The Journal of Experimental Biology 216, 800-808

doi:10.1242/jeb.072868

RESEARCH ARTICLE

Phenotypic flexibility in migrating bats: seasonal variation in body composition, organ sizes and fatty acid profiles

Liam P. McGuire*, M. Brock Fenton and Christopher G. Guglielmo

Department of Biology, University of Western Ontario, 1151 Richmond Street N., London, Ontario, Canada, N6A 5B7

*Author for correspondence at present address: Department of Biology, University of Winnipeg, 515 Portage Avenue, Winnipeg, Manitoba, Canada, R3B 2E9 (l.mcguire@uwinnipeg.ca)

SUMMARY

Many species of bats migrate long distances, but the physiological challenges of migration are poorly understood. We tested the hypothesis that migration is physiologically demanding for bats by examining migration-related phenotypic flexibility. Both bats and birds are endothermic, flying vertebrates; therefore, we predicted that migration would result in similar physiological trade-offs. We compared hoary bats (*Lasiurus cinereus*) during spring migration and summer non-migratory periods, comparing our results with previous observations of birds. Migrating bats had reduced digestive organs, enlarged exercise organs, and fat stores had higher proportions of polyunsaturated fatty acids (PUFAs). These results are consistent with previous studies of migrating birds; however, we also found sex differences not typically associated with bird migration. Migrating female hoary bats increased the relative size of fat stores by reducing lean body components, while males maintained the same relative amount of fat in both seasons. The ratio of n-6 to n-3 PUFA in flight muscle membrane increased in migrating males and decreased in migrating females, consistent with males using torpor more frequently than females during spring migration. Enlarged exercise organs, reduced digestive organs and changes in adipose tissue composition reflect the elevated energetic demands of migration. Sex-specific patterns of fat storage and muscle membrane composition likely reflect challenges faced by females that migrate while pregnant. Our results provide some of the first insights into the physiological demands of bat migration and highlight key differences between bats and birds.

Key words: Chiroptera, digestive organ, exercise organ, lipids, hoary bat, *Lasiurus cinereus*, migration.

Received 22 March 2012; Accepted 8 November 2012

INTRODUCTION

Many species of bats migrate long distances between breeding and wintering areas [>1000 km (Fleming and Eby, 2003)], but the physiological adaptations and trade-offs involved have been poorly studied (McGuire and Guglielmo, 2009). Some species of bats can fly continuously for long durations [e.g. >6 h (Barclay, 1989)] and travel great distances [e.g. >50 km (Best and Geluso, 2003)] just for daily foraging, and consequently the degree to which migration poses additional flight-related physiological challenges to bats is unknown. A night of migratory flight may be no more demanding than a long night of foraging. With the exception of aerial foraging insectivores such as swallows, the endurance flights required for migratory movements of most birds far exceed the typical amount of time spent flying at any other time of the year (e.g. Portugal et al., 2011). In birds, the high energy demands of migration are often reflected in dramatic changes in physiology, and lead to seasonal changes in numerous characters at the molecular, biochemical, tissue, organ and whole-animal levels (e.g. Guglielmo and Williams, 2003; McWilliams et al., 2004; Guglielmo, 2010). Such reversible changes are referred to as phenotypic flexibility (Piersma and Drent, 2003; Piersma and van Gils, 2011).

We examined migration-related phenotypic flexibility in migrating and non-migrating bats. We hypothesized that bats and birds, despite being distantly phylogenetically related, would face similar physiological challenges during migration (McGuire and Guglielmo, 2009). Mammalian exercise physiology has been studied extensively (e.g. Roberts et al., 1996; Weber et al., 1996a; Weber et al., 1996b; Weibel et al., 1996; McClelland, 2004), but the high metabolic rates required to sustain flight [~15 times basal metabolic rate (Speakman and Thomas, 2003)] and selection for high energy density substrates (fat, not carbohydrates) (Jenni and Jenni-Eiermann, 1998) make it unclear whether the 'mammalian exercise model' (McWilliams et al., 2004) is appropriate for migrating bats. Migratory birds may serve as a better group for comparison. Bats and birds are both vertebrate endotherms that have evolved the capacity for true powered flight (Rayner, 1988; Maina, 2000), and thus may have converged on similar solutions to some of the physiological challenges of migration. If bat migration is indeed more physiologically demanding than normal foraging flight, we predicted that we would observe phenotypic changes similar to those previously shown in migratory birds. Specifically, we examined seasonal variation in body composition (fat and lean stores), organ sizes (exercise and digestive organs) and the fatty acid (FA) composition of adipose tissue and flight muscle membranes.

Several studies have assumed that fat is the primary fuel for migratory flight in bats (O'Shea, 1976; Fleming and Eby, 2003; McGuire et al., 2012), but there are few detailed studies of fuel metabolism in bat migration. Bats can fuel flight with recently ingested nutrients (Welch et al., 2008; Voigt et al., 2010a) and use a mixed fuel strategy, relying on both recently ingested nutrients and endogenous fat stores (Voigt et al., 2012). When exogenous nutrients are not available, fasted bats can fuel short periods of flight,

and possibly endurance migratory flights as well (Voigt et al., 2012), exclusively with stored fat (Welch et al., 2008; Voigt et al., 2010a). Seasonal fat deposition is common in many species of bats that migrate to and from hibernacula (e.g. Krulin and Sealander, 1972; Ewing et al., 1970; Kunz et al., 1998), but it is difficult to distinguish between fat deposited to fuel migration and that deposited to survive months of hibernation. Studies of non-hibernating migratory bat species are rare. Brazilian free-tailed bats (*Tadarida brasiliensis*) do not hibernate and deposit fat stores for spring and autumn migration (O'Shea, 1976). Fat deposition consistent with the timing of migration has also been reported in several West African species (O'Shea and Vaughan, 1980) and lesser long-nosed bats (*Leptonycteris yerbabuenae*) (Ceballos et al., 1997). In the current study, we predicted that fat stores would be larger in bats during spring migration than in summer when they are not migrating.

Migrating bats and birds face two seemingly opposed activities, flight and refuelling, each of which may require increased capacities of different physiological systems (although the difference may be less pronounced in aerial compared wtih terrestrial foraging species). The contrasts between high-intensity exercise during flight and hyperphagia during refuelling have led numerous studies of birds to examine changes in 'exercise organs' or 'flight machinery' (e.g. flight muscles, heart, lungs) and 'digestive' or 'nutritional organs' (stomach/gizzard, intestines, kidneys, liver) (Hume and Biebach, 1996; Piersma, 1998; Piersma and Gill, 1998; Piersma et al., 1999; Battley et al., 2001; Guglielmo and Williams, 2003; Landys-Ciannelli et al., 2003; Bauchinger et al., 2005). Increases in flight machinery may increase exercise performance on long migratory flights (Pennycuick, 1998; Guglielmo and Williams, 2003), or may provide extra protein to be catabolized in flight to maintain blood glucose, replenish metabolite pools for the Krebs cycle, or provide metabolic water (Jenni and Jenni-Eiermann, 1998; Gerson and Guglielmo, 2011). Thus, we predicted that migrating bats would have enlarged exercise organs.

