meticulously scrutinizing these fundamental aspects, the study aimed to shed light on the intricate mechanisms underlying the migratory phenomenon in avian organisms. The primary objective of this study was to elucidate and gain a deeper understanding of the intricate molecular mechanisms that underlie migratory adaptations by means of a comprehensive comparison of the responses exhibited by two distinct species to alterations in photoperiodic conditions.

The experimental design encompassed the inclusion of two distinct groups, each comprising individuals of the blackheaded bunting species (Emberiza melanocephala) and the Indian weaver bird species (Ploceus philippinus). The avian specimens under investigation were initially procured from their natural habitats and subsequently subjected to a process of acclimatization within a controlled environment, specifically an open-air aviary, for a duration of one week. After undergoing a period of acclimation, the subjects were subsequently subjected to a regimen of non-stimulatory short days, characterized by a photoperiod consisting of 8 hours of light and 16 hours of darkness, for a duration of 1.5 weeks. This experimental manipulation was carried out within the confines of an indoor aviary, providing a controlled environment conducive to the study of the subjects' behavioral and physiological responses. The experimental conditions involved the careful maintenance of the ambient temperature at a consistent level of approximately 24°C, ensuring a stable and controlled environment for the duration of the study. Similarly, the light intensity was meticulously regulated and maintained at a constant level throughout the entirety of the experimental procedure. By meticulously controlling these crucial variables, the researchers aimed to minimize any potential confounding factors that could potentially influence the outcomes of the study, thereby enhancing the validity and reliability of the obtained results.

In order to ensure the ethical integrity of our research, we diligently adhered to the established guidelines set forth by the esteemed Institutional Animal Ethics Committee at the renowned University of Lucknow. By meticulously following these ethical considerations, we aimed to uphold the highest standards of moral responsibility and ensure the welfare and well-being of the animals involved in our study.

- Both of these photoperiodic settings were included as primary experimental controls since they were thought to have the most impact on the outcomes of the study.
- First, we tried a scenario called "long days" (LD), in which the birds would experience 16 hours of light and 8 hours of darkness to mimic their natural migratory schedule.
- Second, non-migratory circumstances were represented by "short days" (SD), with 8 hours of daylight and 16 hours of darkness.

- The purpose of this research was to learn more about the physiological changes that occur in migrating songbirds, with a focus on the blackheaded bunting. It was determined that the observed alterations were related to migration by comparing them to the behavior of the Indian weaver bird, a non-migratory species that uses the same habitat during the overwintering season.

- During the experiment, several different readings and analysis were taken:

- The size of the testes and the total mass of the body were measured to track the physiological changes that occur during migration and reproduction.

- To ensure the birds had the proper nourishment, we fed them a mixture of cereal grains and supplemental food.

- Metabolites and triglyceride levels were analyzed from the serum that was isolated from the blood samples that were taken.

- The research looked at variations in the expression of proteins in the muscles used for flying. Proteins in the wing muscles of both species were separated and analyzed using SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Two-Dimensional Gel Electrophoresis (2D-GE) methods.

    - Quantitative Real-Time Polymerase Chain Reaction (qPCR): qPCR is a molecular method used to assess changes in gene expression in response to photoperiod, and tissue samples were kept for future examination.

The study's overarching objective was to learn about the physiological and biochemical changes that occur in migrating birds as they get ready for the long journey ahead. The goal of this study was to discover essential proteins and molecular pathways involved in migration-related adaptations by comparing the responses of migratory and non-migratory species.

- The birds were killed after the experiment was over, and samples of their blood and flying muscle tissue were taken for study. During this phase, the following actions were taken:

- Triglyceride levels in the serum were tested since they are a good predictor of how much energy is being stored and how much is being used.

    - Protein Separation: Extracts of proteins were obtained by homogenizing and centrifuging tissue from the flying muscles. The Lowry technique was used to quantify the amount of protein present. Differences in the protein profiles of the blackheaded buntings' flight muscles were identified between short-day (SD) and long-day (LD) circumstances when the proteins were separated using SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Researchers can now see the different protein expression levels side by side.

