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PROTEIN REQUIREMENTS OF AN OMNIVOROUS AND A GRANIVOROUS SONGBIRD DECREASE DURING MIGRATION

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Abstract.—Many songbirds are seasonally frugivorous and eat primarily fruit during migration and insects or seeds during nonmigratory periods. Previous work has suggested that most wild fruits may have inadequate protein for birds. Assessing the nutritional adequacy of fruit requires knowing the protein requirements of birds in relation to the composition of available fruits. We tested predictions of two hypotheses: (1) interspecific differences in protein requirements of birds are related to their foraging strategy; and (2) protein requirements of birds increase with demand, for example during migratory periods of the annual cycle. We measured the protein requirements of the omnivorous Hermit Thrush (*Catharus guttatus*) and the granivorous White-throated Sparrow (*Zonotrichia albicollis*) during nonmigratory and migratory stages of the annual cycle and compared the results with published estimates for other songbird species. In the nonmigratory state both species ate less, lost body mass, and had more negative nitrogen balance as dietary protein decreased. In the migratory state Hermit Thrushes lost body mass and had lower nitrogen balance but did not reduce food intake as dietary protein decreased, whereas White-throated Sparrows did not change body mass, food intake, or nitrogen balance as dietary protein decreased. Both species had lower protein requirements during migration (9.3 mg N day^{-1} and 15.8 mg N day^{-1}, respectively) than during nonmigratory periods (53.1 mg N day^{-1} and 46.0 mg N day^{-1}, respectively) when fed a diet containing 15.9 kJ g^{-1}. These findings may partially explain how birds can adequately refuel on protein-limited foods such as fruits during migration. *Received 17 December 2008, accepted 6 April 2010.*

Key words: *Catharus guttatus*, fruit, Hermit Thrush, migration, nitrogen balance, protein requirements, songbirds, White-throated Sparrow, *Zonotrichia albicollis*.

Los Requerimientos Proteicos de un Ave Canora Omnívora y una Granívora Disminuyen Durante la Migración

Resumen.—Muchas aves canoras son frugívoras estacionalmente: se alimentan principalmente de frutos durante la migración y de insectos o semillas durante los períodos no migratorios. Estudios previos han sugerido que la mayoría de los frutos silvestres podrían tener un contenido proteico inadecuado para las aves. Para evaluar el valor nutricional de los frutos es necesario conocer los requerimientos proteicos de las aves en relación con la composición de los frutos disponibles. Evaluamos las predicciones de dos hipótesis: (1) las diferencias interespecíficas en los requerimientos de proteína de las aves están relacionadas con sus estrategias de forrajeo y (2) los requerimientos proteicos de las aves aumentan con la demanda, por ejemplo durante los períodos migratorios del ciclo anual. Medimos los requerimientos proteicos de la especie omnívora *Catharus guttatus* y de la granívora *Zonotrichia albicollis* durante estadíos migratorios y no migratorios del ciclo anual y comparamos los resultados con estimados publicados para otras especies de aves canoras. En el estadío no migratorio, ambas especies comieron menos, perdieron masa corporal y presentaron un balance de nitrógeno más negativo al reducirse el contenido proteico en la dieta. En el estadío migratorio, los individuos de *C. guttatus* perdieron masa corporal y presentaron un balance de nitrógeno menor, pero no disminuyeron la ingesta de alimento con las disminuciones en la proteína. Los individuos de *Z. albicollis* no cambiaron su masa corporal, ingesta de alimento, ni balance de nitrógeno al reducirse la proteína. Ambas especies presentaron menores requerimientos proteicos durante la migración (9.3 mg N día^{-1} y 15.8 mg N día^{-1}, respectivamente) que durante los períodos no migratorios (53.1 mg N día^{-1} and 46.0 mg N día^{-1}, respectivamente) al ser alimentadas con una dieta que contenía 15.9 kJ g^{-1}. Estos hallazgos podrían explicar parcialmente cómo es que las aves pueden reaprovisionarse ingiriendo alimentos limitados en proteína, como los frutos, durante la migración.

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Birds rest and refuel at stopover sites during migration, and the quality and quantity of foods at these sites can influence the rate at which fat and protein stores are replenished (Schaub and Jenni 2001a, Bairlein 2002, Piersma 2002, Pierce and McWilliams 2004) and, hence, the pace of migration (Schaub and Jenni 2001b). Many songbirds in temperate eastern North America and western Europe switch from eating mostly insects during spring and summer to eating mostly fruit during autumn migration (Herrera 1984, Levey and Karasov 1989, Bairlein 1996, Klasing 1998). Seasonal frugivory may benefit birds during autumn migration because fruits are often abundant at stopover sites and are a relatively high-energy food (Herrera 1987, Bairlein 1990, Bairlein and Simons 1995, Smith et al. 2007). Songbirds select habitats with greater fruit densities while at stopover sites during autumn migration (Parrish 1997, Herrera 1998, Takanose and Kamitani 2003), and songbirds that ate more fruit fattened at faster rates while at stopover sites with greater fruit densities (Parrish 1997, Smith and McWilliams 2010). However, wild fruits offer consumers mostly simple carbohydrates or fats, relatively little protein, much seed bulk and water content, and a mixture of secondary compounds (Herrera 1984, Johnson et al. 1985, Witmer 1998). Thus, fruits may provide adequate energy but inadequate protein for birds during migration, especially compared with alternative foods such as insects, seeds, and grains (Herrera 1987, Jordano 1988, Levey and Karasov 1989). A primary focus of the present study is to determine whether wild fruits can satisfy the protein requirements of migratory songbirds.

Berthold (1976) proposed that the protein content of fruit may limit its consumption by omnivorous songbirds. For example, captive Garden Warblers fed only fruit were unable to maintain body mass, whereas those fed fruit supplemented with protein maintained or increased body mass (Berthold 1976; scientific names of all bird species are given in Table 4). Many other experiments have also shown that fruit may be nutritionally inadequate in that birds were unable to maintain body mass while eating only fruit for several days (Izhaki and Safriel 1989, Levey and Karasov 1989, Izhaki 1992, Bairlein 1996). Witmer (1998) demonstrated that highly frugivorous birds such as Cedar Waxwings were able to maintain body mass on synthetic diets with only 1.5% protein but that more omnivorous thrush species were unable to maintain body mass on the same diets. Pryor et al. (2001) and Singer (2003) also suggested that frugivorous birds may have lower nitrogen requirements as adaptations to their low-protein diets. Therefore, interspecific differences in the protein requirements of songbird species may be related to their feeding strategy.

Protein requirements of birds may also depend on their physiological state (Koutsos et al. 2001a). For example, the protein requirements of female birds increase during egg laying (Robbins 1981), and chicks need more protein during growth than adults require for maintenance (Roudybush and Grau 1986, Koutsos et al. 2001b). Murphy and King (1992) estimated that the protein requirement of White-crowned Sparrows during molt was 45% above their minimum maintenance requirement. Protein requirements may increase during migration because protein can be catabolized as a fuel and water source, especially when fat reserves are depleted (Klaassen 1996, Bauchinger and Biebach 1998, Jenni and Jenni-Eiermann 1998). Thus, protein requirements are unlikely to be fixed for a given bird but instead may change with nutritional demands across the annual cycle.

Our study tested predictions of two related hypotheses: (1) interspecific differences in protein requirements of birds is related to their foraging strategy; and (2) protein requirements of birds increase with demand, for example during migratory periods of the annual cycle. To test these hypotheses, we measured the protein requirements of two passerine species, the White-throated Sparrow and the Hermit Thrush, during nonmigratory and migratory periods of their annual cycles and compared these estimated protein requirements with published estimates for other songbird species. We selected the White-throated Sparrow and Hermit Thrush as focal species for our study because these birds are similar in body mass (on average, 26 g and 31 g, respectively), both are seasonally frugivorous but to different extents (fruits composed 80% of diet during autumn migration in Hermit Thrushes and 60% in White-throated Sparrows; Parrish 1997), and they differ in general foraging strategy during nonmigratory periods (omnivorous Hermit Thrush, granivorous White-throated Sparrow). We predicted that the more seasonally frugivorous Hermit Thrush (Jones and Donovan 1996) would have lower protein requirements than the less seasonally frugivorous White-throated Sparrow (Falls and Kopachena 1994) but that the protein requirements of both species would be higher than those of other migratory birds that eat mostly fruit year-round. We also predicted that the protein requirements of both species would increase during the migratory period as protein turnover (Pennycuick 1998, Schwilch et al. 2002) and metabolic rate increased (Scott et al. 1994, Piersma 2002). We also determined the extent to which the protein availability of wild fruits in southern New England satisfies the estimated protein requirements of these two migratory songbirds and other species.