Digestive organs can be catabolized in flight, but are needed to maximize fuelling rate and minimize time spent at stopover ['migration takes guts' (McWilliams and Karasov, 2005)]. In some cases, digestive organs appear to represent surplus mass that can be eliminated for a more energy efficient flight ['guts don't fly' (Piersma and Gill, 1998)]. Consequently, changes in the size of digestive organs can indicate migratory strategy. Birds that migrate long distances without stopping to refuel (typically crossing ecological barriers such as oceans or deserts) reduce the size of digestive organs prior to or during flight, and rebuild these organs upon arrival at stopover sites (Hume and Biebach, 1996; Piersma, 1998; Piersma and Gill, 1998; Piersma et al., 1999; Battley et al., 2000; Battley et al., 2001; Bauchinger et al., 2005). Alternatively, species that stop frequently along the migratory route may maintain enlarged digestive organs to minimize refuelling time at each stopover [e.g. western sandpipers, *Calidris mauri* (Guglielmo and Williams, 2003)]. Most bats remain nocturnal during migration and therefore must complete all foraging and migratory flight within the hours of darkness, unlike nocturnally migrating songbirds, which forage by day and fly at night. We predicted that the additional time pressure faced by migratory bats would select for enlarged digestive organs to facilitate rapid refuelling.

Migrants may alter the FA composition of adipose stores and cell membranes to facilitate migration (McWilliams et al., 2004; Price, 2010). FA chain length and degree of unsaturation affect the potential energy derived from fat stores (more ATP from longer FAs with fewer double bonds) (Price, 2010). However, these same factors affect the rate of mobilization: short FAs with more double bonds are preferentially mobilized (Price et al., 2008) and preferentially oxidized (Leyton et al., 1987; Price et al., 2011). Consequently, adipose FA profiles present a trade-off between rapid mobilization and energy density. Adipose FA composition is largely determined by diet (Price, 2010), and although bats generally do not demonstrate the same degree of dietary plasticity as birds (an insectivorous bat will not suddenly switch to a diet of seeds or berries), there is some limited evidence that bats select insect prey based on FA content (Schalk and Brigham, 1995). If migrating bats are able to alter the FA composition of adipose stores either through dietary selection or by preferentially retaining particular FAs, we predict a shift towards shorter chain length and more unsaturated FAs, resulting in more rapid mobilization.

Variation in the FA profiles of cell membrane phospholipids (PLs) has been suggested to affect whole-animal exercise performance. A pervasive theme in both mammalian and avian literature is the importance of essential dietary polyunsaturated FAs (PUFAs; n-3 and n-6), though the pattern is not consistent. Various studies have found increased exercise performance (or increased proportion in migrants compared with non-migrants) with high n-6 PUFA (Pierce et al., 2005; Ayre and Hulbert, 1997; Ruf et al., 2006), high n-3 PUFA (Maillet and Weber, 2007), a high ratio of n-6:n-3 (Klaiman et al., 2009) or a low ratio of n-6:n-3 (Guglielmo et al., 2002). Alternatively, muscle PL composition may not affect exercise performance at all; rather, differences may simply arise due to differences in adipose FAs (Price and Guglielmo, 2009). Therefore, the role of muscle PL composition in whole-animal exercise performance remains unclear.

Muscle PL composition has also been implicated in torpor and hibernation studies. Munro and Thomas (Munro and Thomas, 2004) described improved torpor performance (e.g. torpor depth, torpor bout duration, metabolic rate) with high membrane PUFA content. Based on the Munro and Thomas (Munro and Thomas, 2004) framework, migrating insectivorous bats should seek to maximize PUFAs. However, another review suggested that a high n-6:n-3 ratio is important for maintaining membrane function at low body temperatures (Ruf and Arnold, 2008). Male hoary bats are more likely to use torpor during spring migration than females (Cryan and Wolf, 2003), and therefore we predicted that sexes would differ in muscle PL composition during migration. Specifically, we predicted that males would have either greater total PUFAs or greater n-6:n-3 if the Munro and Thomas (Munro and Thomas, 2004) or Ruf and Arnold (Ruf and Arnold, 2008) hypotheses were supported, respectively.

MATERIALS AND METHODS

Study species

The hoary bat [*Lasiurus cinereus* (Beauvois 1796)] is the most widely distributed bat species in North America (Shump and Shump, 1982), and is believed to migrate longer distances than other migratory species (Cryan et al., 2004). Hoary bats exhibit sexual size dimorphism, where females are ~3% larger than males (Williams and Findley, 1979). Hoary bats are solitary and roost in exposed foliage (Willis and Brigham, 2005; Carter and Menzel, 2007; Cryan and Veilleux, 2007). In summer the sexes are largely segregated, with males more commonly found in mountainous regions in the western part of North America, whereas females are widespread throughout the eastern part of the continent (Cryan, 2003). Mating occurs during autumn migration (Cryan, 2008) and females delay pregnancy until spring, migrating north while pregnant (Cryan and Wolf, 2003). The winter distribution is not well understood, though it is thought that most individuals overwinter in southern California and Mexico (Cryan, 2003).

Animal collection

Migrating *L. cinereus* were captured from 5 to 17 May 2009 by setting mist nets across creeks in Bernalillo County, New Mexico, USA (35°12′N, 106°18′W), or around water pools in the Manzano Mountains, Cibola National Forest, New Mexico, USA [34°59′N, 106°21′W; see Cryan and Wolf (Cryan and Wolf, 2003) for a description of the region]. Non-migrating bats were captured between 20 July and 1 August 2008 and 2009 in mist nets set across creeks in Cypress Hills Interprovincial Park, Saskatchewan, Canada [49°34′N, 109°53′W; see Willis and Brigham (Willis and Brigham, 2005) for description]. For the non-migrants, we determined age (sub-adult or adult) by the degree of ossification of the metacarpal-phalanges joint (Anthony, 1988). By autumn the metacarpal-phalanges joint is fully ossified, and thus it was not possible to determine the age of bats during spring migration. Lactating females were identified by manually expressing milk from the mammary glands. Sub-adults and lactating females were released immediately upon capture. We collected 15 female and 15 male migrants (New Mexico), and eight female and seven male non-migrants (Saskatchewan). All non-migrating females were post-lactating and all females collected during spring migration were pregnant. We euthanized the bats immediately following capture by cervical dislocation under isoflurane anaesthesia. We recorded body mass (±0.1 g) and forearm length (±0.05 mm) and immediately excised samples of pectoral muscle, liver and adipose tissue, which we transferred to pre-weighed individual vials (for liver and muscle; 2 ml Cryotube, Cryo.S, Grenier Bio-One) or 600 µl O-ring-sealed screw cap microcentrifuge tubes (for adipose; Fisherbrand, Thermo Fisher Scientific, Pittsburgh, PA, USA) and froze in a liquid nitrogen cooled dry-shipper (Taylor-Wharton CX-100, Theodore, AL, USA). The remainder of the carcass was frozen in a sealed plastic bag at –20°C. Samples were transported back to the laboratory either in liquid nitrogen cooled cryoshippers or packed in dry ice. Pectoralis, liver and adipose tissue samples were stored at –80°C; the remaining tissues were stored at –20°C.

All animal collection and experimental protocols were approved by the University of Western Ontario Animal Use Sub-committee (protocol no. 2008-003-04) and conducted under permits from the New Mexico Department of Game and Fish (permit no. 3424), the United States Department of Agriculture-Forest Service (permit no. SND502), Saskatchewan Ministry of Environment (permit nos 08FW080 and 09FW045) and the Saskatchewan Ministry of Tourism, Parks, Culture and Sport (permit nos SP-CHPP-02-08 and SP-CHPP-01-09). Samples from New Mexico were imported to Canada under the approval of the Canadian Food Inspection Agency (permit no. A-2009-01022-3).

