



## SYMPOSIUM

# During Stopover, Migrating Blackcaps Adjust Behavior and Intake of Food Depending on the Content of Protein in Their Diets

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From the symposium “Environment, Energetics, and Fitness: A Symposium Honoring Donald W. Thomas” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2011, at Salt Lake City, Utah.

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**Synopsis** During migration, birds undergo alternating periods of fasting and re-feeding that are associated with dynamic changes in body mass ( $m_b$ ) and in organ size, including that of the digestive tract. After arrival at a migratory stopover site, following a long flight, a bird must restore the tissues of its digestive tract before it can refuel. In the present study we examined how the availability of dietary protein influences refueling of migrating blackcaps (*Sylvia atricapilla*) during a migratory stopover. We tested the following predictions in blackcaps deprived of food and water for 1–2 days to induce stopover behavior: (1) birds provided with a low-protein diet will gain  $m_b$ , lean mass and fat mass, and increase in pectoral muscle size slower than do birds fed a high-protein diet; (2) since stopover time is shorter in spring, birds will gain  $m_b$  and build up fat tissue and lean tissue faster than in autumn; and (3) if low dietary protein limits a bird's ability to gain  $m_b$  and fat reserves, then birds that do not obtain enough protein will initiate migratory restlessness (*Zugunruhe*) earlier than will birds with adequate dietary protein. These predictions were tested by providing captured migrating blackcaps with semisynthetic isocaloric diets differing only in their protein content. Each day, we measured  $m_b$ , and food intake; also lean mass and fat mass were measured using dual energy X-ray absorptiometry. In addition, we monitored nocturnal activity with a video recording system. In both spring and autumn, birds fed diets containing either 3 or 20% protein increased in  $m_b$ , lean mass and fat mass at similar rates during the experiment. However, the group receiving 3% protein ate more than did the group receiving 20% protein. In support of our predictions,  $m_b$ , lean mass, fat mass, and intake of food all were higher in spring than in autumn. We also found that in spring all birds had higher levels of migratory restlessness, but birds fed 3% protein were less active at night than were birds fed 20% protein, possibly an adaptation conserving energy and protein. We conclude that protein requirements of migrating blackcaps during stopover are lower than expected, and that birds can compensate for low dietary protein by behavioral responses, i.e. hyperphagia and decreased migratory restlessness, that ensure rapid refueling.

## Introduction

Every year, migrating birds make long seasonal journeys from their breeding grounds to their wintering grounds and back. These sustained flights, which may be hundreds or even thousands of kilometers at a stretch (Berthold 1996), pose huge physiological challenges for birds, and require much preparation before the journey, and refueling along the way.

During migration, relatively short periods of endurance flight alternate with longer periods of feeding at stopover sites, where energy and nutrient reserves are rebuilt in preparation for the next leg of the journey (Hedenström and Ålerstam 1997). These alternating periods of flight and stopover result in dynamic changes in body mass ( $m_b$ ).

The spring and autumn migratory seasons differ in many respects. In spring, when migrating to their breeding grounds, birds fly towards higher latitudes, where day length is longer (Bauchinger and Klaassen 2005). Also, in spring, migrants are constrained by time, since early arrival at the breeding grounds increases the bird's probability of finding a territory and hence its likelihood of reproductive success (Smith and Moore 2005). In autumn, birds migrate towards the southern hemisphere where days are shorter and they do not always acquire territories at the final destination (Newton 2008). Therefore, in spring, stopover time is minimized and  $m_b$  is gained as fast as possible (Alerstam and Lindström 1990). In contrast, in autumn migration, there is apparently little benefit from very early arrival at the wintering grounds (Newton 2008). Therefore, during this season migrants are less constrained by time. However, before crossing an ecological barrier, such as the Sahara desert, birds must accumulate large reserves of energy. Thus, birds spend more time at stopovers in autumn than in spring (Stutchbury et al. 2009) and gain more  $m_b$ , but do so at a slower rate than in spring (Safriel and Lavee 1988; Lavee et al. 1991; Frumkin et al. 1995; Izhaki and Maitav 1998).

In flight, most birds cannot forage and must therefore rely on endogenous energy resources and metabolic water. Two main types of oxidative fuels are used by birds: lipids, stored as triglycerides in adipose tissue, and protein stored in various organs. About 95% of the energy required for flight comes from stored fat and the remaining ~5% from protein (Bauchinger and Biebach 1998; Jenni and Jenni-Eiermann 1998).