METHODS

Capture and maintenance of birds.—White-throated Sparrows ($n = 22$) and Hermit Thrushes ($n = 20$) were captured using mist nets during fall migration in 2006 in Kingston, Rhode Island (41°28′N, 71°31′W). The birds were transferred to indoor facilities and housed individually in stainless-steel cages (59 × 45 × 36 cm) at constant temperature (23°C) and a constant photoperiod representative of natural photoperiod at capture (12 h light:12 h dark [12L:12D]; lights on at 0800 hours). During the 10-week acclimation period, birds were provided ad libitum food and water along with about 8 to 10 mealworms (*Tenebrio molitor*) each day.

The nutrient composition of the acclimation diet (diet A) was similar to that of many natural high-carbohydrate fruits (59.5% carbohydrates, 12.8% protein, and 8.0% fats; Table 1). Casein and L-crystalline amino acids were the sole source of dietary protein. This agar-based mash diet is similar to the semisynthetic banana-based diet of Denslow et al. (1987), with modifications as described in Afik et al. (1997) and Podlesak and McWilliams (2006). The composition of the essential amino acids was based on the semisynthetic diets described in Murphy and King (1982) that satisfy the maintenance requirements of White-crowned Sparrows (Murphy 1993). Body mass was measured daily (±0.1 g), and birds remained in good health.

Maintenance diets and experiment.—After the 10-week acclimation period, we offered birds only the acclimation diet (no mealworms) for the next 6 weeks. We randomly assigned birds to one

Table 1. Composition of the acclimation diet (diet A). The other three experimental diets were the same as this acclimation diet except that glucose replaced casein as dietary protein was decreased.

Ingredients	Dry mass (%)	Wet mass (%)
Glucose (g)	59.5	17.9
Casein[a] (g)	10.0	3.0
Amino acid mix[b] (g)	2.8	0.8
Vitamin and mineral mix[c] (g)	2.5	0.8
Salt mix[d] (g)	5.5	1.7
Cellulose[e] (g)	5.0	1.5
$NaHCO_3$[f] (g)	1.5	0.5
Choline chloride[f] (g)	0.2	0.1
Olive oil[g] (g)	8.0	2.4
Agar[h] (g)	5.0	1.5
Water (g or mL)	0	70.0

[a] Casein (high N): U.S. Biochemical, Cleveland, Ohio.
[b] Amino acid mix: Fisher Scientific, Pittsburg, Pennsylvania; Murphy and King 1982.
[c] AIN-76 Vitamin and Mineral Mix, ICN Biomedicals, Irvine, California.
[d] Salt mix: Briggs-N Salt mixture, ICN Biomedicals.
[e] Celufil-hydrolyzed: U.S. Biochemical.
[f] $NaHCO_3$ and choline chloride supplied by Fisher Scientific.
[g] Botticelli brand olive oil.
[h] Agar bacteriological grade: U.S. Biochemical.

of four diet groups that differed in dietary protein and carbohydrate concentration (diet A: 12.8%, 59.5%; diet B: 9.7%, 62.2%; diet C: 6.7%, 65.6%; and diet D: 3.6%, 68.7%, respectively). The dietary protein concentrations were chosen so that birds in some groups (diets A and B) were fed diets with adequate protein whereas birds in other groups (diets C and D) were fed diets with inadequate protein given the protein requirements of White-crowned Sparrows (Murphy 1993). Diets were isocaloric because we replaced proteins (casein; Table 1) with carbohydrates (glucose) and these two macronutrients have similar energy density (protein: 17.8 kJ g^{-1}, carbohydrate: 17.6 kJ g^{-1}; Schmidt-Nielsen 1997).

We conducted 3-day total-collection trials (Murphy 1993, Robbins 1993) for each diet group for both species during the 17th week of captivity. Birds were transferred from the maintenance diet (diet A) to their respective group diets on day 1 of the total-collection trials. At 0800 hours each day during these 3-day trials we measured each bird's body mass, provided each bird with ad libitum fresh food, and weighed the food that remained from the previous day. We also collected all feces produced by each bird during the previous 24 h, along with samples of food offered and remaining. All samples were stored frozen at −20°C for later analysis.

We dried the samples of remaining food at 47°C until sample mass was constant (~1 week). The samples of offered food and feces that were used for measuring protein content were freeze-dried to constant mass (2 days) to ensure that nitrogen was not lost during drying. Dried samples were homogenized using mortar and pestle. Nitrogen concentration in 1.5- to 3.5-mg subsamples was measured using a Carlo-Erba NA 1500 Series II elemental analyzer attached to a continuous-flow isotope-ratio Micromass Optima spectrometer (F-IRMS). Urea and powdered dogfish muscle (DORM-2) reference material (National Research Council, Institute for Environmental Chemistry, Ottawa) were used as working standards for nitrogen concentration analysis. Additional replicates (2–8) were measured as needed until each sample's coefficient of variation (CV) was <20% for samples with <5% nitrogen (N) or until CV was <15% for samples with >5% N. We measured energy content of food and feces using a Parr 1266 Isoperibol Oxygen Bomb Calorimeter.

We estimated daily apparent metabolizable energy (AME; kJ day^{-1}) as follows: $AME = (F \times FE) - (D \times DE)$, where F is amount of food consumed (g dry mass [DM] day^{-1}), FE is food energy density (kJ g^{-1} DM), D is amount of feces (g DM day^{-1}), and DE is feces energy density (kJ g^{-1} DM). The efficiency of dietary energy utilization was estimated as AME divided by gross energy content of ingested food (Servello et al. 2005). We estimated nitrogen balance (mg day^{-1}) as the N intake minus N lost in urine and feces; the equation is as follows: $N\ balance = (F \times FN) - (D \times DN)$, where F is amount of food consumed (g DM day^{-1}), FN is nitrogen content of the food (mg N g^{-1} DM), D is amount of feces (g DM day^{-1}), and DN is nitrogen content of the feces (mg N g^{-1} DM) (Murphy 1993).

Photoperiod stimulation and migration experiment.—Because birds have a strong circadian and circannual rhythm, we used both photoperiod stimulation (exposure to longer days) and time of year to induce the migratory state (Bairlein 1990, Bairlein and Gwinner 1994, Gauthreaux 1996, Helm and Gwinner 2005). In general, the migratory state is associated with a significant increase in nightly activity, hyperphagia, and body mass (Bairlein and Gwinner 1994). On 23 March 2007, four Hermit Thrushes and four White-throated Sparrows were randomly assigned to a control group, placed in a separate room, and maintained on the same constant light levels (12L:12D; lights on at 0800 hours). Three birds were excluded prior to the migratory-state experiment because they did not maintain body mass. Light levels for the treatment birds (14 Hermit Thrushes and 17 White-throated Sparrows) were then incrementally changed over 11 days from 12L:12D to a photoperiod representative of spring at the Rhode Island latitude (16L:8D; lights on at 0800 hours). Treatment and control birds were fed the acclimation diet (diet A), and we measured body mass and food intake daily. This allowed us to determine when treatment birds increased body mass and food intake as expected for birds in the migratory state. We determined that a bird was in the migratory state when its body mass increased by at least 7% within 5–7 days (King and Farner 1965). For simplicity we designated birds that underwent premigration hyperphagia and fattening as "in migratory state," because actual migration did not take place. The control birds (4 Hermit Thrushes and 4 White-throated Sparrows) exposed to constant light levels did not significantly change in body mass ($t = -1.63$, df = 3, $P = 0.202$ and $t = -2.42$, df = 3, $P = 0.094$, respectively), and so, as expected, they never transitioned into the migratory state. However, these nonsignificant results must be cautiously interpreted because of the small sample size.