Organ size measurement and body composition analysis

Carcasses were thawed overnight at 4°C prior to dissection. We removed the remaining pectoral muscle, intestines (large and small combined), stomach, remaining liver tissue, kidneys, heart and lungs. Intestines and stomach were opened to remove all contents, rinsed in 0.9% NaCl and blotted dry. We recorded the wet mass of each organ or tissue (±0.0001 g), correcting for subsamples taken in the field. We dried organs to a constant mass at 70°C, then placed them in pre-weighed filter paper envelopes (Whatman #1) and extracted the lipid fraction with petroleum ether (boiling point 30–60°C) for 6 h in a Soxhlet apparatus. Similarly, we dried the remainder of the carcass at 70°C, and homogenized it with a heavy-duty blender (model CB15l Waring Commercial, Torrington, CT, USA). We divided the homogenate into two to three pre-weighed filter paper envelopes (Whatman #1) for Soxhlet extraction with petroleum ether.

Fatty acid analysis of adipose and muscle tissue

Total lipids were extracted by adding the sample (75–120 mg muscle or 6–12 mg adipose) to 8 ml chloroform:methanol (1:1 v/v) containing butylated hydroxytoluene (25 mg l^{-1}), homogenizing for 2×10 s (Polytron PT 10-35, Kinematica, Bohemia, NY, USA), adding 4 ml chloroform and homogenizing for an additional 1×10 s. The homogenizer was rinsed with an additional 6 ml chloroform methanol (2:1 v/v) to ensure complete transfer of the sample to the sample tube. The sample was then centrifuged for 15 min at 2056 ***g***, and gravity filtered (Whatman #1) into a new tube. The previous sample tube was rinsed with 12 ml chloroform:methanol (2:1 v/v), which was also filtered into the new tube. To separate aqueous solutes, we added 7.5 ml 0.25% KCl, incubated in a 70°C water bath for 10 min, and discarded the aqueous layer. The remaining organic phase was transferred to a pear flask (25 ml) and evaporated under vacuum (Rotovapor, Buchi, Switzerland) at 60°C. Dried samples were either suspended in 1–2 ml chloroform:methanol (1:1 v/v) under nitrogen for overnight storage at –20°C, or immediately resuspended in 100 µl chloroform for separation of the different lipid fractions. PL, neutral lipid (NL; primarily triglycerides) and non-esterified FA (NEFA) fractions were separated with Supelclean solid phase extraction tubes (LC-NH_2, 100 mg; Supelco, Sigma-Aldrich Canada, Oakville, ON, Canada). The columns were conditioned with 2 ml hexane prior to addition of the samples. After each elution the samples were centrifuged for 1 min at 1370 ***g***. NLs were eluted with 1.8 ml chloroform:isopropanol (2:1 v/v). NEFAs were eluted with 1.6 ml isopropyl ether:acetic acid (98:2 v/v). PLs were eluted with 3 ml methanol. We added heptadecanoic acid (17:0; 3 mg ml^{-1} in hexane) as an internal standard to the NL and PL fractions.

For transesterification, the NL and PL fractions were dried at 70°C under a stream of N_2, resuspended in 2 ml of 1 mol l^{-1} acetyl chloride in methanol and incubated at 90°C for 2 h. The samples were then dried under N_2, resuspended in 1 ml methanol and dried under N_2 again to remove any residual HCl and H_2O. Finally, the samples [now fatty acid methyl esters (FAMEs)] were resuspended in dichloromethane for analysis by gas chromatography (Agilent Technologies 6890N, Hewlett Packard, Palo Alto, CA, USA). We used a J&W Scientific high-resolution gas chromatography column (DB-23, Agilent Technologies), a flame ionization detector and He as a carrier gas [as described in Klaiman et al. (Klaiman et al., 2009)]. The temperature programme was 2 min at 80°C, increase 5°C min^{-1} for 20 min, hold 180°C for 3 min, increase 1.5°C min^{-1} for 13.3 min, hold 200°C for 0 min, increase at 10°C min^{-1} for 4 min, and hold 240°C for 4 min. Fatty acids were identified by comparing relative retention time (retention time/retention time of internal standard) to known standards (Supelco C8–C24 FAME mix, Supelco 37 component FAME mix, and Supelco PUFA no. 3 from menhaden oil). For analysis, we did not consider short chain fatty acids (less than 16 carbons) or any fatty acids that comprised less than 0.5% of the total FA content.

Statistical analysis

All analyses were completed with the software R (version 2.9.2) (R Development Core Team, 2009). In all cases where we considered body mass, we used empty mass (capture mass – stomach content mass). We first used a linear model to test for sex and migration effects on forearm length to confirm that there was no systematic size difference between the migrating and non-migrating bats. We then tested for sex and migration effects on body mass, dry lean mass and fat mass. For all linear models, we started with a full model including main effects and all two-way interactions. We then removed non-significant terms and re-evaluated the model until only

Table 1. Summary statistics for body composition and organ size measurements of migrating and non-migrating hoary bats

	Migrating		Non-migrating		
	Female	Male	Female	Male	*P*
Forearm length (mm)	54.90±0.26	52.98±0.35	54.84±0.20	53.25±0.42	Sex: <0.0001
Body mass (g)	29.39±0.80	22.35±0.49	33.01±1.00	26.39±0.87	Sex: <0.0001; migration: <0.0001
Dry lean mass (g)	7.15±0.15	6.09±0.19	7.65±0.15	6.54±0.24	Sex: <0.0001; migration: 0.02
Fat mass (g)	4.67±0.42	2.51±0.15	3.78±0.41	3.13±0.22	Sex × migration: 0.017
Dry lean pectoralis (g)	0.31±0.009		0.29±0.02		n.s.
Dry lungs (g)	0.12±0.006		0.099±0.006		Migration: 0.0057
Dry lean heart (g)	0.078±0.003		0.081±0.005		n.s.
Wet intestine (g)	0.52±0.03		0.92±0.11		Migration: 0.0001
Dry stomach (g)	0.036±0.002		0.037±0.003		n.s.
Dry kidneys (g)	0.075±0.002	0.062±0.001	0.082±0.002	0.066±0.002	Sex: 0.0045; migration: 0.026
Dry lean liver (g)	0.22±0.01	0.14±0.008	0.25±0.02	0.14±0.01	Sex: <0.001

Values presented are means ± s.e.m. Significant sex and migration effects are indicated in the final column (n.s., not significant). See Results, 'Body composition', for analysis details.

significant terms remained. All masses were $\log_e$-transformed prior to analysis.

For statistical analysis of organ sizes, we considered wet intestine mass, dry lung, kidney and stomach masses, and dry lean (extracted) liver, heart and pectoralis masses as in Guglielmo and Williams (Guglielmo and Williams, 2003). When comparing organ sizes we originally considered forearm length as a measure of body size but found that organ sizes were more strongly correlated with body mass. Thus, we calculated corrected body mass as $\log_e$(body mass – organ mass) as a measure of body size accounting for part whole correlation, substituting total wet mass, total dry mass or total dry lean mass as appropriate. For each organ, we used general linear models to test for the effects of sex and migration controlling for corrected body mass (including all two- and three-way interactions). We sequentially removed non-significant terms and re-evaluated the model until only significant terms remained. Whether considering models correcting for forearm length or body mass, the results were qualitatively the same.