Differentially expressed proteins in flying muscle tissue were further characterized using Two-Dimensional Gel Electrophoresis (2D-GE), a more sophisticated approach. Separation of the proteins by molecular weight and charge gave a more complete picture of the protein make-up.

Mass spectrometry (MS-MS) was used to analyze the differentially expressed protein spots discovered using 2D-GE. MS-MS is a method for determining a protein's identity based on its mass and charge. This method aids in identifying proteins and elucidating their probable functions in the body.

During the course of this scientific investigation, the primary objective was to discern and delineate the intricate and intricate physiological and molecular alterations that transpire within avian species engaged in migratory behavior when subjected to extended periods of daylight, as opposed to their non-migratory counterparts existing within the confines of abbreviated daylight cycles. The primary objective of the researchers was to discern and elucidate the

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fundamental proteins and intricate molecular pathways that are intricately linked to the physiological alterations associated with migration in the blackheaded bunting, a species of avian known for its long-distance migratory behavior. In order to achieve this goal, the researchers conducted a comparative analysis by juxtaposing the aforementioned migratory species with the Indian weaver bird, a non-migratory avian species. This comparative approach allowed the researchers to discern and isolate the key proteins and molecular pathways that are specifically implicated in the migration-related physiological changes observed in the blackheaded bunting. These physiological changes encompassed a wide array of intricate processes, including but not limited to energy storage and utilization, muscular adaptations, and reproductive activity. By meticulously investigating and scrutinizing these molecular mechanisms, the researchers aimed to gain a comprehensive understanding of the intricate interplay between the aforementioned physiological alterations and the underlying molecular processes, thereby contributing to the existing body of knowledge in the field of avian migration.

The primary objective of the experiment was to discern and elucidate the intricate physiological and molecular alterations that transpire within avian species engaged in the process of preparing for their arduous migratory journeys. Through the utilization of a comparative approach, this study aimed to elucidate the intricate mechanisms that underlie migratory adaptations, energy storage dynamics, muscle modifications, and reproductive activity in both migratory and non-migratory species, all under varying photoperiodic conditions. By delving into these multifaceted aspects, a deeper understanding of the intricate interplay between these physiological processes can be attained, shedding light on the fascinating phenomenon of migration in the animal kingdom. The utilization of this all-encompassing methodology facilitated the acquisition of profound insights into the intricate mechanisms implicated in the phenomenon of avian migration, thereby engendering a heightened comprehension of the physiological alterations that endow birds with the capacity to embark upon their extraordinary voyages.

Quantitative real - time PCR (qPCR)

The relative mRNA expression of FABP, myoglobin, and Creatine kinase was measured by performing qPCR at a 1:5 dilution on an optical 96 - well reaction plate (cat no. # 4306737) on an ABI 7500 thermal cycler, normalized to - actin. The approach is outlined in great length in the "General Materials and Methods" section. Target and reference gene forward (F) and reverse (R) primer sequences were as follows:

Myoglobin (127 bp):	F (5'AAGGGTAATCATGAGGCCGAGTTG3'),
	R (5'GCAGCGTGTTTTTCAGCAAGGAC3')
FABP (157 bp):	F (5'GCACCTTCAAGAACACCGAGATCAC3'),
	R (5'TCGTCTCCTTCCCCTCCCACTTC3')
Creatine Kinase (154bp):	F (5'GCGCACGGGGAGAAGCATTAAG 3'),
	R (5'CCTGCTCCGTCATGGCCTTCA 3')
β - actin (162 bp):	F (5'GCCCTGGCACCTAGCACAATGA3'),
	R (5'TGCGGTGGACAATGGAGGGT3')