Nineteen of 31 birds (9 Hermit Thrushes and 10 White-throated Sparrows) exposed to longer day length increased their body mass by, on average, 10.6 ± 2.8% within 5–7 days (or, on average, 2.9 ± 1.8 g for Hermit Thrushes, $t = -4.77$, df = 8, $P = 0.001$; and 2.8 ± 1.0 g for White-throated Sparrows, $t = -9.01$, df = 9, $P < 0.001$). The 12 birds that did not increase body mass did not participate in the remainder of the experiment. Once we determined that birds were in the migratory state, we assigned them sequentially to one of the three diet groups used during the maintenance experiment (B, C, D; Table 1). The acclimation diet (diet A) was not tested during the migratory experiment, to increase sample size for the other three diet groups. We conducted 3-day total-collection trials on experimental birds in the migratory state while simultaneously conducting total-collection trials on control birds of the same species. This procedure resulted in the 19 experimental birds starting their 3-day total-collection trials 15–24 days after the change in day length. All birds were fed diet A prior to the start of the 3-day total-collection trials, and diet B, C, or D thereafter. We conducted total-collection trials on control birds over the same period in order to compare treatment and control birds on the same days.

Statistical analysis.—We used paired *t*-tests to determine the effect of photoperiod stimulation on body mass and food intake in control and treatment birds. General-linear-model repeated-measures analysis was used to estimate changes in body mass, food intake, and N balance in control birds throughout the migratory-state experiment to determine whether they remained in the nonmigratory state. Linear mixed modeling (LMM) was used to determine the effect of treatment, day, and their interaction on N balance for all birds in the nonmigratory and migratory states. Multiple covariance structures of LMM were analyzed to determine the most accurate model on the basis of Hurvich and Tsai's information criterion (Lindsey and Jones 1998). For simplicity, we report degrees of freedom rounded to the nearest whole number. We used analysis of variance (ANOVA) to determine the effect of bird species, physiological state, and dietary protein on N balance, food intake, change in body mass, feces excretion, percent N in feces, and AME for each species on day 3 of the migratory- and nonmigratory-state experiments. We also used ANOVA to compare percent energy utilization among birds fed protein-sufficient diets (diet B) for both species in the nonmigratory and migratory states. We used a conservative P value (0.01) for Levene's test of homogeneity of variance because of the robustness of the ANOVA model; all reported statistical analyses satisfied the assumptions of normality and homogeneity of variance among treatment groups. We estimated protein requirements on the basis of linear regressions of N balance on N intake (mg) for birds on all four diets in the nonmigratory-state experiment and diets B, C, and D in the migratory-state experiment. We used *t*-tests to compare slope and elevation (y-intercept) parameters from these regression equations for the two species in different physiological states (Sokal and Rohlf 1981). Values are reported as means ± SD, and the significance level was set at $P \leq 0.05$. All statistical analyses were conducted with SPSS, version 16.0, for Macintosh (SPSS, Chicago, Illinois).

RESULTS

Confirmation of experimental design.—We first determined whether the birds were acclimated to the experimental diets by day 3 of the 3-day trials. As the birds acclimated to the diets, the N balance of Hermit Thrushes in the nonmigratory and migratory states changed over the 3 days ($F = 3.40$, df = 2 and 46, $P = 0.042$ and $F = 4.71$, df = 2 and 23, $P = 0.019$, respectively), whereas the N balance of White-throated Sparrows changed while in the migratory state ($F = 4.95$, df = 2 and 20, $P = 0.018$) but not while in the nonmigratory state ($F = 2.29$, df = 2 and 43, $P = 0.114$). Given these changes in N balance across the 3-day trials, we focus the remainder of our analyses for both the nonmigratory- and migratory-state experiments on the third day of the 3-day trials because this reduces the potential effects of previous diets and because the birds had, by then, been acclimated to the experimental diets for several days. Murphy (1993) measured protein requirements in birds for a 15- to 18-day period and concluded that the first 3 days were the most accurate because birds fed low-protein diets thereafter catabolized body protein. We assume that any bias associated with the birds not being fully acclimated to the experimental diets by day 3 was equally represented across treatment groups and therefore would not change the relative differences in the estimated protein requirements of these groups.

We increased day length for treatment birds and then monitored their body mass and food intake to verify that this change in daily photoperiod induced the migratory state. As expected, body mass and daily food intake of treatment birds significantly increased within 20 days from the onset of increased day-length exposure (Hermit Thrush: $t = -4.77$, df = 8, $P = 0.001$ and $t = -3.03$, df = 8, $P = 0.016$, respectively; White-throated Sparrow: $t = -9.01$, df = 9, $P < 0.001$ and $t = -7.44$, df = 9, $P < 0.001$, respectively; Table 2), whereas body mass and daily food intake of control birds exposed to constant day length did not change significantly over the same period (Hermit Thrush: $t = -1.63$, df = 3, $P = 0.202$ and $t = -2.30$, df = 3, $P = 0.105$, respectively; White-throated Sparrow: $t = -2.42$, df = 3, $P = 0.094$ and $t = -1.31$, df = 3, $P = 0.282$, respectively; Table 2). We calculated the statistical power to detect a 6-g change in food intake and a 2.8-g change in body mass (treatment effects reported

Table 2. Body mass and food intake (means ± SD) of control (nonmigratory-state) and treatment (migratory-state) Hermit Thrushes and White-throated Sparrows before and after we changed the daily photoperiod to induce the migratory state.

		Body mass (g)		Wet food intake (g)	
Species		Before	After[a]	Before	After[a]
Hermit Thrush	Control ($n = 4$)	30.6 ± 0.7	31.3 ± 1.2	21.1 ± 3.5	24.4 ± 0.8
	Treatment ($n = 9$)	32.1 ± 2.7	34.9 ± 2.7**	22.1 ± 3.6	28.3 ± 4.4*
White-throated Sparrow	Control ($n = 4$)	25.6 ± 2.0	27.3 ± 2.2	21.6 ± 1.9	22.9 ± 1.0
	Treatment ($n = 10$)	24.9 ± 2.0	27.7 ± 1.8**	17.5 ± 2.2	25.7 ± 1.7*

Note: *Significant paired *t*-test ($P < 0.05$) comparing body mass and food intake before and after change in photoperiod.

**Significant paired *t*-test ($P < 0.001$) comparing body mass and food intake before and after change in photoperiod.

[a] Control birds were kept at 12L:12D while treatment birds were changed from 12L:12D to 16L:8D.

in Table 2), assuming $\alpha = 0.05$ and using the estimated within-group variance from our experiment. The power in this case was 84–99% for food intake and 79–99% for body-mass change in control Hermit Thrushes and White-throated Sparrows. Thus, we can be 79–99% certain of detecting this magnitude of change in food intake and body mass for the two species at the 5% level of significance. Because we conducted migration experiments in series over 20 days, we compared body mass, food intake, and N balance of control birds between days 1, 10, and 20 to verify that control birds remained in the nonmigratory state. As expected, control birds did not significantly change in body mass (Hermit Thrush: $F = 3.552$, df = 2 and 6, $P = 0.096$; White-throated Sparrow: $F = 0.644$, df = 2 and 6, $P = 0.558$), food intake (Hermit Thrush: $F = 1.432$, df = 2 and 6, $P = 0.310$; White-throated Sparrow: $F = 3.49$, df = 2 and 6, $P = 0.099$), or N balance (Hermit Thrush: $F = 2.22$, df = 2 and 6, $P = 0.190$; White-throated Sparrow: $F = 4.27$, df = 2 and 6, $P = 0.070$) during the 20-day experimental period. However, sample sizes of control birds were small, so these results should be interpreted cautiously.