We also used principal components analysis (PCA) to conduct a multivariate analysis of migration-related changes in muscle and organ sizes. We entered all organ masses and total fat mass into a PCA and used MANOVA to test for effects of sex, migration and body mass on the retained PC axes.

We compared the fatty acid profiles of adipose NLs and muscle PLs as described in Klaiman et al. (Klaiman et al., 2009). We arcsin square root transformed the proportions of each FA and conducted two-way ANOVA to test for effects of sex, migration and sex×migration interaction. Furthermore we calculated the double bond index as DBI=Σ[(proportion FA_i)(number of double bonds per FA_i)]. We also calculated the n-6:n-3 ratio, and the total proportions of MUFA, PUFA and saturated FA.

RESULTS

Body composition

Body composition is summarized by sex and migratory status in Table 1. Female hoary bats were 3.4% larger than males (forearm length: $F_{1,42}$=30.91, P<0.0001) but there was no effect of migratory status on body size ($F_{1,42}$=0.081, P=0.78). Body mass was greater in females ($F_{1,42}$=88.55, P<0.0001) and migrating bats weighed ~15% less than non-migrants ($F_{1,42}$=23.91, P<0.0001). Dry lean mass was lower in migrants ($F_{1,42}$=5.73, P=0.021) and males ($F_{1,42}$=32.87, P<0.0001). The effect of migratory status on fat storage was sex dependent (sex × migration: $F_{1,41}$=6.24, P=0.017; Fig. 1). Absolute fat mass decreased during migration for males ($F_{1,20}$=4.92, P=0.038), but when controlling for fat-free mass (accounting for overall decrease in body mass) there was no difference in fat content of migrating and non-migrating males ($F_{1,19}$=1.31, P=0.27). For females, there was no difference in the absolute fat mass between migrating and non-migrating bats ($F_{1,21}$=2.19, P=0.15), but when controlling for fat-free mass the relative fat content increased during migration ($F_{1,20}$=5.53, P=0.029), accounting for as much as 24% of body mass in one individual. In summer, there was no difference in the relative fat content of males and females ($F_{1,12}$=0.0034, P=0.95).

Organ sizes

Organ masses are summarized by sex and migration in Table 1. Upon visual inspection of the untransformed organ masses, we noted obvious outliers for intestine and pectoralis mass (Fig. 2). There were three female non-migrants with much larger intestines than any other bats. These were the first three females we captured in the summer. These same individuals and one other non-migrant bat (a male) had much smaller pectoralis muscles than would have been expected

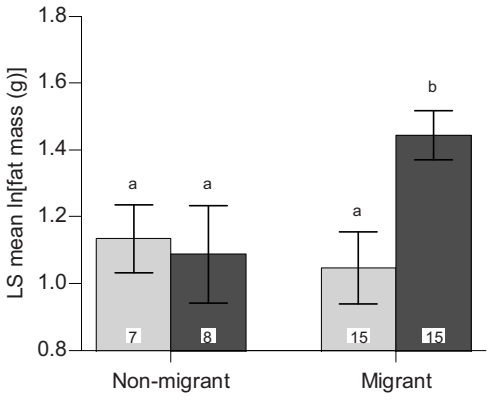

Fig. 1. Fat storage in relation to migration was sex dependent. Male hoary bats (light bars) decreased body and fat mass proportionally during migration, and consequently relative fat mass was the same in summer and spring migration. Females (dark bars) maintained the same absolute fat mass in both samples, but a decrease in overall body mass during migration resulted in higher relative fat mass for migrants. Bars indicate least square means predicting fat mass controlling for fat-free mass. Error bars indicate ±s.e.m. Sample sizes are indicated inside the bars. Different letters indicate significant (P<0.05) differences between groups.

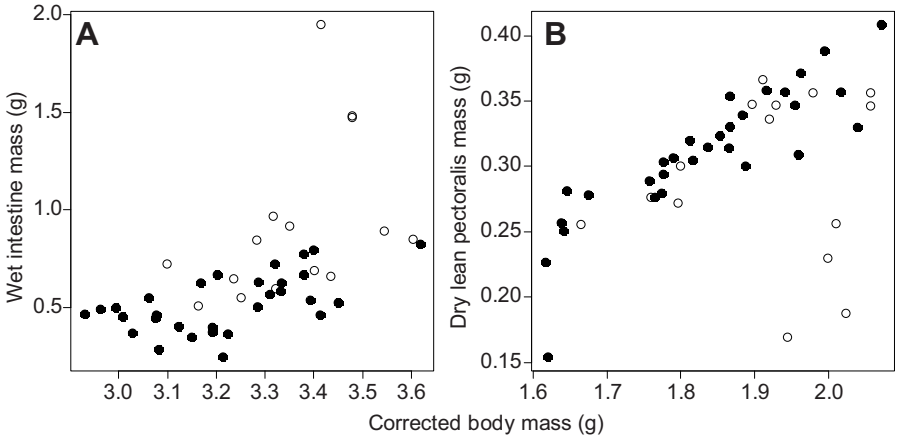

Fig. 2. (A) Wet intestine mass relative to corrected body mass for spring migrating (●) and summer non-migrating (○) hoary bats. Note the three outliers (two overlaid) among the non-migrants. These three individuals are females and were captured earlier in the summer than the other females. (B) Dry lean pectoralis mass relative to corrected body mass for spring migrating (●) and summer non-migrating (○) hoary bats. Note the four outliers among the non-migrants, three of which are the same individuals as the outliers noted in A.

for their size. After applying the $\log_e$ transformation to intestine mass, the outliers were no longer problematic. Migrating bats had smaller intestines than non-migrating bats ($F_{1,42}$=14.97, P<0.0001). However, the pectoralis mass outliers were still apparent even after data transformation and thus were excluded from the analysis. Excluding outliers, there was no effect of migratory status on pectoralis mass ($F_{1,38}$=1.09, P=0.30). However, it appears that the pectoralis muscles in four of the non-migrants were much smaller than expected for bats of their size.

No other outliers were apparent in the data set. Migrating bats had larger lungs than non-migrants ($F_{1,42}$=8.49, P=0.0057) but there was no difference in heart size ($F_{1,42}$=0.13, P=0.72). There was no difference between migrants and non-migrants in the size of stomach ($F_{1,42}$=0.15, P=0.70). Similarly, liver size was not affected by migratory status ($F_{1,41}$=0.36, P=0.55), though females had larger livers than males ($F_{1,41}$=33.01, P<0.0001). Kidney size was affected by both sex (females had larger kidneys; $F_{1,41}$=9.03, P=0.0045) and migratory status ($F_{1,41}$=5.33, P=0.026), with smaller kidneys in migrating bats.

Excluding the three outliers among the non-migrant females, the results of the PCA largely reflected the conclusions of the individual organ analyses. The first two principle components (PC1 and PC2) were retained in the analysis, accounting for 43.1 and 13.4% of the total variance, respectively (Table 2). All loadings in PC1 were positive and approximately equal, suggesting that this PC reflects body size. This is further confirmed by the strong correlation between PC1 and body mass (Pearson's r=0.87). Lungs, heart and pectoralis loaded positively on PC2, while intestines, stomach, kidneys and fat had negative loadings (Table 2), suggesting that this PC represented an axis of exercise machinery and digestive machinery.