3.1 STATISTICS

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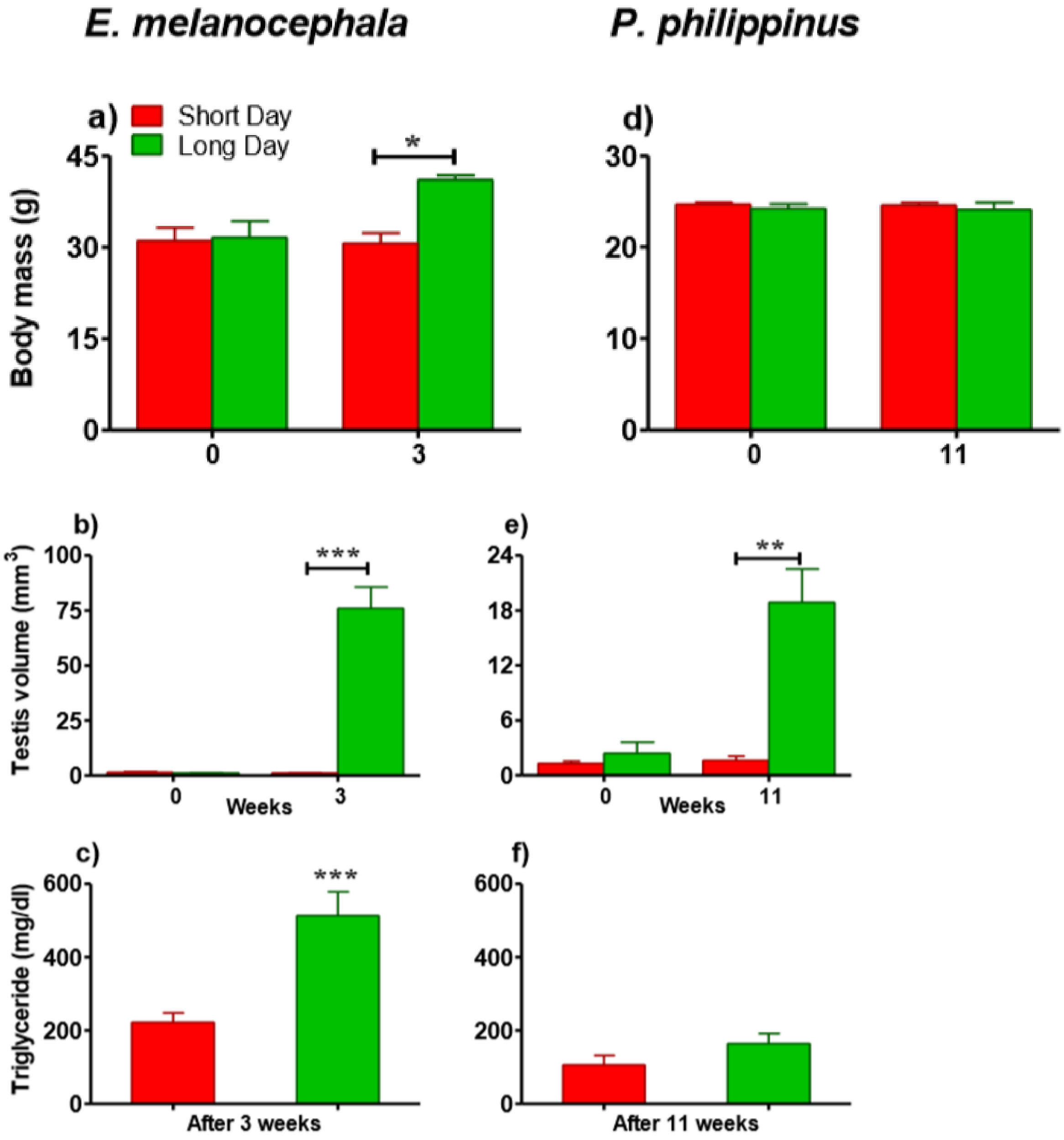

Figure 6: Mean (SEM; n = 5 or 6) changes in body mass (a, d), testis volume (b, e) and serum triglycerides levels (c, f) in the blackheaded bunting (E. melanocephala; left panel, a-c) and Indian weaver bird (P . philippinus; right panel, d-f) exposed to short days (SD, 8L: 16D) and long days (LD, 16L: 8D).The significance of difference between SD and LD groups at final measurements has been shown by asterisks at P < 0.05 (*), P< 0.001 (**) and P < 0.0001(***) levels (Student's t-test).

Introduction to Migration Physiology: Birds undergo physiological changes during migration, a phenomenon seen in songbirds undertaking latitudinal migrations. These migrations involve long journeys twice a year between breeding and wintering grounds. The physiological changes are adaptations to optimize energy utilization and cover migration routes efficiently.