Bauchinger et al. (2005) measured the masses of organs of migrating garden warblers (*Sylvia borin*) before and after they crossed the Sahara, and found a decrease in lean mass of the digestive organs, heart, liver, kidneys, and muscles. Similar changes were found for dry lean mass of songbirds before and after long-distance migration and simulated in-flight starvation (Biebach 1998; Schwilch et al. 2002; Biebach and Bauchinger 2003; Bauchinger and Biebach 2005).

Since birds do not eat in flight the digestive system is superfluous, but at a stopover birds need to refuel as fast as possible and the digestive system becomes crucial (Karasov et al. 2004). Fasting and restriction of food reduces the size of the digestive organs of passerine birds and limits their initial food intake upon re-feeding (McWilliams and Karasov 2005). For example, Hume and Biebach (1996) found a reduction of 44% in the digestive tract

and small intestine in migratory garden warblers after 48 h of starvation designed to simulate in-flight fasting. In studies on blackcaps done under laboratory conditions as well as in the field, it was found that food intake in previously fasted birds did not start to increase substantially until the second day of re-feeding as tissues began to be rebuilt (Hume and Biebach 1996; Gannes 1999; Karasov and Pinshow 2000; Bauchinger et al. 2009).

Low rates of food intake may lower the rate of renewal of tissue and reduce the rate of increase in  $m_b$ , thereby affecting the length of the stopover that may, in turn, cause late arrival at the final destination (Alerstam and Lindström 1990; Gannes 2002).

Birds that are held in captivity during a migratory season often exhibit intense activity at night. Wagner (1930) called this phenomenon "Zugunruhe" which translates to "migratory restlessness." It has been shown that the pattern of migratory restlessness reflects the pattern of actual migration in several species of song birds (including Sylvid warblers) that migrate long distances (Gwinner 1986). Migratory restlessness is influenced by the bird's  $m_b$  and fat levels (Fusani et al. 2009). In the laboratory, migratory restlessness in birds first deprived of food and then re-fed increased during food deprivation, was then depressed when feeding began, and subsequently re-appeared as  $m_b$  and fat levels increased (Biebach 1985; Gwinner et al. 1985). In the field, birds arriving at a stopover site where there was little food usually do not stay for more than a day, resuming flight that same night (Biebach 1985; Yong and Moore 1993), which indicates that expression of migratory restlessness may be related to variations in quantity of food at stopover sites along the migratory route.

Since a limiting factor for a bird's readiness to leave a stopover site is its ability to restore the tissues of its digestive tract and maximize its food intake rapidly (McWilliams and Karasov 2005), it must obtain a sufficient amount of protein in its diet. Therefore, we hypothesized that the proportion of protein in the diet will affect the rate of restoring lean mass and subsequently the rate of increase in fat reserves

Furthermore, given that during spring, stopover times are minimized because birds are heading towards their breeding grounds, we hypothesized that birds gain  $m_b$  and build up fat and lean tissue faster than in autumn. In addition, we predicted that the intensity of migratory restlessness is influenced by a bird's ability to renew its fuel supplies successfully. We further predicted that nocturnal activity is higher in spring than in autumn. In order to test

these predictions, during both spring and autumn migrations, we used freshly caught migrating blackcaps that were re-fed after a period of food deprivation intended to simulate migratory in-flight starvation.

## Methods

### Bird and maintenance

Blackcaps are one of the most common small Palearctic passerine migrants that stopover in the Negev desert after crossing the Sahara and Sinai deserts (Shirihai et al. 1996). Twenty-five blackcaps were captured at Midreshet Ben-Gurion (E 34°57'; N 30°51'), using mist nets, during the spring and autumn migration seasons (March–April and October) of 2009. All birds were banded for individual identification, weighed to  $\pm 0.1$  g and transferred to individual metal cages (35.5 × 24.7 × 37 cm) with two perches, and two bowls containing *ad libitum* water and food. Birds were kept in a temperature-controlled animal room maintained at  $35 \pm 2^\circ\text{C}$  by day and at  $15 \pm 2^\circ\text{C}$  by night, with a light cycle that simulated the natural light cycle, thereby ensuring the birds retained migratory behavior.