Including both PCs, MANOVA indicated significant effects of migratory status (Wilks' λ=0.764, $F_{2,37}$=5.73, P=0.0068), sex (Wilks' λ=0.199, $F_{2,37}$=74.64, P<0.0001) and body mass (Wilks' λ=0.408, $F_{2,37}$=26.84, P<0.0001). PC1 was related to body mass ($F_{1,38}$=48.76, P<0.0001) and sex ($F_{1,38}$=137.92, P<0.0001), but not migratory status ($F_{1,38}$=0.098, P=0.76), further confirming this PC as an indication of body size (the inclusion of sex reflects sexual size dimorphism). PC2 was related to migratory status ($F_{1,38}$=10.21, P=0.0028), but not body mass ($F_{1,38}$=0.24, P=0.63) or sex ($F_{1,38}$=0.27, P=0.61). Therefore, migrating bats were shifted towards increased exercise machinery and reduced digestive machinery.

Fatty acid profiles

Adipose NLs

We found migration-related differences in the proportions of all adipose NL FAs except 18:0 (Fig. 3). Although some sex × migration interactions indicated that the proportions of certain FAs changed for one sex but not the other, the general pattern was a decrease in 16:0 and increases in 18:2n-6 and 18:3n-3 during migration. This pattern is further supported by decreased saturated FAs ($F_{1,42}$=35.53, P<0.0001) and increased PUFAs. A sex × migration interaction indicated a greater PUFA increase for males ($F_{1,20}$=15.12, P=0.00091) than females ($F_{1,21}$=8.89, P=0.0071). There was no overall change in MUFAs ($F_{1,42}$=0.44, P=0.51). DBI was greater in migrants ($F_{1,42}$=45.57, P<0.0001) and in males ($F_{1,42}$=5.91, P=0.019). There was no difference in the n-6:n-3 ratio of migrating and non-migrating bats ($F_{1,42}$<0.0001, P=0.99).

Muscle PLs

There were differences in FA composition between migrants and non-migrants for five of the nine FAs comprising muscle PLs

Table 2. Eigenvectors of the first two principal components of organ sizes in hoary bats

	PC1 (body size)	PC2 (digestive/exercise organs)
Pectoralis	0.390	0.113
Lungs	0.332	0.415
Heart	0.253	0.688
Intestines	0.300	–0.465
Kidneys	0.451	–0.267
Liver	0.416	0.023
Stomach	0.237	–0.098
Adipose	0.389	–0.212
Proportion of total variance explained	0.431	0.134

The first principal component (PC1) represents body size, while the second principal component (PC2) reflects a trade-off between digestive organs and exercise organs.

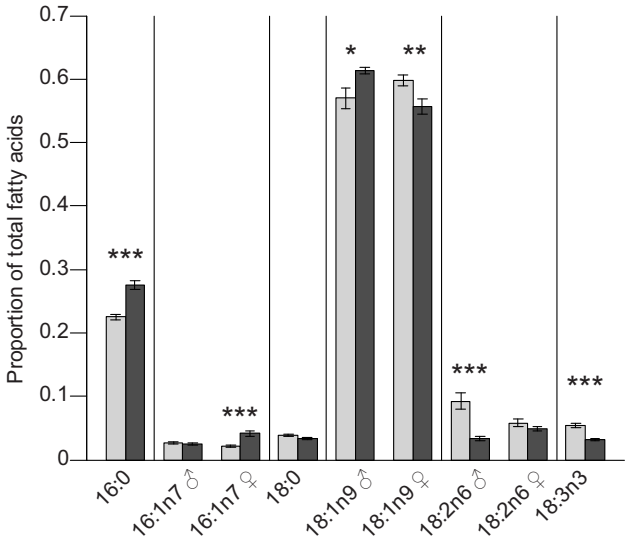

Fig. 3. Fatty acid composition of adipose neutral lipids of migrating (light bars) and non-migrating (dark bars) hoary bats. In cases of sex × migration interaction, males and females are presented separately. Vertical lines separate individual fatty acids. Bars indicate means ± s.e.m. *P<0.1, **P<0.05, *** P<0.01.

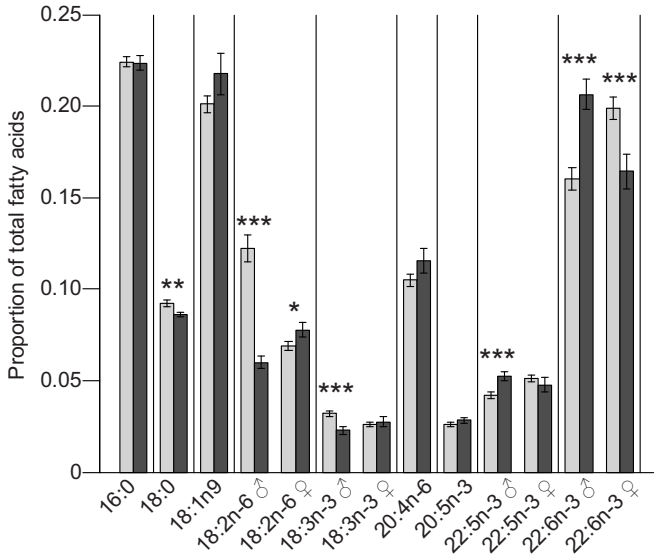

Fig. 4. Fatty acid composition of muscle phospholipids of migrating (light bars) and non-migrating (dark bars) hoary bats. In cases of sex×migration interaction, males and females are presented separately. Vertical lines separate individual fatty acids. Bars indicate means ± s.e.m. *P<0.1, **P<0.05, ***P<0.01.

(Fig. 4). Sex × migration interactions indicated sex-specific responses for several FAs, notably 18:2n-6 and 22:6n-3. Males had more 18:2n-6 and less 22:6n-3 during migration, while the pattern was reversed for females. This led to a significant change in the n-6:n-3 ratio, which was higher during migration for males (migrants: 0.89±0.06; non-migrants: 0.56±0.02; $F_{1,20}$=12.4, P=0.0022) and lower during migration for females (migrants: 0.59±0.03; non-migrants: 0.74±0.05; $F_{1,21}$=8.98, P=0.0069). DBI was similarly affected, decreasing during migration for males (migrants: 2.25±0.04; non-migrants: 2.48±0.05; $F_{1,20}$=14.08, P=0.0013) and increasing marginally for females (migrants: 2.43±0.03; non-migrants: 2.31±0.08; $F_{1,21}$=3.15, P=0.091). The composition of MUFAs, PUFAs and total saturated FAs was not affected by migratory status (all P>0.05).

DISCUSSION

We found migration-related changes in all aspects of our study, suggesting that for these bats, as in birds, migration presents distinct physiological challenges compared with other periods of the annual cycle (e.g. Gwinner, 1990; Dingle, 1996; Jenni and Jenni-Eiermann, 1998; Guglielmo, 2010). Sex was important for every aspect of physiology we examined, a pattern not typically observed in migratory birds. Migrating females carried relatively larger fat stores during migration, but males did not. In summer, some females stood out as outliers with larger intestines and smaller pectoralis muscles. Separate male and female effects were evident in adipose FA composition, and males and females changed muscle PL composition in opposite directions. Below we discuss these findings in the context of previous studies of migratory birds, and consider bat life-history factors that may contribute to the observed sex differences.