Interspecific and physiological-state comparisons.—White-throated Sparrows had more positive N balance and higher food intake, and lost less body mass on day 3 of the total-collection trials, than Hermit Thrushes fed the same diets (species effect: $F = 7.77$, df = 1 and 39, $P = 0.008$; $F = 16.69$, df = 1 and 39, $P < 0.001$; and $F = 5.82$, df = 1 and 39, $P = 0.021$, respectively). In general, birds of both species in the migratory state had more positive N balance and higher food intake than, and similar change in body mass as, birds in the nonmigratory state (state effect: $F = 213.91$, df = 1 and 39, $P < 0.001$; $F = 186.16$, df = 1 and 39, $P < 0.001$; and $F = 0.16$, df = 1 and 39, $P = 0.694$, respectively). However, interpretation of species and physiological-state differences in N balance was complicated because we detected significant interactions among species, physiological state, and diet (species*diet effect: $F = 5.10$, df = 2 and 39, $P = 0.011$; state*diet interaction: $F = 4.10$, df = 2 and 39, $P = 0.024$). By contrast, interpretation of species and physiological-state differences in food intake and body mass were simpler because these interactions were not significant ($P > 0.20$ in all cases). Therefore, we report the remainder of the results separately for Hermit Thrushes and White-throated Sparrows in the migratory and nonmigratory states, so that we can consider these interactions and also because this allows us to include data from all diet groups (four diets for the nonmigratory-state experiment and three diets for the migratory-state experiment).

Nonmigratory-state Experiment

Nitrogen balance, food intake, and body mass for birds in the nonmigratory state.—In general, birds lost body mass, ate less, and were in negative N balance as dietary protein decreased (Fig. 1). Hermit Thrushes and White-throated Sparrows lost, on average, 1.7 ± 1.3 g and 1.8 ± 0.6 g and ate 1.5 ± 1.6 g and 3.1 ± 0.6 g, respectively, when fed diets with <4% protein, whereas the two species gained or maintained body mass and ate significantly more when fed the highest-protein diets (diet effect on body mass: $F = 3.61$, df = 3 and 16, $P = 0.037$ and $F = 20.62$, df = 3 and 18, $P < 0.001$, respectively; diet effect on food intake: $F = 11.94$, df = 3 and 16, $P < 0.001$ and $F = 12.81$, df = 3 and 18, $P < 0.001$, respectively; Fig. 1). Hermit Thrushes and White-throated Sparrows that were fed <4% protein also produced less feces ($F = 5.65$, df = 3 and 16, $P = 0.008$ and $F = 18.14$, df = 3 and 18, $P < 0.001$, respectively) and had lower percent N in their feces ($F = 37.21$, df = 3 and 16, $P < 0.001$ and $F = 24.99$, df = 3 and 18, $P < 0.001$, respectively) than those fed higher-protein diets. However, this reduction in N excretion did not compensate for the lower N intake, in that Hermit Thrushes and White-throated Sparrows that were fed <4% protein had a more negative N balance than those fed the higher dietary protein ($F = 25.75$, df = 3 and 16, $P < 0.001$ and $F = 6.75$, df = 3 and 18, $P = 0.003$, respectively; Fig. 1C, F).

Protein requirements, minimum nitrogen requirements, and total endogenous nitrogen losses of birds in the nonmigratory state.—Protein requirements of birds in the nonmigratory state were estimated from the regression of N balance on N intake for all four diet groups on day 3 of the experiment (Fig. 1C, F). We estimated that the protein requirement of Hermit Thrushes in the nonmigratory state was 332 mg protein day^{-1} (53.1 mg N × 6.25), or ~9.63% protein for a diet containing 14.1 kJ AME g^{-1} DM. Minimum nitrogen requirement (MNR) was calculated from the regression equation as the dietary N intake that supports N equilibrium (Allen and Hume 2001). The MNR of Hermit Thrushes was 718.7 mg N kg$^{-0.75}$ day^{-1}. We estimated that the protein requirement of White-throated Sparrows in the nonmigratory state was 287 mg protein day^{-1} (46.0 mg N × 6.25), or ~6.89% protein for a diet containing 14.5 kJ AME g^{-1} DM, and that the MNR of White-throated Sparrows was 710.4 mg N kg$^{-0.75}$ day^{-1}. Total endogenous nitrogen loss (TENL) was calculated by regressing N balance (N input − N output) against N intake and extrapolating to zero N intake. Our estimates of TENL in Hermit Thrushes and White-throated Sparrows were 308.9 mg N kg$^{-0.75}$ day^{-1} and 254.0 mg N kg$^{-0.75}$ day^{-1}, respectively. We detected no significant difference between regressions (Fig. 1C, F) estimating the protein requirements of Hermit Thrushes and White-throated Sparrows in the nonmigratory state (slope: $t = 0.78$, df = 41, $P = 0.438$; elevation: $t = 0.81$, df = 41, $P = 0.424$), so these results for each species are reported only for comparative purposes. In a separate section below we report estimates of MNR from a common regression for these birds in the nonmigratory state because we detected no significant differences in MNR between species.

Energy utilization and metabolized energy of birds in the nonmigratory state.—Hermit Thrushes and White-throated Sparrows in the nonmigratory state that were fed 12.8% dietary protein had significantly higher AME than birds fed lower dietary protein ($F = 8.43$, df = 3 and 14, $P = 0.002$ and $F = 12.11$, df = 3 and 18, $P < 0.001$, respectively; Table 3). Energy density of feces was less in Hermit Thrushes fed the highest-protein diet ($F = 4.27$, df = 3 and 16, $P = 0.021$), whereas it did not differ among dietary groups in White-throated Sparrows ($F = 0.12$, df = 3 and 18, $P = 0.946$). Hermit Thrushes fed the lowest dietary protein had significantly lower energy utilization (AME/gross energy content of ingested food) than birds fed higher levels of protein ($F = 7.20$, df = 3 and 14, $P = 0.004$), although this was the one comparison for which there was heterogeneity in variance between diet groups (Levene's test, $P = 0.001$). Energy utilization in White-throated Sparrows differed significantly between diet groups ($F = 16.67$, df = 3 and 18, $P < 0.001$). Overall, AME was similar between species ($F = 1.36$, df = 1 and 38, $P = 0.251$), whereas energy utilization was greater in White-throated Sparrows than in Hermit Thrushes ($F = 9.96$, df = 1 and 38, $P = 0.003$). We excluded from these estimates of AME