### Experimental procedure

Prior to the experiment, birds were habituated to laboratory conditions for 7 days and provided with a standard diet for insectivorous birds: 25% (by dry mass) commercial insect-based food (Fettmischung fein, Aleckwa, Tiernahrung, Altrip Germany), 30% minced hard boiled eggs, 21% curd, 15% bread crumbs, 6% ground egg shells, 3% minced beef heart and 0.53% vitamins (Vitakalk and moult vitamins) (Gwinner et al. 1988), supplemented with 20–50 mealworms per day until they attained a  $m_b$  of 17–21 g. They were then deprived of food and water. These were removed at lights-out and birds remained deprived of food and water for 1 or 2 more days in order to induce stopover behavior (Hume and Biebach 1996, Gannes 1999, Karasov and Pinshow 2000) and to bring all birds to a similar low body mass between 13 and 14 g, i.e. the mass of a bird arriving after crossing an ecological barrier. Since they were unable to exercise, we assumed that the fatter birds used up their excess fat before they began to metabolize significant protein (Cherel et al. 1988; Lindgård et al. 1992; Schilch et al. 2002)

After the fast, birds were randomly assigned to one of two groups that differed in the protein content of their semi-synthetic isocaloric diets: high-protein (20% protein, 55% carbohydrate, 10% fat) spring:  $n=5$ , autumn:  $n=6$ ; low-protein (3% protein,

71% carbohydrate, 10% fat) spring:  $n=8$ , autumn:  $n=7$ . *Ad libitum* food and water were provided for 6 days, to simulate feeding at a stopover. Birds that lost  $m_b$  to below 13 g were removed from the experiment. At the end of the experiment, birds were allowed 3–4 days to recover before release.

During the simulated stopover, food was replaced three times each day to ensure the birds always had access to fresh food. All food offered, and orfts, were weighed ( $\pm 0.02$  g) for each bird, and gross food intake (by wet mass) per day was calculated. Food was supplied after birds were weighed in the morning and then at  $\sim 4$  h intervals, and was finally removed 10–15 min before lights-out at night. To account for evaporation, two food bowls were placed between the cages; these bowls were also weighed and replaced three times per day. Fresh batches of food were prepared every 3–4 days and kept in a refrigerator at  $6^\circ\text{C}$ .

In order to test the effects of dietary protein content on accumulation of lean and fat tissue and  $m_b$ , we measured body composition by dual energy X-ray absorptiometry (DEXA) using a Lunar PIXImus™ 2 (General Electric Medical, Fitchburg, WI) that was previously calibrated for small birds (Korine et al. 2004). The birds were lightly anaesthetized with Isoflurane for the duration of the scan. Birds were scanned twice on the morning of the first, third and last days of the experiment before they were allowed to feed. In addition, every morning, immediately at lights-on, we weighed all birds ( $\pm 0.02$  g).

In order to observe nocturnal activity and evaluate the birds' readiness to resume migratory flight, we used an infrared video camera. Each day the camera was activated one hour before lights-off and activity was recorded for the entire night until lights-on the following morning. We quantified migratory restlessness by monitoring activity for the first 2 min of every 20-min interval during the entire night. For each 2 min, the observer assigned either a 1 for "active" or a 0 for "inactive." Next, the activity score of each bird was calculated by summing the number of 2-min periods during which it was active and dividing by the total number of 20-min periods for the night.

### Statistical analysis

To analyze and compare data from spring and autumn, we used two-way RM ANOVA with sampling day as the within-subject variable, and group and season as the between-subject factors. Food deprivation time was included as a covariant in all analyses, but since it was not significant in any, we

removed it as a variable. Nocturnal behavior was analyzed by ANCOVA in a general linear model with the birds'  $m_b$  on the first day of re-feeding as a covariant, sampling day as the within-subject variable, and groups receiving different protein content as the between-subject factor. Before running RM ANOVA and ANCOVA, residuals were tested for normality of distribution (Kolmogorov–Smirnov tests), and all data were tested for homoscedasticity (Levene's test) and sphericity. The Huynh-Feldt Epsilon (H-F) correction was used when the assumption of sphericity was not met. When the data were not normally distributed or the assumption of homoscedasticity was violated, either a log a logit transformation was applied which remedied the situation. Tukey's *post hoc* test was used to compare differences between the days and groups and seasons. Values are presented as means  $\pm$  SD ( $n$ ). All statistical analyses were done using STATISTICA 7®.