Photoperiodic Regulation of Migration: The timing of migration is closely linked to photoperiod,the length of daylight hours. Migratory birds respond differently to changing day lengths, with springmigration occurring when

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day lengths increase. Previous studies have shown that migratory phenotypes like body fattening, weight gain, and migratory restlessness (Zugunruhe) are induced bylong days.

Energy Metabolism during Migration: Migratory birds accumulate energy reserves before migration, stored as carbohydrates, lipids (triglycerides), and proteins. Carbohydrates provide initial energy, while lipids are the primary energy source, and proteins help with tissue maintenance. Enzymes like creatine kinase play a role in energy refueling.

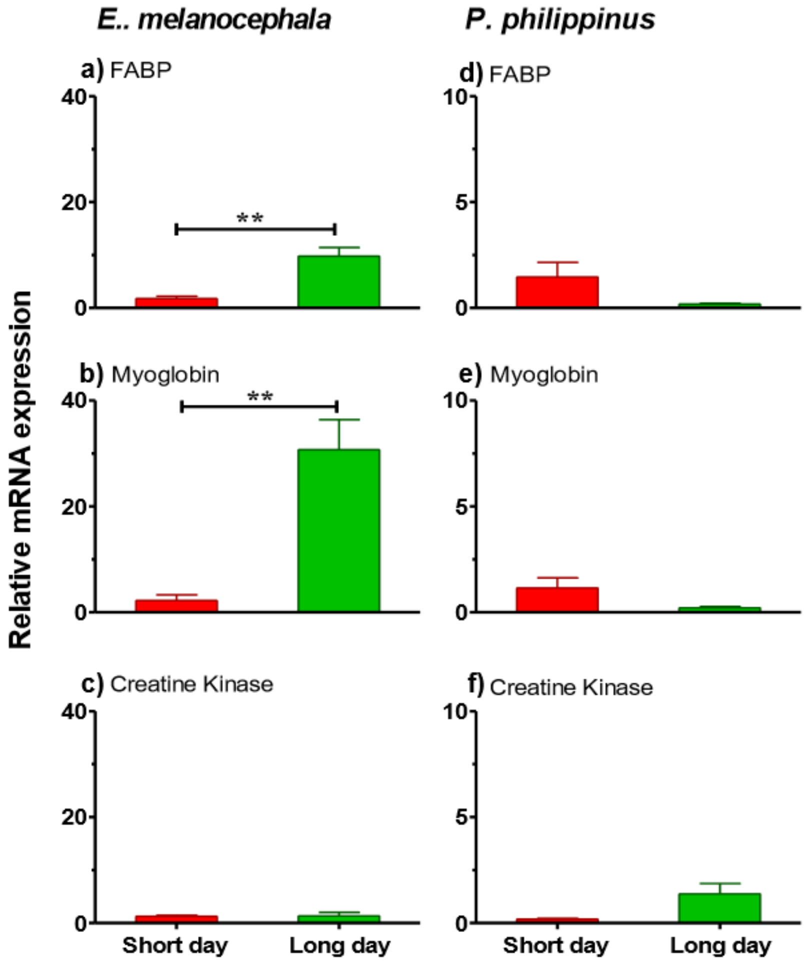

Figure 7: SEM of fatty acid binding protein (FABP), myoglobin, and creatine kinase (CK) mRNA expressions in flight muscle after exposure to short (8L: 16D) and long (16L: 8D) days in blackheaded bunting (E. melanocephala, n = 5) and Indian weaverbird (P. philippinus, n = 3). Buntings' expression levels change between short and long days, as shown by the use of an asterisk (**) to denote statistical significance (P 0.001; Student's t-test).

Proteins Involved in the Uptake and Binding of Fatty Acids Fatty acids (FAs) are a crucial source of energy for migrating birds. Essential for bringing FAs into cells are proteins called fatty acid binding proteins (FABPs). FABP at the plasma membrane (FABPpm) aids in FA uptake. Utilization of FAs, maintenance of intracellular metabolic pathways, and defense of cellular structures are only a few of the many functions performed by FABPs.