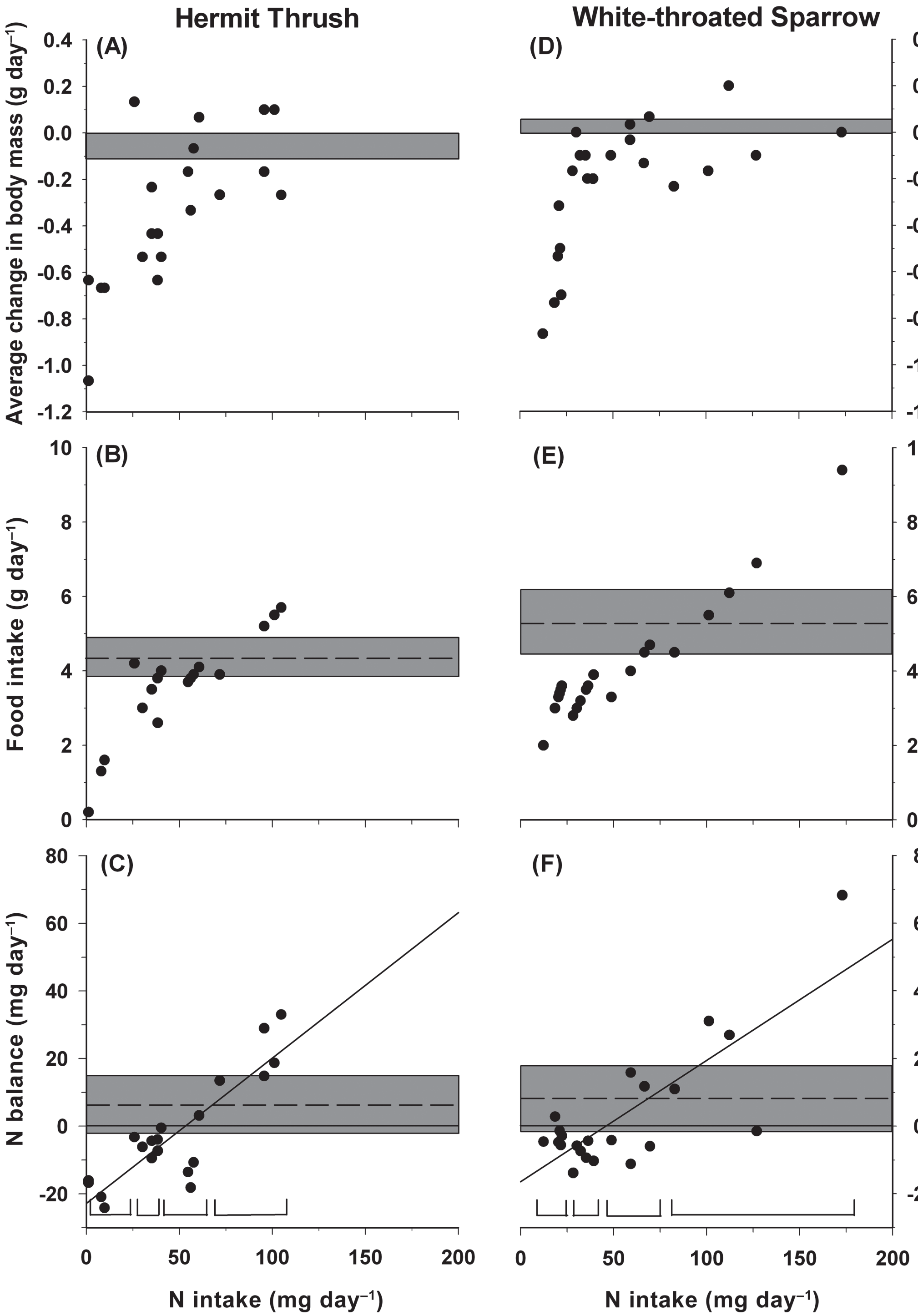

Fig. 1. Average changes in body mass, food intake, and nitrogen (N) balance in Hermit Thrushes and White-throated Sparrows in the nonmigratory state ($n = 20$ and $n = 22$, respectively) that ingested different amounts of protein. Brackets along the x-axis show the four dietary protein groups (3.6%, 6.7%, 9.7%, and 12.8%) that were used to produce this range in dietary N intake. Changes in N balance as a function of N intake by Hermit Thrushes and White-throated Sparrows are described by the equations $y = 0.430x - 22.82$ ($R^2 = 0.75$, $n = 20$) and $y = 0.358x - 16.44$ ($R^2 = 0.63$, $n = 22$), respectively. (A) Horizontal shaded area depicts the average (± 90% confidence interval [CI]) change in body mass of birds in steady state (Hermit Thrush, $n = 20$; White-throated Sparrow, $n = 22$) during the 14 days prior to the start of total-collection trials. Horizontal dashed lines and surrounding shaded area depict (B and E) food intake and (C and F) N balance (± 90% CI) of birds in the nonmigratory state that consumed adequate dietary protein (≥9.7% protein) during the total-collection trials.

TABLE 3. Apparent metabolizable energy intake (AME ± SD, kJ day^{-1}) and energy utilization (percentage ± SD, kJ day^{-1}) of Hermit Thrushes and White-throated Sparrows in the nonmigratory and migratory states when fed one of four diets with different amounts of protein.

	Nonmigratory state[a]				Migratory state	
Dietary protein (%)	Hermit Thrush		White-throated Sparrow		Hermit Thrush	White-throated Sparrow
	Apparent metabolizable energy intake					
12.8	70.8 ± 9.6	A	93.1 ± 28.5	A	—	—
9.7	48.4 ± 8.5	B	57.8 ± 7.8	B	96.1 ± 12.6	119.5 ± 13.8
6.7	46.8 ± 5.5	B	44.3 ± 5.9	B	101.8 ± 6.4	124.3 ± 22.3
3.6	28.3 ± 24.9	B	45.8 ± 8.8	B	82.9 ± 16.3	115.4 ± 31.8
	Energy utilization					
12.8	84.1 ± 1.3	A	86.6 ± 2.1	A	—	—
9.7	78.2 ± 1.6	A	82.7 ± 1.4	B	88.5 ± 0.7	90.1 ± 0.7
6.7	79.3 ± 1.7	A	80.2 ± 2.7	B	90.4 ± 1.3	89.1 ± 4.1
3.6	64.9 ± 14.7	B	86.9 ± 0.5	A	90.3 ± 0.8	91.6 ± 1.7

[a] For Hermit Thrushes and White-throated Sparrows in the nonmigratory state, rows with different letters denote significant differences between diets for each species (Tukey's test, $P < 0.05$). There were no significant differences in AME between diet groups for Hermit Thrushes and White-throated Sparrows in the migratory state.

and energy utilization two Hermit Thrushes in diet group D that were not in energy balance on day 3 because of low food intake.

Migratory-state Experiment

Nitrogen balance, food intake, and body mass of birds in the migratory state.—Body mass of Hermit Thrushes fed diets with $<4\%$ protein dropped, on average, 2.5 ± 0.6 g, whereas those fed more dietary protein maintained body mass ($F = 8.80$, df = 2 and 6, $P = 0.016$; Fig. 2A). White-throated Sparrows lost, on average, 1.3 ± 1.2 g of body mass when fed the same range of dietary protein and changed similarly across diet groups ($F = 1.58$, df = 2 and 7, $P = 0.271$; Fig. 2D). On each of the three diets the birds had similar food intake (Hermit Thrush: $F = 1.88$, df = 2 and 6, $P = 0.232$; Fig. 2B; White-throated Sparrow: $F = 0.33$, df = 2 and 7, $P = 0.728$; Fig. 2E) and produced similar amounts of feces (Hermit Thrush: $F = 2.57$, df = 2 and 6, $P = 0.156$; White-throated Sparrow: $F = 4.37$, df = 2 and 7, $P = 0.059$). Percent N in feces decreased in White-throated Sparrows fed $<4\%$ protein ($F = 4.88$, df = 2 and 7, $P = 0.047$), whereas percent N in feces did not change in Hermit Thrushes fed the same range of dietary protein ($F = 3.92$, df = 2 and 6, $P = 0.082$). Hermit Thrushes fed the lowest dietary protein had a less positive N balance than Hermit Thrushes fed the higher dietary protein ($F = 70.49$, df = 2 and 6, $P < 0.001$; Fig. 2C). The same trend was apparent in White-throated Sparrows, but the difference in N balance between diet groups was not significant ($F = 4.28$, df = 2 and 7, $P = 0.061$; Fig. 2F).

Protein requirements, minimum nitrogen requirements, and total endogenous nitrogen losses of birds in the migratory state.—Protein requirements of birds in the migratory state were estimated from the regression of N balance on N intake for the three diet groups (B, C, and D) on day 3 of the experiment (Fig. 2C, F). The protein requirement of Hermit Thrushes in the migratory state was 57.8 mg protein day^{-1} (9.25 mg N × 6.25), or ~0.93% protein for a diet containing 14.8 kJ AME g^{-1} DM. The MNR of Hermit Thrushes was 125.2 mg N kg$^{-0.75}$ day^{-1}. We estimated that the protein requirement of White-throated Sparrows in the migratory state was 98.8 mg protein day^{-1} (15.81 mg N × 6.25), or ~1.26% protein for a diet containing 15.1 kJ AME g^{-1} DM. The MNR of White-throated Sparrows was 244.2 mg N kg$^{-0.75}$ day^{-1}. Our estimate of TENL in Hermit Thrushes and White-throated Sparrows was 71.1 mg N kg$^{-0.75}$ day^{-1} and 146.3 mg N kg$^{-0.75}$ day^{-1}, respectively. We detected no difference in either the slopes ($t = 1.02$, df = 18, $P = 0.320$) or the elevations ($t = 0.38$, df = 18, $P = 0.706$) of the regression equations (Fig. 2C, F) that estimated the protein requirements of Hermit Thrushes and White-throated Sparrows in the migratory state, so these results for each species are reported only for comparative purposes. In a separate section below we report estimates of MNR from a common regression for these birds in the migratory state and compare them with those of birds in the nonmigratory state.