## Results

### Body mass

Body mass of blackcaps increased during the stopover (Fig. 1; day effect:  $F_{2,43} = 0.32$ ,  $P < 0.00001$ ), and this increase was similar for birds on high- and on low-protein diets (day  $\times$  group effect:  $F_{2,43} = 0.32$ ,  $P = 0.35$ ) and for birds in spring and in autumn (day  $\times$  season effect:  $F_{2,43} = 0.32$ ,  $P = 0.39$ ).

### Lean and fat mass

During the 6 days of re-feeding, lean mass increased significantly in both groups (Fig. 2; day effect:  $F_{2,40} = 0.96$ ,  $P < 0.00001$ ); furthermore lean mass was higher in birds fed the 20% protein diet (group effect:  $F_{1,21} = 8.09$ ,  $P = 0.001$ ) and overall lean mass was higher in spring than in autumn (season effect:  $F_{1,21} = 13.18$ ,  $P = 0.002$ ). We found no significant interaction between day  $\times$  group ( $F_{2,40} = 0.96$ ,  $P = 0.45$ ) or day  $\times$  season ( $F_{2,40} = 0.96$ ,  $P = 0.49$ ). Fat mass increased significantly in both groups (Fig. 3; day effect:  $F_{2,36} = 0.86$ ,  $P < 0.00001$ ), but only after the second day of re-feeding (from  $1.99 \pm 0.99$  g to  $3.07 \pm 0.1$  g; Tukey's *post hoc* test,  $P > 0.001$ ). Also, overall fat mass was higher during spring migration (season effect:  $F_{1,21} = 20.8$ ,  $P < 0.001$ ). We found no significant interaction between day  $\times$  group ( $F_{2,36} = 0.86$ ,  $P = 0.21$ ) or day  $\times$  season ( $F_{2,36} = 0.86$ ,  $P = 0.17$ ).

### Intake of food

Mass-specific food consumption was significantly higher in spring than in autumn (Fig. 4; day  $\times$  season effect:  $F_{4,89} = 0.84$ ,  $P < 0.00001$ ). In addition, mass-

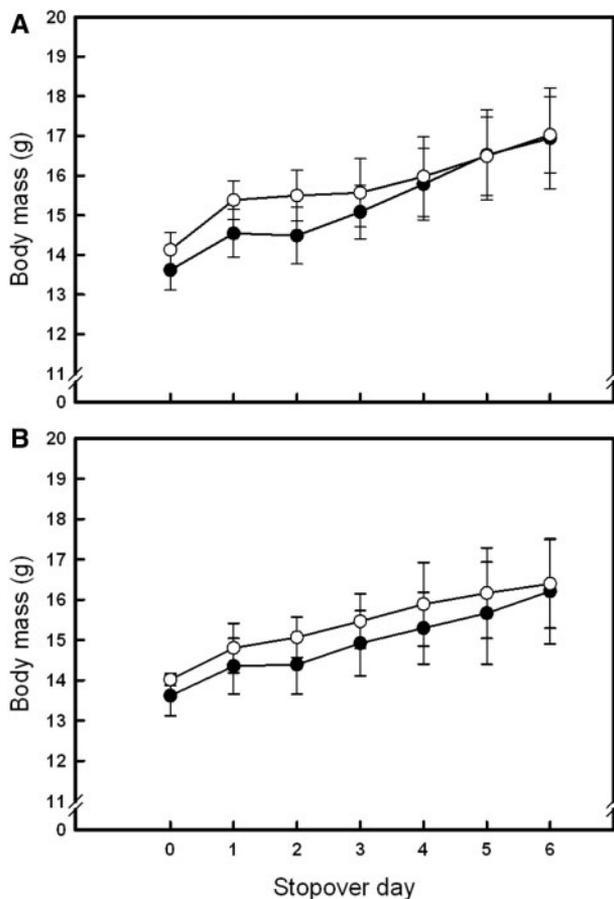
specific food consumption was higher in the group receiving 3% protein than in the group receiving 20% protein (day  $\times$  group effect:  $F_{4,89} = 0.84$ ,  $P = 0.0001$ ).

### Migratory restlessness

Nocturnal behavior was significantly affected by the quantity of protein in the diet. Initial  $m_b$  was found to be a significant co-variant for the level of nocturnal activity (co-variant:  $F_{1,8} = 8.84$ ,  $P = 0.02$ ). In both groups, nocturnal behavior significantly increased during the 6 days of stopover (Fig. 5; day effect:  $F_{5,40} = 1$ ,  $P < 0.001$ ). In both seasons, the group fed 20% protein had a higher level of nocturnal activity (group effect:  $F_{1,8} = 7.35$ ,  $P = 0.02$ ) and in spring their nocturnal activity was higher than in autumn (season effect:  $F_{1,8} = 14.18$ ,  $P < 0.01$ ). We found no significant interaction between day  $\times$  group ( $F_{5,40} = 1$ ,  $P = 0.5$ ) or day  $\times$  season ( $F_{5,40} = 1$ ,  $P = 0.1$ ).