Myoglobin, which is present in muscle cells, is responsible for storing oxygen in working tissues and facilitating its transit. Aerobic activities like flight rely on its presence since it has a stronger affinity for oxygen than hemoglobin.

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The blackheaded bunting (Emberiza melanocephala) is the subject of this investigation; it is a long-distance migrant that spends the winter in India but spends its breeding seasons in Western Asia and Europe. Buntings exhibit migratory characteristics when the days are long.

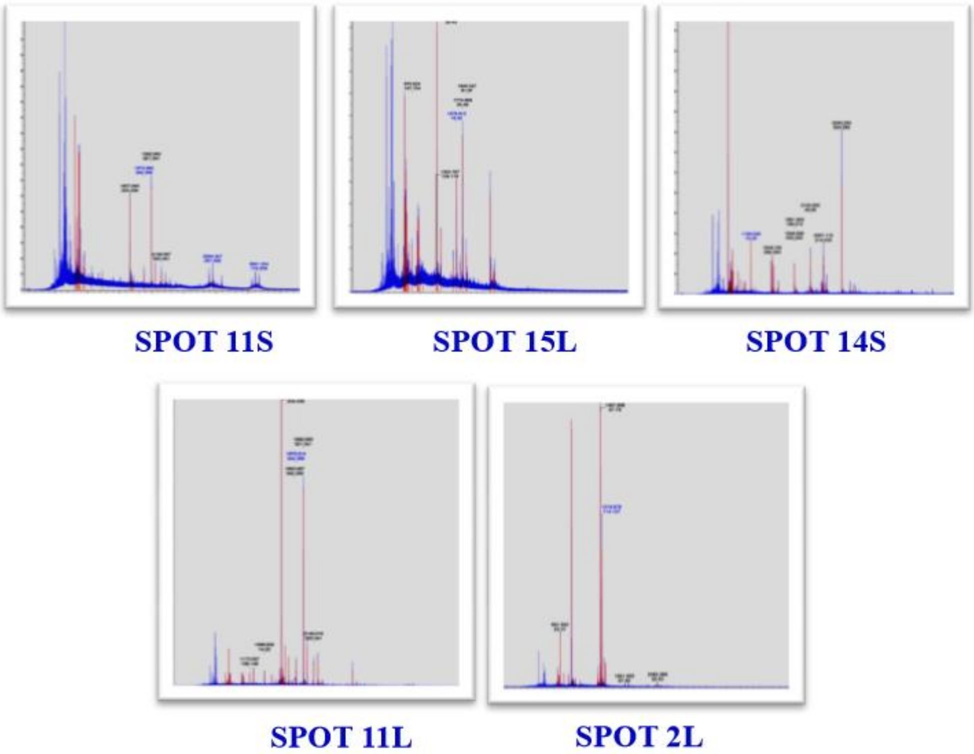

Figure 8: Mass spectra representation of spots identified by 2D gel electrophoresis showing differential expression under short days and long days in migratory blackheaded bunting (spot 11S and 11L: creatine kinase; spot 15L: myoglobin; spot 2L: putative fatty acid binding protein; spot 14S: hair keratin).

- The experimental design and protocol included exposing male wild-caught buntings and weaver birds to both short (SD) and long (LD) days to acclimatize them. Serum triglyceride levels, body fat percentage, and testicular volume were also assessed. SDS-PAGE and 2D-GE were used to examine protein profiles in flight muscles.

- Bunting Physiological Alterations: Compared to SD buntings, buntings given LD had larger bodies, testes, and serum triglycerides. Buntings, but not weaver birds, have different protein profiles in their flying muscles when tested with SDS-PAGE under SD and LD conditions.

- Differentially expressed proteins, like as fibroblast activation protein (FABP) and myoglobin, were found in the flying muscles of the bunting using mass spectrometry. enhanced FABP and myoglobin gene expression in buntings under LD was verified by semi-quantitative and quantitative PCR tests, suggesting higher energy needs and enhanced oxygen supply during migration.

- Results and Discussion: The physiological alterations seen in LD-exposed buntings are consistent with their increased readiness for migration. A high myoglobin level indicates strenuous physical activity during migration, whereas an elevated FABP level suggests higher fatty acid metabolism for energy. Muscle injury may be indicated by elevated CK levels, although further research is needed into how CK is regulated at the transcriptional level.