Energy utilization and metabolized energy of birds in the migratory state.—Both Hermit Thrushes and White-throated Sparrows in the migratory state that were fed different levels of dietary protein had similar AME and energy utilization (Hermit Thrush: $F = 1.81$, df = 2 and 6, $P = 0.243$ and $F = 3.55$, df = 2 and 6, $P = 0.096$, respectively; White-throated Sparrow: $F = 0.11$, df = 2 and 7, $P = 0.898$ and $F = 0.90$, df = 2 and 7, $P = 0.448$, respectively; Table 3). Overall, White-throated Sparrows had greater AME than Hermit Thrushes ($F = 7.06$, df = 1 and 25, $P = 0.014$), whereas energy utilization did not differ between species ($F = 4.05$, df = 1 and 25, $P = 0.055$). These differences in AME (kJ day^{-1}) between species are accentuated when the smaller body mass and, hence, higher mass-specific metabolic rate of White-throated Sparrows are considered (e.g., for the 9.7% protein diet in birds in the nonmigratory state, the adjusted AME [kJ day^{-1} body mass$^{-0.75}$] was 45% higher in White-throated Sparrows [5.19] than in Hermit Thrushes [3.59], compared with the 19% higher AME [kJ/day] shown in Table 3).

Comparison of protein requirements of birds in the nonmigratory and migratory states.—As noted above, because there were no significant differences in slope and elevation from the regressions for each species in the nonmigratory or migratory state, we

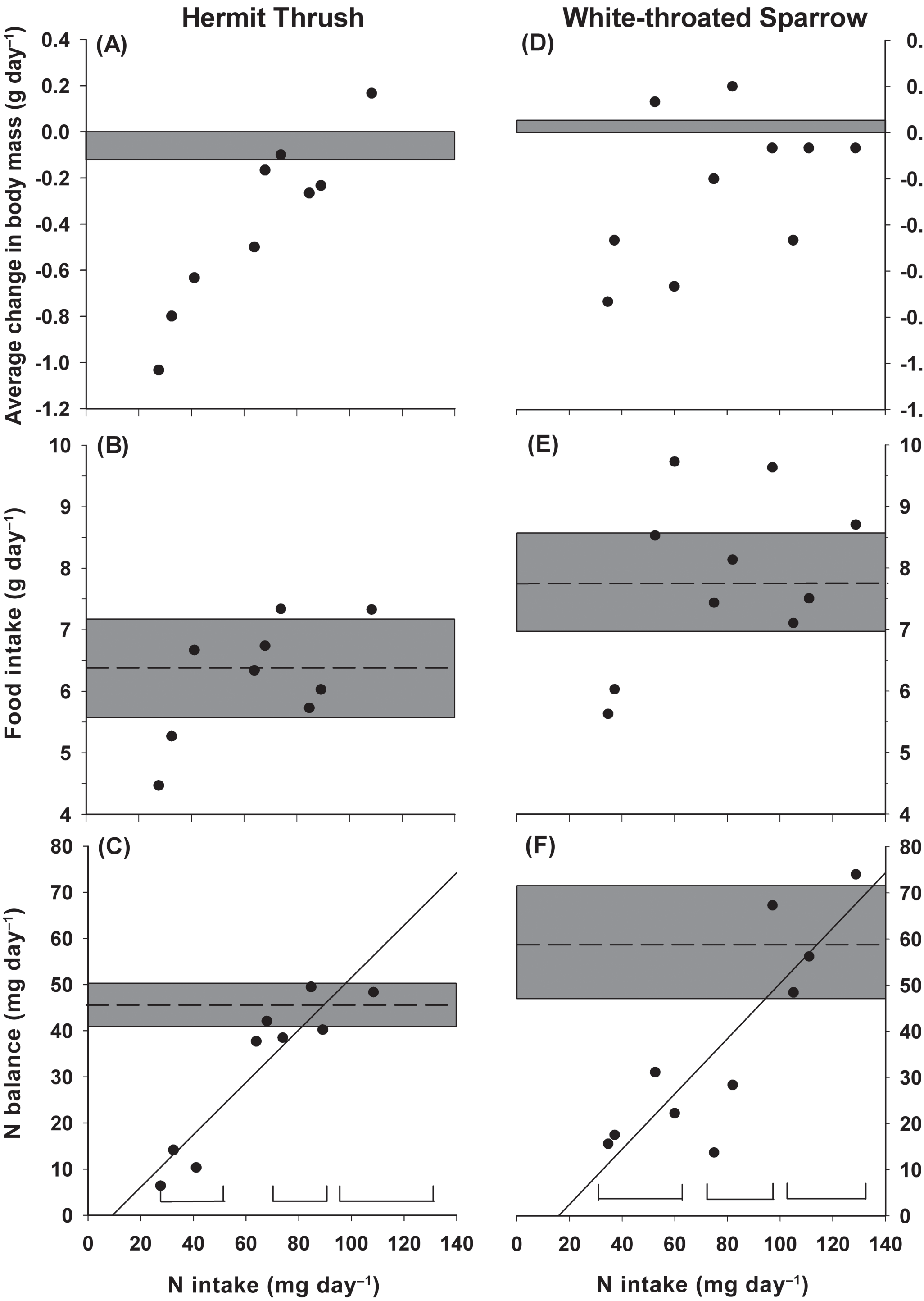

FIG. 2. Average changes in body mass, food intake, and nitrogen (N) balance in Hermit Thrushes and White-throated Sparrows in the migratory state ($n = 9$ and $n = 10$, respectively) that ingested different amounts of protein. Brackets along the x-axis show the three dietary protein groups (3.6%, 6.7%, and 9.7%) that were used to produce this range in dietary N intake. Changes in N balance as a function of N intake by Hermit Thrushes and White-throated Sparrows are described by the equations $y = 0.567x - 5.25$ ($R^2 = 0.85$, $n = 9$) and $y = 0.599x - 9.47$ ($R^2 = 0.74$, $n = 10$), respectively. (A) Horizontal shaded area depicts the average (± 90% confidence interval [CI]) change in body mass of birds in steady state (Hermit Thrush, $n = 20$; White-throated Sparrow, $n = 22$) during the 14 days prior to the start of total-collection trials in the nonmigratory-state experiments. Horizontal dashed lines and surrounding shaded area depict (B and E) food intake and (C and F) N balance (± 90% CI) of birds in the migratory state that consumed adequate dietary protein (9.7% protein) during the total-collection trials.