## Discussion

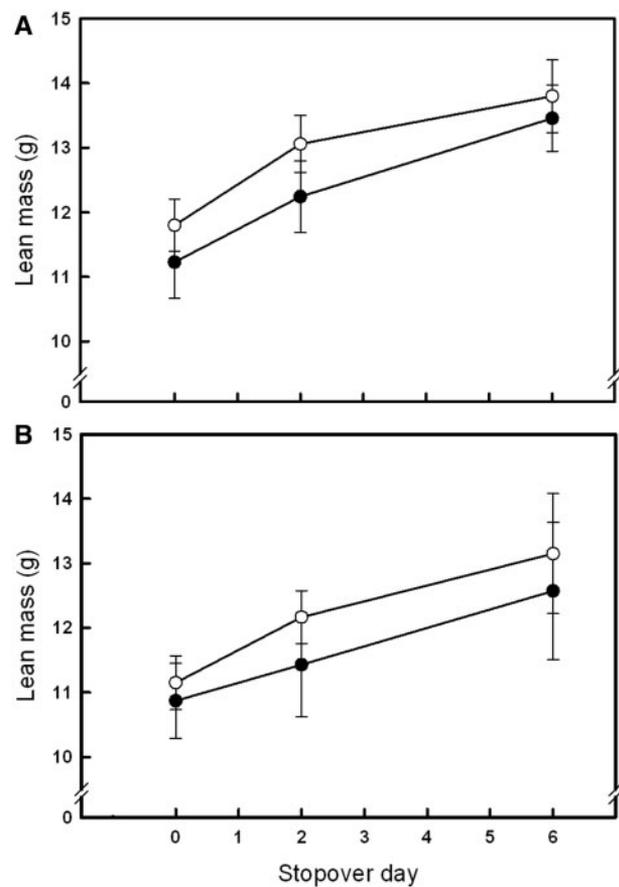
During both seasons, the group of blackcaps fed the 3% protein diet had a higher rate of food intake and lower levels of migratory restlessness than did those fed 20% protein. However, we found no differences in  $m_b$ , lean or fat mass between the two groups, which strongly suggests that the behavioral responses, i.e. hyperphagia and reduced migratory restlessness, in the group fed 3% protein compensated for the low dietary protein. This increased food intake is a known compensatory response of migratory songbirds faced with low-protein diets (Bairlein 1987, 1991; Pierce and McWilliams 2004) and has been invoked to explain how frugivorous birds compensate for the nutrient deficiency of fruits (Bairlein 1987; Witmer 1998; Witmer and Van Soest 1998); fleshy fruits being generally regarded as a poor nutritional source, containing mostly carbohydrates and little protein or fat (Izhaki and Safriel 1989; Karasov 1996; McWilliams and Karasov 2001). It was found that songbirds, artificially switched from an insect diet to a fruit diet, can indeed gain in  $m_b$  and fat reserves by increasing food intake (Parrish 1997; McWilliams and Karasov 2001; Bairlein 2002). Furthermore, Goymann et al. (2010) reported that birds in better body condition leave a stopover site earlier, indicating that body mass and fat deposition affects migratory restlessness in a way similar to that found under caged conditions (Fusani et al. 2009). In the present study, we show for the first time that migratory songbirds rebuild energy and nutrient stores by compensating for low protein in their



**Fig. 1** Mean body mass ( $m_b$ )  $\pm$  SD of blackcaps during migration in spring (**A**) and autumn (**B**). Birds were fed either a high (20%) or low (3%) protein diet for 6 days, after being deprived of food for 1–2 days. Average  $m_b$  for each diet group for the two seasons is shown: 3% (filled circle, spring  $n=7$ ; autumn  $n=8$ ), 20% (open circle, spring  $n=5$ ; autumn  $n=6$ ). No differences were found in  $m_b$  between the groups or between seasons. See text for statistical details.

diet in two complementary ways: increased food intake and decreased migratory restlessness.