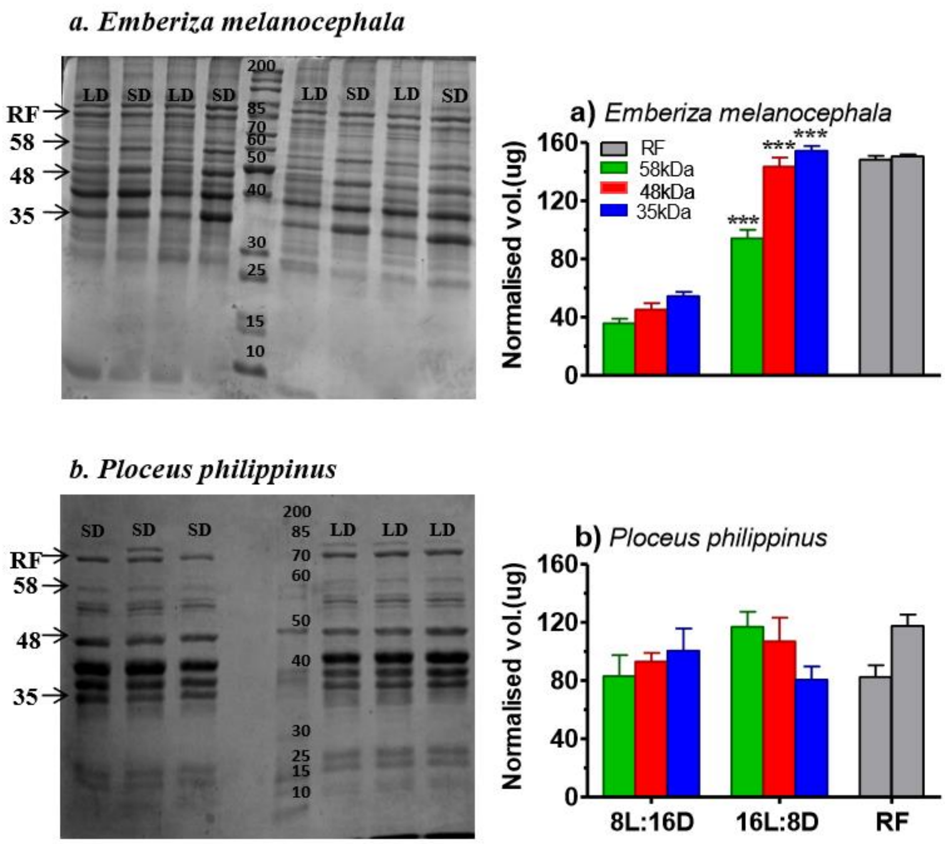

Figure 9: Protein profiles of flying muscles were compared between short-day (8L:16D) and long-day (16L:8D) conditions in blackheaded buntings (E. melanocephala; n = 4) and weaver birds (P. philippinus; n = 3), respectively (left panel). Densitometric quantification (in g for n = 4 gel replicates) of differentially expressed proteins is shown in the right panel. Differentially expressed proteins (58kDa, 48kDa, and 35kDa) change significantly (P 0.0001; Student's ttest) between short and long days.

These findings shed light on the physiological adaptations that enable migratory birds to undertake their arduous journeys, and the study provides valuable insights into the regulatory mechanisms behind these changes.

Photoperiodic Response and Reproductive State: The study indicates that the response to photoperiod differs between species due to their different life history strategies. Bunting's rapid response to long days reflects their need to synchronize activities with the highly seasonal temperateenvironment, while weaver birds' slower response might be due to the flexibility allowed by their less predictable tropical habitat.

Testis Recrudescence and Reproductive Timing: Testis recrudescence (reproductive development)was induced in both species under LD, with buntings showing faster and more pronounced response.Testis size gradation indicated reproductive maturation stages. LD-induced testis development confirms the role of photoperiod in regulating breeding activities in both species.