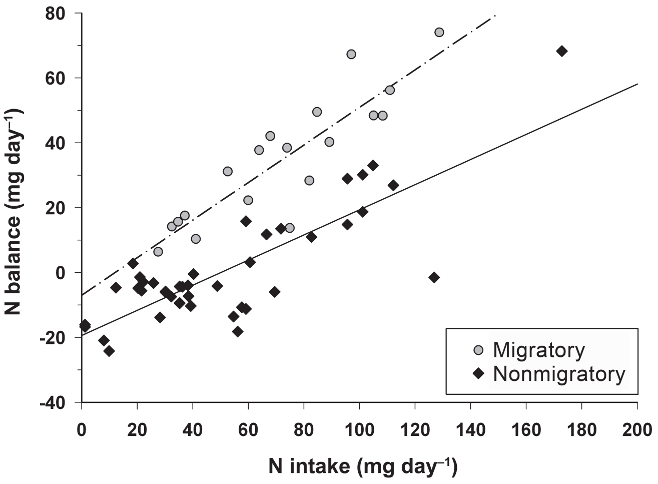

FIG. 3. Nitrogen (N) balance as a function of N intake by Hermit Thrushes and White-throated Sparrows in the nonmigratory state (solid line) is described by the equation $y = 0.387x - 19.36$ ($R^2 = 0.68$, $n = 42$), and N balance as a function of N intake by Hermit Thrushes and White-throated Sparrows in the migratory state (broken line) is described by the equation $y = 0.579x - 7.01$ ($R^2 = 0.78$, $n = 19$).

calculated common regression equations for birds in each state. The regressions differed significantly between states and demonstrated that birds had lower protein requirements in the migratory state than in the nonmigratory state (slope: $t = 2.13$, df = 60, $P = 0.037$; elevation: $t = 7.97$, df = 60, $P < 0.001$; Fig. 3). We estimated that the protein requirement of White-throated Sparrows and Hermit Thrushes in the nonmigratory state was 312.5 mg protein day^{-1} (50.0 mg N × 6.25), or ~8.17% protein for a diet containing 14.3 kJ AME g^{-1} DM. We estimated that the protein requirement of White-throated Sparrows and Hermit Thrushes in the migratory state was 75.6 mg protein day^{-1} (12.1 mg N × 6.25), or ~1.07% protein for a diet containing 14.9 kJ AME g^{-1} DM.

A comparison of birds fed adequate protein (diet B) indicated that birds in the migratory state had greater dietary energy utilization than those in the nonmigratory state ($F = 201.48$, df = 1 and 13, $P < 0.001$) and that White-throated Sparrows had greater dietary energy utilization than Hermit Thrushes ($F = 20.57$, df = 1 and 13, $P < 0.001$). Greater dietary energy utilization in migratory-state birds was associated with higher food intake ($F = 65.53$, df = 1 and 67, $P < 0.001$), whereas percent N in feces and amount of feces produced did not differ between states ($F = 2.60$, df = 1 and 67, $P = 0.111$ and $F = 0.001$, df = 1 and 67, $P = 0.974$, respectively).

Changes in body mass of birds during 3-day trials in both physiological states were compared to determine whether protein accretion might account for the differences in protein requirements of birds in the migratory versus the nonmigratory state. Hermit Thrushes and White-throated Sparrows lost, on average, 0.8 ± 6.1 g body mass during the 3-day collection trails (day effect: $F = 21.99$, df = 3 and 141, $P < 0.001$), although the extent of mass loss differed by bird species (day*species interaction: $F = 2.90$, df = 3 and 141, $P = 0.037$) and physiological state (day*state interaction: $F = 7.77$, df = 3 and 141, $P < 0.001$). In the nonmigratory state, Hermit Thrushes lost, on average, 1.2 ± 1.0 g during the 3-day trials, and White-throated Sparrows lost, on average, 0.8 ± 0.8 g. In the migratory state, Hermit Thrushes lost only 0.7 ± 0.9 g during the 3-day trials, and White-throated Sparrows lost only 0.1 ± 0.5 g. These results simplify the interpretation of N requirements across species and physiological state because they demonstrate that birds in the nonmigratory and migratory states were not accumulating protein or fat stores while we measured N balance.

DISCUSSION

Protein requirements of birds in relation to their foraging strategy.—Our results were not consistent with the hypothesis that the protein requirements of the more seasonally frugivorous Hermit Thrushes were lower than those of the less frugivorous White-throated Sparrows. Foeken et al. (2008) similarly found that a more frugivorous hornbill species in the genus *Aceros* did not differ in protein requirements from a more carnivorous hornbill species in the genus *Bucorvus*. We found that MNR was similar in Hermit Thrushes and White-throated Sparrows in the nonmigratory state and was within the range of 403–1,188 mg kg$^{-0.75}$ day^{-1} estimated for other passerines in previous studies (Table 4). Our MNR estimates for the Hermit Thrush were higher than published estimates for other omnivores such as the American Robin, yet lower than that for the Wood Thrush. Our MNR estimates for the White-throated Sparrow were higher than those for other granivores such as the White-crowned Sparrow, yet lower than that for the granivorous American Tree Sparrow. Likewise, our estimated TENL for the Hermit Thrush was similar to those for the Wood Thrush and European Starling, and our estimated TENL for the White-throated Sparrow was similar to that for the White-crowned Sparrow. In general, the studies of highly frugivorous birds published to date (Table 4) have consistently shown that their protein requirements are lower than those of omnivores and granivores, including our estimates for Hermit Thrushes and White-throated Sparrows in the nonmigratory state.

Our estimates of the protein requirements of Hermit Thrushes (53.1 mg N day^{-1}) and White-throated Sparrows (46.0 mg N day^{-1}) in the nonmigratory state are higher than those predicted from allometric equations that were based on 10 to 15 species of songbirds (range in body mass: 12–8,290 g) other than Hermit Thrushes and White-throated Sparrows. Tsahar et al.'s (2006) allometric equation for omnivorous birds (MNR = 575.4 mg N kg$^{-0.75}$ day^{-1}) predicts MNRs of 41.1 mg N day^{-1} and 35.9 mg N day^{-1} for Hermit Thrushes and White-throated Sparrows, respectively. Robbins's (1993) equation for birds in general (MNR = 430 mg N kg$^{-0.75}$ day^{-1}) predicts MNRs of 31.8 mg N day^{-1} and 27.8 mg N day^{-1} for Hermit Thrushes and White-throated Sparrows, respectively. This suggests a need for more studies such as ours that empirically measure protein requirements of a diversity of birds so that more accurate allometric models can be developed.

Protein requirements of birds change with physiological state.—Birds ate significantly more food in the migratory state than in the nonmigratory state, whereas N excreted per day was similar in both states. Consequently, birds in the migratory state had a more positive N balance than birds in the nonmigratory state. Hyperphagia is well documented in birds during premigratory fattening periods and migration (King and Farner 1965). Previous captive studies found that migratory birds increased their

Table 4. Maintenance nitrogen requirements (MNR; mg N $kg^{-0.75}$ day^{-1}) and total endogenous nitrogen losses (TENL; mg N $kg^{-0.75}$ day^{-1}) of bird species during the nonmigratory state.

Species	Body mass (g)	MNR	TENL	Source
Granivores				
Budgerigar (*Melopsittacus undulatus*)	42	381	260	Pryor 2003
White-crowned Sparrow (*Zonotrichia leucophrys*)	28	563	215	Calculated from Murphy 1993
House Sparrow (*Passer domesticus*)	27	1,141	884	Weglarczyk 1981
White-throated Sparrow (*Zonotrichia albicollis*)[a]	26	710	253	Present study
American Tree Sparrow (*Spizella arborea arborea*)[b]	18	1,146	—	Calculated from Martin 1968
Zebra Finch (*Taeniopygia guttata*)	12	403	153	Allen and Hume 2001
Omnivores				
African Gray Parrot (*Psittacus erithacus erithacus*)[b]	500	491	—	Kamphues et al. 1997
Amazon parrots (*Amazona* spp.)	413	304	173	Westfahl et al. 2008
European Starling (*Sturnus vulgaris*)	72	584	319	Tsahar et al. 2005a
American Robin (*Turdus migratorius*)	66	484	197	Witmer 1998
Wood Thrush (*Hylocichla mustelina*)	47	911	258	Witmer 1998
Hermit Thrush (*Catharus guttatus*)[c]	31	719	309	Present study
Garden Warbler (*Sylvia borin*)[b]	20	1,188	—	Calculated from Bairlein 1987
Frugivores				
Hornbill (*Aceros* sp.)[b]	2,399	387	—	Foeken et al. 2008
Pesquet's Parrot (*Psittrichas fulgidus*)	757	320	50	Pryor 2003
Tristram's Grackle (*Onychognathus tristrami*)	115	125	101	Tsahar et al. 2005b
Yellow-vented Bulbul (*Pycnonotus xanthopygos*)	36	99	75	Tsahar et al. 2005b
Cedar Waxwing (*Bombycilla cedrorum*)	35	264	69	Witmer 1998