Although lean mass was higher in birds fed the 20% protein diet, no difference was found in the rate of increase in adipose tissue; this may be ascribed to the increased nocturnal activity observed in this group. In the present study the low-protein diet contained 3% protein, a value that is lower than the requirements for maintenance in white crowned sparrows (5.3% *Zonotrichia leucophrys*) (Murphy 1993), or garden warblers ( $\sim$ 5% *Sylvia borin*) (Bairlein 1998), suggesting that protein requirements during migratory stopovers are not as high as we predicted. This concurs with the data of Langlois and McWilliams (2010) who found that the protein requirements of hermit thrushes (*Catharus guttatus*) and white-throated sparrows (*Zonotrichia albicollis*) decreased during the migratory season, although

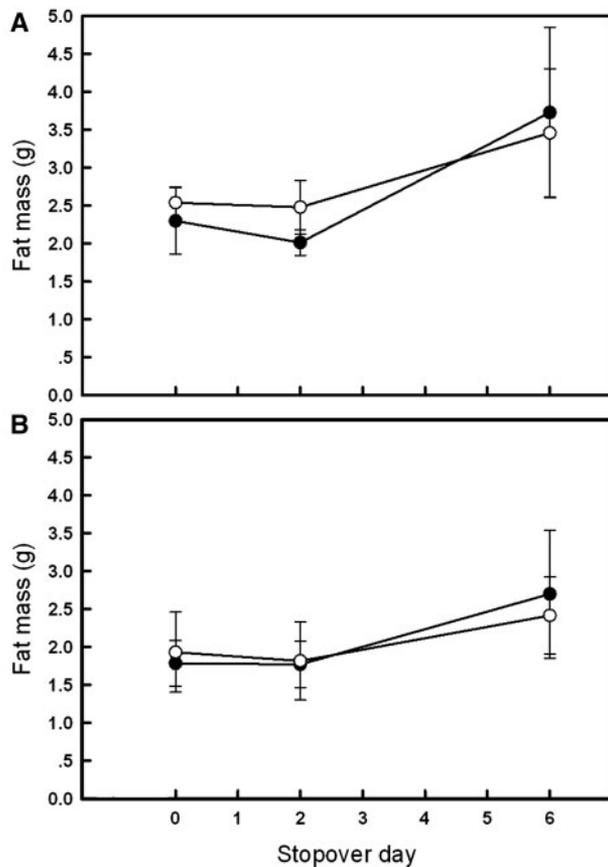


**Fig. 2** Mean lean mass  $\pm$  SD of blackcaps during spring (**A**) and autumn (**B**). Birds were fed either a high (20%) or low (3%) protein diet for 6 days, after being deprived of food for 1–2 days. Average  $m_b$  for each diet group for the two seasons is shown: 3% (filled circle, spring  $n=7$ ; autumn  $n=8$ ), 20% (open circle, spring  $n=5$ ; autumn  $n=6$ ). Lean mass was higher during spring, increased significantly during the experiment and was higher in birds fed the 20% protein diet. See text for statistical details.

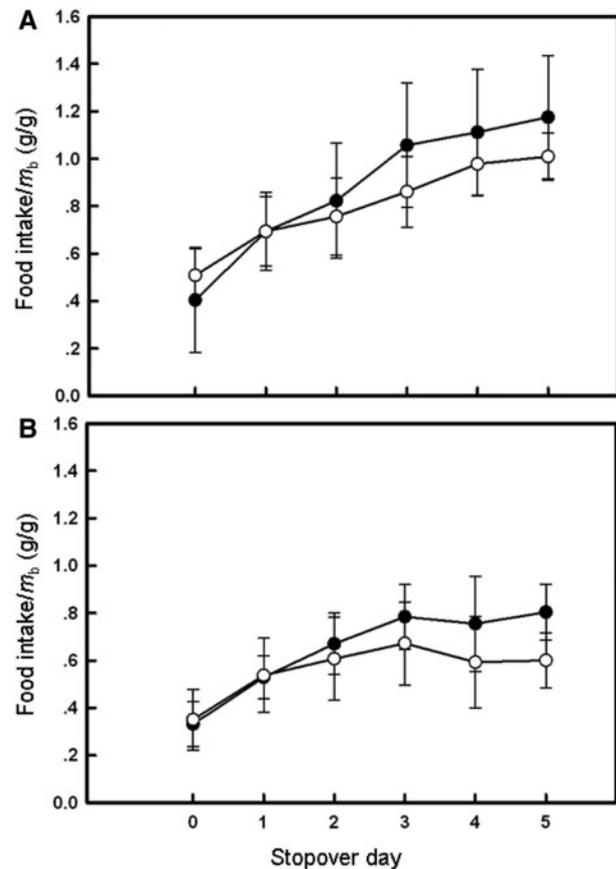
Langlois and McWilliams (2010) studied pre-migratory birds, not birds at stopover.