Serum Triglycerides and Migration: Elevated serum triglyceride levels under LD in buntings correlate with their pre-migratory phenotype. Triglycerides likely provide energy for migration, butthe exact role needs further investigation. ANCOVA analysis suggests a strong link between triglyceride levels and body mass gain, possibly both serving migration preparation.

Role of FABP in Migration: Fatty acid binding proteins (FABPs) play a significant role in transporting fatty acids into cells. The increased expression of FABP under LD conditions suggestsa direct link to the utilization of fatty acids for energy during migration. This increased energy demand aligns with the body fattening and weight

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gain observed in migratory buntings under long days.

Myoglobin and Oxygen Supply: Elevated myoglobin expression under LD conditions indicates a greater oxygen demand, likely due to intensified physical activity during migration. Myoglobin's roleas an oxygen buffer is crucial during long flights and potential oxygen deficits, particularly at higher altitudes, ensuring efficient energy metabolism during migratory flights.

Creatine Kinase and Muscle Damage: Creatine kinase (CK) levels serve as a marker for muscle damage during migration. While protein levels of CK increased under LD conditions, transcript levels did not differ between SD and LD. This suggests that CK expression might be regulated at thetranslational level rather than transcriptional regulation.

Implications and Significance: The study provides crucial insights into the physiological mechanisms underlying the preparation for migration in migratory birds. It highlights the role of photoperiodic cues in regulating various physiological changes that are necessary for the successful execution of long-distance migrations.

Limitations and Future Directions: While the study sheds light on various physiological changes associated with migration, there are some limitations. Further research could delve into the detailedmolecular mechanisms behind the observed changes in FABP, myoglobin, and CK expression. Investigating the specific metabolic pathways affected by these proteins during migration could provide deeper insights into energy utilization and muscle function.

Comparative Approach and Conservation Implications: The comparative approach, contrastingmigratory and non-migratory species, adds to the study's significance. Understanding how different species respond to photoperiodic cues and the physiological changes they undergo could have broader implications for conservation strategies, especially for migratory species facing environmental changes and habitat loss.

Methodological Considerations: The study's methodology, including capturing birds from the wild,acclimatizing them to captivity, and using controlled light conditions, was designed to mimic natural conditions as closely as possible. However, some inherent limitations of captivity studies, such as potential stress effects, should be acknowledged.

Integrated Approach for Holistic Understanding: The study integrates physiological, molecular,and behavioral aspects to provide a holistic understanding of migration preparation. Combining thesedifferent levels of analysis offers a more comprehensive picture of how birds adapt to seasonal changes and long migrations.

Future Studies and Conservation Implications: Future research could explore the role of other proteins and metabolic pathways in migratory preparation. Additionally, studying the effects of anthropogenic factors, such as light pollution, on migratory phenotypes could have implications forbird conservation and management.

Contributions to Avian Migration Research: Overall, this study contributes significantly to the field of avian migration research by uncovering the intricate physiological changes associated with migration preparation. It adds to our understanding of how birds respond to environmental cues andadapt to challenging long-distance migrations.

Synthesis of Findings: The study provides strong evidence that long photoperiods induce physiological changes in migratory buntings, preparing them for migration. These changes include body fattening, elevated serum triglycerides, increased expression of FABP and myoglobin, and upregulated CK levels. Such changes collectively facilitate successful migration.

4. CONCLUSION

The study of photoperiod-induced physiological and biochemical changes in migratory and non-migratory birds, specifically focusing on the blackheaded bunting and the Indian weaver bird, reveals crucial insights into the adaptations necessary for successful migration. The research demonstrates that long-day photoperiods trigger significant increases in body mass, serum triglycerides, and testis size in migratory buntings, preparing them for their arduous journeys. These changes are accompanied by differential expression of key proteins such as fatty acid binding protein (FABP) and myoglobin in flight muscles, indicating heightened energy demands and oxygen transport efficiency during migration. The study underscores the vital role of photoperiod as a trigger for these physiological changes, which are absent in the non-migratory Indian weaver bird, highlighting the species-specific nature of these adaptations. This research contributes to our understanding of how migratory birds optimize their physiology to meet the extreme demands of long-distance travel, providing valuable insights for conservation strategies in the face of environmental changes.

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