[a] MNR and TENL for White-throated Sparrows in the migratory state: 244.2 mg $kg^{-0.75}$ day^{-1} and 146.3 mg $kg^{-0.75}$ day^{-1}, respectively.
[b] TENL was not reported.
[c] MNR and TENL for Hermit Thrushes in the migratory state: 125.2 mg $kg^{-0.75}$ day^{-1} and 71.1 mg $kg^{-0.75}$ day^{-1}, respectively.

energy intake by 25–30%, on average, when hyperphagic, compared with the nonmigratory state (Bairlein 1990). We found that daily energy intake in migratory-state Hermit Thrushes and White-throated Sparrows increased by 53.4% and 55.5%, respectively, compared with the nonmigratory state. Because increased daily food and energy intake also increased N intake and was associated with no change in dry mass of feces excreted or percent N in feces, birds in the migratory state were able to retain more nitrogen, and, hence, their dietary protein requirements decreased. In other words, for a given N intake, birds in the migratory state were able to extract more N and had a more positive N balance than birds in the nonmigratory state (Fig. 3). To our knowledge, the present study is the first to quantify protein requirements of the same songbird species in both the migratory and the nonmigratory states.

Lower protein requirements of birds in the migratory state were associated with an increase in energy utilization in birds fed adequate protein diets (diet B). Furthermore, energy utilization increased, on average, 10.3% in Hermit Thrushes and 7.4% in White-throated Sparrows from the nonmigratory to the migratory state. Increases in energy utilization have been documented in birds during migration (Bairlein 1985, Johnson et al. 1985). Energy utilization may play an important role in migrant birds that subsist on protein-deficient diets such as wild fruits.

Our estimates of MNR and TENL were remarkably lower for Hermit Thrushes and White-throated Sparrows in the migratory state than for those in the nonmigratory state. Both species were in positive N balance during the migratory state, so we must interpret these estimates of protein requirements with caution given that this involved extrapolating to zero N balance. However, in support of our findings, Roxburg and Pinshow (2000) found no significant difference between estimates of endogenous N loss extrapolated from regression of N intake versus N excretion and the actual values measured. Furthermore, because several birds fed diets with the lowest dietary protein approached N equilibrium (N balance ≤10 mg N day^{-1}) and the regression equations explained the majority of the variance (Hermit Thrush, $R^2 = 0.85$; White-throated Sparrow, $R^2 = 0.74$), we are confident that the protein requirements of these two species were lower in the migratory state than in the nonmigratory state. Dietary N intake that supports a zero N balance is the standard method of MNR measurement (Murphy 1993, Allen and Hume 2001), although birds are not always able to maintain body mass at N equilibrium (Brice and Grau 1991, Murphy 1993). Protein accretion and N loss other than in urine and feces can explain a portion of this variation and, thus, contribute to N requirements (Murphy 1993); however, it is unlikely that our birds accrued protein stores given that they lost, on average, 0.1–1.2 g body mass during the 3-day trials.

Ecological implications.—Contrary to our prediction that dietary nutrient requirements would increase during migration, we found that birds in the migratory state had lower protein requirements than those in the nonmigratory state. Our findings support Bairlein's (2002) and Murphy's (1994) hypothesis that the protein requirements of birds may change seasonally and Klasing's (1998) statement that birds with high rates of food intake are able to satisfy daily protein requirements on diets with lower protein concentrations. Our results are also similar to those of Bairlein (2002), who found that Garden Warblers in the nonmigratory

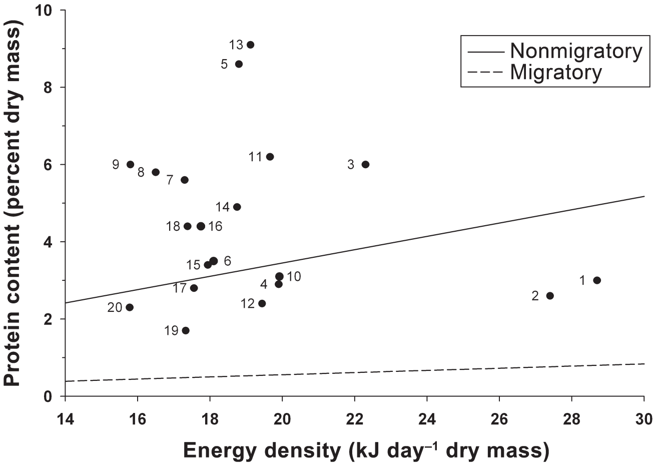

FIG. 4. Energy density and protein content of 20 fruit species in the northeastern United States (from Smith et al. 2007) in relation to estimated dietary protein content required to satisfy the daily protein requirements of migratory birds in the nonmigratory state (solid line) and in the migratory state (broken line). Fruits below the lines contain insufficient protein to satisfy a bird's daily requirements. Daily nitrogen requirements were estimated from the regressions in Figure 3 and then converted to daily protein requirements, assuming a 4.4 conversion efficiency (Witmer 1998). Field metabolic rate (FMR, kJ day^{-1}) for free-living birds with body mass of ~28 g in the nonmigratory state was estimated using Log FMR = 1.145 + (0.53 × log body mass, g) (Koteja 1991). Daily energy requirement (DER, kJ day^{-1}) was estimated as FMR/0.64, assuming a 64% efficiency of conversion of dietary energy (Karasov 1990). We assumed that DER of migratory birds was ~50% higher than that of nonmigratory birds, following Wikelski et al. (2003). We also assumed that birds in the nonmigratory and migratory states ate enough fruit of a given energy density each day to satisfy their respective DER, and we estimated the dietary protein content required to satisfy their respective daily protein requirements given these daily intake rates. Key: 1 = *Myrica pensylvanica*, 2 = *Viburnum dentatum*, 3 = *Parthenocissus quinquefolia*, 4 = *Ilex verticillata*, 5 = *Celastrus orbiculatus*, 6 = *Aronia melanocarpa*, 7 = *Rosa* sp., 8 = *Phytolacca americana*, 9 = *Elaeagnus umbellata*, 10 = *Viburnum acerifolium*, 11 = *Smilax rotundifolia*, 12 = *Aronia prunifolia*, 13 = *Sambucus canadensis*, 14 = *Vitis labrusca*, 15 = *Prunus virginiana*, 16 = *Lonicera japonica*, 17 = *Rosa virginiana*, 18 = *Aronia arbutifolia*, 19 = *Vaccinium corymbosum*, and 20 = *Rubus occidentalis*.

state were unable to maintain body mass when fed only fruits yet were able to maintain body mass when fed the same fruits during the premigratory fattening period. Lower protein requirements of birds during migration may enable them to adequately refuel when feeding on low-protein fruits during migration and could explain, in part, why so many migratory birds seasonally switch from insect to fruit consumption during migration (Berthold 1976).

Wild fruits vary in energy and protein composition (Fig. 4), and only some of these fruits may satisfy the protein requirements of birds in the nonmigratory state, whereas most would satisfy protein requirements during migration. Smith et al. (2007) used equations for Wood Thrush (Witmer 1998) and for 10 species of omnivores (Tsahar et al. 2006) to estimate protein requirements of migratory songbirds and concluded that many fruits available at stopover sites cannot satisfy their need for protein. However, our results suggest that migrant birds have lower protein requirements than those estimated for the same birds in the nonmigratory state. Therefore, fruits previously considered protein-deficient resources for migratory birds may satisfy their protein requirements. However, our conclusions ignore the potentially complex interactions between secondary compounds in fruits and protein requirements (Witmer 2001), and these should be taken into consideration. Our findings help resolve the apparent paradox of seasonal frugivory: birds during migration may switch to eating fruits with less protein because the hyperphagia associated with the migratory state in birds functionally reduces their dietary protein requirements.

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