We also found that lean mass increased throughout the experiment, whereas fat mass increased only after an increase in lean mass took place on the second day of re-feeding, which indicates that protein reserves need to be recovered before fat reserves can be replenished.

As predicted, in spring migration  $m_b$ , lean mass and fat mass were higher than in autumn. This may be due to the difference in the nature of the two migratory seasons. An optimal migration strategy during spring would be to minimize stopover time and gain  $m_b$  as fast as possible, since an early arrival at the breeding grounds increases the chance of finding a successful territory and thereby would improve reproductive success (Alerstam and Lindström 1990). In contrast, in autumn there is little benefit gained



**Fig. 3** Mean fat mass  $\pm$  SD of blackcaps during spring (A) and autumn (B). Birds were fed either a high (20%) or low (3%) protein diet for 6 days, after being deprived of food for 1–2 days. Average  $m_b$  for each diet group for the two seasons is shown: 3% (filled circle, spring  $n=7$ ; autumn  $n=8$ ), 20% (open circle, spring  $n=5$ ; autumn  $n=6$ ). Fat mass was higher during spring and increased only after the second day. See text for statistical details.

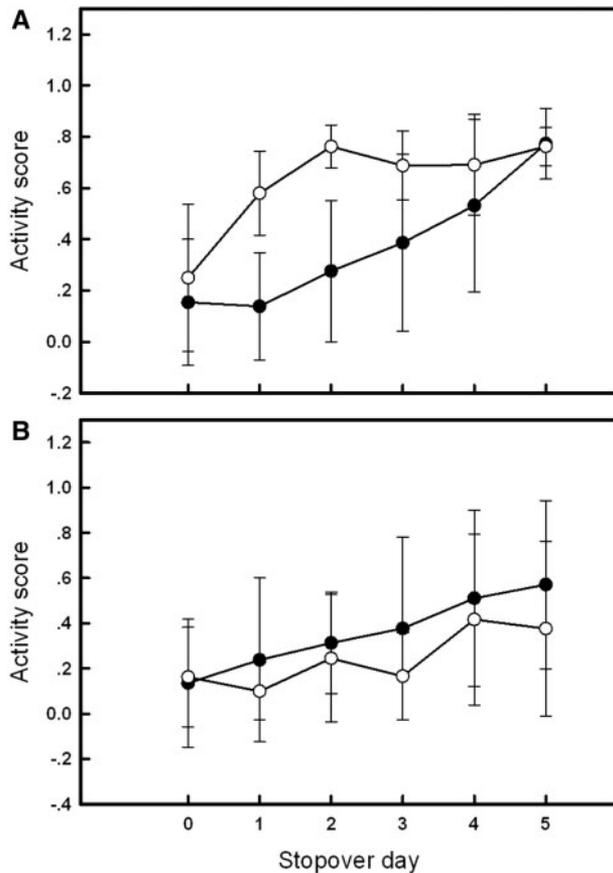


**Fig. 4** Mass-specific food consumption  $\pm$  SD of blackcaps during spring (A) and autumn (B). Birds were fed either a high (20%) or low (3%) protein diet for 6 days, after being deprived of food for 1–2 days. Average  $m_b$  for each diet group for the two seasons is shown: 3% (filled circle, spring  $n=7$ ; autumn  $n=8$ ), 20% (open circle, spring  $n=5$ ; autumn  $n=6$ ). The rates of food intake were different between the groups and were higher during spring; see text for statistical details.

by an early arrival at the wintering grounds. Therefore, the autumn journey is less constrained by time, but the crossing of broad ecological barriers requires large reserves of energy. Hence, birds can refuel more slowly, but need to depart the last stopover site preceding the barrier with higher  $m_b$  (Safriel and Lavee 1988; Lavee et al. 1991; Frumkin et al. 1995; Izhaki and Maitav 1998). Further, the rate of increase in intake of food in spring was higher than in autumn. This too may be because during spring birds are time-constrained, whereas during autumn they are not and, therefore, can consume food at a lower rate (Alerstam and Lindström 1990; Lavee et al. 1991; Izhaki and Maitav 1998).