Body composition

We predicted that migrating bats would weigh more than non-migrating bats due to the increased mass of fat stores and hypertrophy of muscles and digestive organs. Contrary to our expectations, body mass was reduced in migrating bats. Particularly surprising was the overall reduction of lean mass in migrants (see discussion of digestive organs below). A reduction in body mass reduces wing loading and consequently lowers the energetic cost of flight (Bowlin and Wikelski, 2008; Voigt et al., 2010b). The wing loading effect is in addition to the efficient flight that hoary bats achieve due to a high aspect ratio. Of 81 bats measured in the family Vespertilionidae, hoary bats had the fourth highest aspect ratio (Norberg and Rayner, 1987). High aspect ratio and seasonally lowered wing loading suggest that the energetic costs of migration have played an important role in the evolution of this species (although wing morphology adaptations for an open-space aerial hawking foraging strategy have also surely contributed).

Increases in the relative size of fat stores for migrating females, but not males, may reflect an increased energetic cost of migration for females. Female hoary bats generally migrate greater distances than males, and therefore females may require larger fat stores. Alternatively, the discrepancy may arise as a result of differences in thermoregulatory strategy during migration. Migrating bats could use daily torpor during migration and spare energy stores to fuel migratory flight (McGuire et al., 2012). This strategy could greatly reduce the overall energetic costs of migration; migratory birds incur twice the energy cost during stopover compared with actual migratory flight, largely because of thermoregulatory costs during overnight roosting (Wikelski et al., 2003; but see Carpenter and Hixon, 1988; Wojciechowski and Pinshow, 2009). However, Cryan and Wolf (Cryan and Wolf, 2003) demonstrated (at the same sites we collected our migrants) that during spring migration female hoary bats defend normothermic body temperature, while males readily use torpor when ambient temperature is decreased. Females may not lower body temperature because of the potential detrimental effects on the developing fetuses. If females do not use daily torpor, they would either need larger fat stores to support higher metabolic rates during diurnal roosting periods (compared with torpid males), or they would need to frequently replenish fuel stores that are depleted in both migratory flight and defence of body temperature.

We argue (see below) that the former scenario is more consistent with our observations.

Organ sizes

The general pattern of reduced digestive organs is similar to the pattern observed in numerous studies of migratory birds (e.g. Battley et al., 2000; Bauchinger et al., 2005), consistent with the 'guts don't fly' hypothesis [minimize gut mass to minimize the cost of transport (Piersma and Gill, 1998)]. Individually, intestines and kidneys were smaller in migrating bats (mean intestine mass 43% smaller, mean kidney mass 6–9% smaller; Table 1), and combined digestive organs (except liver) loaded together on the second PC axis, indicating smaller digestive organs in migrants. Most bird species for which reduced guts have been observed migrate vast distances over habitat that does not permit foraging. In the case of bar-tailed godwits (*Limosa lapponica baueri*), non-stop migratory flights may cover >11,000 km and last 9 days as birds fly from Alaska to New Zealand (Gill et al., 2009; Battley et al., 2012). Garden warblers (*Sylvia borin*) migrate >2500 km across the Sahara Desert without feeding (Bauchinger et al., 2005). However, digestive capacity (and digestive tract organ sizes) is typically linked to demand (McWilliams and Karasov, 2005). For a hoary bat migrating from southern California to Canada, there is ample suitable foraging habitat along the route, and therefore it is somewhat surprising that the changes in digestive organs are not similar to the increases observed in western sandpipers (*C. mauri*), which stop to refuel frequently along their migratory route (Guglielmo and Williams, 2003). Instead, it would appear that the bats in our study were more likely to deposit fuel (larger fat stores for the females that could not save energy through the use of daily torpor) prior to migration and minimize time spent foraging along the migratory route. We did not observe sex effects in the reduction of digestive organs, suggesting that both sexes reduce foraging effort similarly. To compensate for reduced foraging during migration, females that do not use torpor could deposit larger fat stores prior to migration, which is indeed what we observed. In the autumn when females are not pregnant, there should be no sex effects in either foraging effort or the size of fat stores. Both of these predictions are consistent with observations of fall migrating silver-haired bats (*Lasionycteris noctivagans*) where few bats foraged and there was no difference in body composition with regards to sex (McGuire et al., 2012). However, only two of the migrants in our study had empty stomachs. Similar ratios of migrating bats with and without stomach contents were observed in a study of hoary and silver-haired bats during autumn migration in Alberta, Canada (Reimer et al., 2010). Therefore, foraging does not cease completely during migration. Bats may forage briefly each night rather than alternating extended periods of refuelling and migratory flight (Voigt et al., 2012).

Increased exercise machinery has been frequently observed in migratory birds, but the individual organ changes differ from the observations of the bats in our study. Birds may increase the size of their heart and flight muscle (Marsh, 1984; Piersma, 1998; Piersma et al., 1999; Portugal et al., 2009), but changes in lung mass are rarely recorded. In the hoary bat PCA, all exercise organs (heart, lungs, pectoralis muscle) loaded together, indicating larger exercise organs in migrating bats. However, the evidence for changes in flight muscle mass is weak (not significant in individual organ comparison, smaller factor loading in PCA), suggesting that flight muscle size varies with body mass, as has been observed in many migratory birds (e.g. Marsh, 1984; Dietz et al., 1999; Lindström et al., 2000). When comparing individual organs, migrating bats had larger lungs (mean lung mass 21% larger; Table 1) but there was no difference in heart or flight muscle size. Lung mass change appears to be a novel component of bat migration physiology that may result from the less rigid structure of the mammalian lung, which enables phenotypic flexibility that is not possible given the design of the avian lung (Maina, 2000). Larger lungs in migrants, independent of body mass, may be associated with increased capacity for aerobic exercise. An intriguing alternative is that increased lung mass may be associated with exposure to low oxygen concentration, as observed in rodents (Burri and Weibel, 1971). Deer mice at high altitudes have been shown to increase lung mass in response to the lower oxygen concentration (Hammond et al., 2001). If bats spend more time flying at high altitudes during migration, and over a long enough period, lung mass may be increased to compensate for lower oxygen concentration. This suggestion is highly speculative and requires further investigation.

The data we collected from some of the non-migrating female hoary bats suggest that all organ size changes we observed may be conservative estimates of seasonal migration-related phenotypic flexibility. Female bats arrive in Cypress Hills in late May or early June (Willis et al., 2006). Parturition occurs shortly after arrival (mid-June), and the young become volant ~5 weeks later (Shump and Shump, 1982). The earliest date we captured post-lactating females (in either year) was 20 July; the latest date we captured lactating females was 22 July. Therefore, some of the earliest females we captured (outliers in Fig. 2) may only have ceased lactating days earlier. The outliers shown in Fig. 2 suggest that there may be a rapid and dramatic change in body composition at weaning. In these early season females, intestines were approximately two times larger and pectoralis muscles were only ~60% of the size that would be expected for their body mass. If these organ size changes are typical, then our late summer female samples may be better considered as pre-migratory samples. Comparison of migrating bats and lactating females may present a more extreme picture than the potentially conservative size changes we documented.

FA profiles

Adipose FA composition was shifted towards increased PUFAs and decreased saturated FAs. Such changes would slightly reduce the net ATP production per gram of triglycerides (Price, 2010), but would greatly increase the potential mobilization of FA stores. In a study of ruffs (*Philomachus pugnax*), the relative mobilization of 18:2n-6 and 18:3n-3 (two FAs that increased in hoary bats; Fig. 3) was ~50% greater than 16:0 (decreased during migration in hoary bats; Fig. 3) (Price et al., 2008). A similar pattern (but less pronounced) has been observed in studies on rats and humans (Raclot and Groscolas, 1993; Raclot, 2003). High-intensity migratory flight demands a continuous supply of energy substrates. Consequently, the trade-off between potential energy and mobilization rate may favour those FAs that permit a high rate of sustained energy substrate delivery to the flight muscles. The reduction in energy density may be compensated by deposition of larger fat stores, or refuelling more frequently.

Interpretation of migration-related changes in the FA composition of muscle PL is notoriously difficult (Price, 2010). Many studies have reported effects of different muscle PL profiles, but these effects may in fact simply reflect differences in adipose composition. This is not likely the case in our study. The most notable differences in muscle PL FA were the changes in the n-6:n-3 ratio, increasing for males and decreasing for females. The only adipose FA where males and females changed in opposite directions was 18:1n-9, which was not affected by sex or migration in muscle PL. Sex-biased differences in muscle PL but not adipose NL suggest that

any differences in muscle PL are not simply consequences of adipose NL composition.

It is difficult to interpret how muscle PL changes might affect exercise performance given that males and females changed in different manners. To interpret exercise performance effects we would need to understand sex differences in the energetic cost or energetic strategies associated with migratory flight. However, sex-based variation in the muscle PL n-6:n-3 ratio is consistent with the Ruf and Arnold (Ruf and Arnold, 2008) hypothesis that an increased n-6:n-3 ratio is associated with torpor use. During spring migration, male hoary bats readily use torpor while females rarely lower their body temperature (Cryan and Wolf, 2003), and accordingly we observed an increased n-6:n-3 ratio in males and a decreased ratio in females. The prediction that migrating males should maximize total PUFAs (Munro and Thomas, 2004) is not supported by our data, as we did not observe any differences in muscle PL total PUFA content.

Conclusions

Although bats and birds represent two phylogenetically distant lineages, they have converged on a number of similar physiological strategies associated with migratory behaviour. We observed patterns consistent with fat being used as the primary fuel for migration, as has been shown in previous studies of migratory birds. Bats are able to deposit large fat stores (as observed in the migrating females), and the FA profile of adipose stores indicates that mobilization of FAs to maintain high delivery rates in flight is more important than the energy density of the fat stores. Furthermore, changes in digestive organ sizes indicate that bats may favour a time-minimizing migration strategy, minimizing time spent foraging *en route*. Like birds, enlarged exercise organs of bats reflect the increased aerobic exercise demands during migratory periods. However, the increase in lung size that we observed has not been previously documented in birds and highlights the fact that the migration physiology of bats is in many ways distinct from that of birds.

The coincidence of spring migration and pregnancy is perhaps the most important contrast between the migration of birds and bats. In birds, migration and breeding are temporally isolated, and thus physiological consequences of migration are relatively easily isolated compared with the situation in bats. Clearly, reproductive physiology is an important consideration when interpreting bat migration-related phenotypic flexibility. Pregnancy and lactation may affect nearly all aspects of migration physiology that we considered. We collected bats during spring migration and summer non-migratory periods because these were the only times of year we could reliably capture hoary bats. Comparison of autumn migrating and wintering bats will help to isolate migration effects from reproductive effects. We were unaware of reliable sites for capturing autumn migrants or overwintering bats. Future work should focus on finding such sites or studying species where such sites are known.

LIST OF SYMBOLS AND ABBREVIATIONS

DBI	double bond index
FA	fatty acid
FAME	fatty acid methyl ester
MUFA	monounsaturated fatty acid
NEFA	non-esterified fatty acid
NL	neutral lipid
PC	principal component
PCA	principal components analysis
PL	phospholipid
PUFA	polyunsaturated fatty acid

ACKNOWLEDGEMENTS

Thanks to Edwin Price and Alex Gerson for helpful discussions and developing laboratory analysis methods. We also thank Mark Brigham, Craig Willis, Robert Barclay, Blair Wolf, Paul Cryan and Ernie Valdez for discussion and assistance in determining field sites and co-coordinating collecting trips. Lauren Hooton, Amanda Adams, Quentin Hays, Julia Kilgour, Erin Gillam and Joe Poissant provided invaluable assistance in the field. Christian Voigt and two anonymous reviewers provided valuable feedback that improved the manuscript.

FUNDING

We gratefully acknowledge funding for this study provided by Bat Conservation International (student scholarship to L.P.M.), the Society for Integrative and Comparative Biology (travel fellowship to L.P.M.), the University of Western Ontario (research grant to L.P.M.) and the Natural Sciences and Engineering Research Council of Canada (Discovery grants to C.G.G. and M.B.F.). L.P.M. was supported by a Canada Graduate Scholarship from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

Anthony, E. L. P. (1988). Age determination in bats. In *Ecological and Behavioral Methods for the Study of Bats* (ed. T. H. Kunz), pp. 47-58. Washington, DC: Smithsonian Institution Press.

Ayre, K. J. and Hulbert, A. J. (1997). Dietary fatty acid profile affects endurance in rats. *Lipids* **32**, 1265-1270.

Barclay, R. M. R. (1989). The effect of reproductive condition on the foraging behaviour of female hoary bats, *Lasiurus cinereus*. *Behav. Ecol. Sociobiol.* **24**, 31-37.

Battley, P. F., Piersma, T., Dietz, M. W., Tang, S., Dekinga, A. and Hulsman, K. (2000). Empirical evidence for differential organ reductions during trans-oceanic bird flight. *Proc. Biol. Sci.* **267**, 191-195.

Battley, P. F., Dietz, M. W., Piersma, T., Dekinga, A., Tang, S. and Hulsman, K. (2001). Is long-distance bird flight equivalent to a high-energy fast? Body composition changes in freely migrating and captive fasting great knots. *Physiol. Biochem. Zool.* **74**, 435-449.

Battley, P. F., Warnock, N., Tibbitts, T. L., Gill, R. E., Jr, Piersma, T., Hassell, C. J., Douglas, D. C., Mulcahy, D. M., Gartell, B. D., Schuckard, R. et al. (2012). Contrasting extreme long-distance migration patterns in bar-tailed godwits *Limosa lapponica*. *J. Avian Biol.* **43**, 21-32.

Bauchinger, U., Wohlmann, A. and Biebach, H. (2005). Flexible remodeling of organ size during spring migration of the garden warbler (Sylvia borin). *Zoology* **108**, 97-106.

Best, T. L. and Geluso, K. N. (2003). Summer foraging range of Mexican free-tailed bats (*Tadarida brasiliensis*) from Carlsbad Cavern, New Mexico. *Southwest. Nat.* **48**, 590-596.

Bowlin, M. S. and Wikelski, M. (2008). Pointed wings, low wingloading and calm air reduce migratory flight costs in songbirds. *PLoS ONE* **3**, e2154.