We found migratory restlessness to be significantly influenced by the bird's  $m_b$  on the first day of stopover; a result similar to other laboratory and field studies that showed that migratory restlessness is strongly influenced by the birds' body condition

( $m_b$  and fat mass) (Biebach 1985; Yong and Moore 1993; Fusani et al. 2009). Contrary to our prediction, we found that when the initial  $m_b$  on the first day of stopover is taken into account (used as a covariant), migratory restlessness was significantly more pronounced in the group fed 20% protein than in the group fed 3% protein. Thus, it seems that migratory restlessness is not only correlated with body condition, but may also be related to the nutritional content of the food. It has been hypothesized that, when faced with a stopover, a migratory bird undergoes a decision-making process that integrates the birds energetic state, environmental conditions *en route*, time in the season, weather conditions and the quality of the stopover site (Biebach et al. 1986; Safriel and Lavee 1988). Our results add to this that the caliber of a stopover site is not only defined by the abundance of food but also by the quality of food;



**Fig. 5** Nocturnal activity (mean  $\pm$  SD) of blackcaps during spring (A) and autumn (B). Birds were fed either a high (20%) or low (3%) protein diet for 6 days, after being deprived of food for 1–2 days. Average  $m_b$  for each diet group for the two seasons is shown: 3% (filled circle, spring  $n=8$ ; autumn  $n=8$ ), 20% (open circle, spring  $n=4$ ; autumn  $n=6$ ). Nocturnal behavior was higher during spring and in the group fed 20% protein. Additionally, in both groups, nocturnal behavior increased during the 6 days of stopover. See text for statistical details.

therefore, a bird may be inclined to leave a site that has an abundance of low-quality food but stay longer at a site that has less food, but it is of high quality. Since birds provided with 3% protein did not differ in  $m_b$ , or fat mass from those fed 20% protein, the lower level of nocturnal activity exhibited by birds fed the 3% protein diet may be a mechanism that conserves energy at night and preserves protein for the re-building of the digestive organs. An alternative explanation is that the groups on 20 and 3% protein diets did not differ from each other in  $m_b$  or fat mass at lights-on when they were weighed because those fed the 20% protein diet were more active during the night after gaining more  $m_b$  during the previous day than was true of birds fed the 3% protein diet (3% having provided them with enough reserves to attempt to resume migratory flight, but which resulted in loss of fat mass and lean mass).

The differences that we found in the level of migratory restlessness between the two seasons is consistent with the time constraints that migrants face during spring, but not during autumn (Alerstam and Lindström 1990).

In summary, our results indicate that protein requirements are relatively low during migratory stopover. During stopover in either spring or autumn dietary protein may be limiting, but if so, birds can compensate behaviorally, especially during the first days when the digestive tract is being re-built. We also found that songbirds, at least under controlled experimental conditions, are able to refuel, not only on diets that are largely insect-based and relatively high in protein, but also on diets that are relatively low in protein which are similar to a diet largely based on fruit. Our study is relevant to the migration of songbirds in the wild because many species switch diet from insects to mostly fruit. A diet of fruit is low in protein but high in carbohydrates and unsaturated fatty acids that can be used to synthesize storage lipids. Compared to insects, fruits are found in patches, are easy to locate and require less time and energy to acquire. Furthermore, fruits are easier to digest owing to their low content of fiber (McWilliams and Karasov 2001; Bairlein 2002; Newton 2008), which makes fruit a favorable food source during re-fueling at stopover sits when time is at a premium and foraging efficiency is essential.

## Acknowledgments

The authors thank Ishai Hoffmann for his technical help; Bruria Gal for help catching blackcaps; and Agusti Muñoz-Garcia and Amos Bouskila for their help with statistical analyses. The authors also thank Agusti Muñoz-Garcia, Marshall McCue, Carmi Korine, Miri Ben-Hamo, Oren Amitai, Shai Pilosof, and Darren Burns for their advice and help along the way. Finally, the authors thank two anonymous reviewers for their constructive criticism and insightful comments.

## Funding

This research was done under permit # 9017/2008 of the Israel Nature and National Parks Protection Authority and was supported by US–Israel Binational Science Foundation grant 2005119 to B.P. and S.R.M., and by Sigma Xi Grant-in-Aid of Research number G200810150517 to O.M. This is publication 735 of the Mitrani Department of Desert Ecology.

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