Burri, P. H. and Weibel, E. R. (1971). Morphometric estimation of pulmonary diffusion capacity. II. Effect of P_{O_2} on the growing lung, adaption of the growing rat lung to hypoxia and hyperoxia. *Respir. Physiol.* **11**, 247-264.

Carpenter, F. L. and Hixon, M. A. (1988). A new function for torpor: fat conservation in a wild migrant hummingbird. *Condor* **90**, 373-378.

Carter, T. C. and Menzel, J. M. (2007). Behaviour and day-roosting ecology of North American foliage roosting bats. In *Bats in Forests* (ed. M. J. Lacki, J. P. Hayes and A. Kurta), pp. 61-82. Baltimore, MD: Johns Hopkins University Press.

Ceballos, G., Fleming, T. H., Chávez, C. and Nassar, J. (1997). Population dynamics of *Leptonycteris curasoae* (Chiroptera: Phyllostomidae) in Jalisco, Mexico. *J. Mammal.* **78**, 1220-1230.

Cryan, P. M. (2003). Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in North America. *J. Mammal.* **84**, 579-593.

Cryan, P. M. (2008). Mating behaviour as a possible cause of bat fatalities at wind turbines. *J. Wildl. Manage.* **72**, 845-849.

Cryan, P. M. and Veilleux, J. P. (2007). Migration and use of autumn, winter, and spring roosts by tree bats. In *Bats in Forests* (ed. M. J. Lacki, J. P. Hayes and A. Kurta), pp. 153-175. Baltimore, MD: Johns Hopkins University Press.

Cryan, P. M. and Wolf, B. O. (2003). Sex differences in the thermoregulation and evaporative water loss of a heterothermic bat, *Lasiurus cinereus*, during its spring migration. *J. Exp. Biol.* **206**, 3381-3390.

Cryan, P. M., Bogan, M. A., Rye, R. O., Landis, G. P. and Kester, C. L. (2004). Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. *J. Mammal.* **85**, 995-1001.

Dietz, M. W., Piersma, T. and Dekinga, A. (1999). Body-building without power training: endogenously regulated pectoral muscle hypertrophy in confined shorebirds. *J. Exp. Biol.* **202**, 2831-2837.

Dingle, H. (1996). *Migration: The Biology of Life on the Move*. New York: Oxford University Press.

Ewing, W. G., Studier, E. H. and O'Farrell, M. J. (1970). Autumn fat deposition and gross body composition in three species of *Myotis*. *Comp. Biochem. Physiol.* **36**, 119-129.

Fleming, T. H. and Eby, P. (2003). Ecology of bat migration. In *Bat Ecology* (ed. T. H. Kunz and M. B. Fenton), pp. 156-208. Chicago, IL: University of Chicago Press.

Gerson, A. R. and Guglielmo, C. G. (2011). Flight at low ambient humidity increases protein catabolism in migratory birds. *Science* **333**, 1434-1436.

Gill, R. E., Jr, Tibbitts, T. L., Douglas, D. C., Handel, C. M., Mulcahy, D. M., Gottschalck, J. C., Warnock, N., McCaffery, B. J., Battley, P. F. and Piersma, T. (2009). Extreme endurance flights by landbirds crossing the Pacific Ocean: ecological corridor rather than barrier? *Proc. Biol. Sci.* **276**, 447-457.

Guglielmo, C. G. (2010). Move that fatty acid: fuel selection and transport in migratory birds and bats. *Integr. Comp. Biol.* **50**, 336-345.
Guglielmo, C. G. and Williams, T. D. (2003). Phenotypic flexibility of body composition in relation to migratory state, age, and sex in the western sandpiper (*Calidris mauri*). *Physiol. Biochem. Zool.* **76**, 84-98.
Guglielmo, C. G., Williams, T. D., Zwingelstein, G., Brichon, G. and Weber, J.-M. (2002). Plasma and muscle phospholipids are involved in the metabolic response to long-distance migration in a shorebird. *J. Comp. Physiol. B* **172**, 409-417.
Gwinner, E. (1990) *Bird Migration: Physiology and Ecophysiology*. Berlin: Springer-Verlag.
Hammond, K. A., Szewczak, J. and Król, E. (2001). Effects of altitude and temperature on organ phenotypic plasticity along an altitudinal gradient. *J. Exp. Biol.* **204**, 1991-2000.
Hume, I. D. and Biebach, H. (1996). Digestive tract function in the long-distance migratory garden warbler, *Sylvia borin*. *J. Comp. Physiol. B* **166**, 388-395.
Jenni, L. and Jenni-Eiermann, S. (1998). Fuel supply and metabolic constraints in migrating birds. *J. Avian Biol.* **29**, 521-528.
Klaiman, J. M., Price, E. R. and Guglielmo, C. G. (2009). Fatty acid composition of pectoralis muscle membrane, intramuscular fat stores and adipose tissue of migrant and wintering white-throated sparrows (*Zonotrichia albicollis*). *J. Exp. Biol.* **212**, 3865-3872.
Krulin, G. S. and Sealander, J. A. (1972). Annual lipid cycle of the gray bat, *Myotis grisescens*. *Comp. Biochem. Physiol.* **42A**, 537-549.
Kunz, T. H., Wrazen, J. A. and Burnett, C. D. (1998). Changes in body mass and fat reserves in pre-hibernating little brown bats (*Myotis lucifugus*). *Ecoscience* **5**, 8-17.
Landys-Ciannelli, M. M., Piersma, T. and Jukema, J. (2003). Strategic size changes of internal organs and muscle tissue in the bar-tailed godwit during fat storage on a spring stopover site. *Funct. Ecol.* **17**, 151-159.
Leyton, J., Drury, P. J. and Crawford, M. A. (1987). Differential oxidation of saturated and unsaturated fatty acids *in vivo* in the rat. *Br. J. Nutr.* **57**, 383-393.
Lindström, Å., Kvist, A., Piersma, T., Dekinga, A. and Dietz, M. W. (2000). Avian pectoral muscle size rapidly tracks body mass changes during flight, fasting and fuelling. *J. Exp. Biol.* **203**, 913-919.
Maillet, D. and Weber, J.-M. (2007). Relationship between n-3 PUFA content and energy metabolism in the flight muscles of a migrating shorebird: evidence for natural doping. *J. Exp. Biol.* **210**, 413-420.
Maina, J. N. (2000). What it takes to fly: the structural and functional respiratory refinements in birds and bats. *J. Exp. Biol.* **203**, 3045-3064.
Marsh, R. L. (1984). Adaptations of the gray catbird *Dumetella carolinensis* to long-distance migration: flight muscle hypertrophy associated with elevated body mass. *Physiol. Zool.* **57**, 105-117.
McClelland, G. B. (2004). Fat to the fire: the regulation of lipid oxidation with exercise and environmental stress. *Comp. Biochem. Physiol.* **139B**, 443-460.
McGuire, L. P. and Guglielmo, C. G. (2009). What can birds tell us about the migration physiology of bats? *J. Mammal.* **90**, 1290-1297.
McGuire, L. P., Guglielmo, C. G., Mackenzie, S. A. and Taylor, P. D. (2012). Migratory stopover in the long-distance migrant silver-haired bat, *Lasionycteris noctivagans*. *J. Anim. Ecol.* **81**, 377-385.
McWilliams, S. R. and Karasov, W. (2005) Migration takes guts. In *Birds of Two Worlds: the Ecology and Evolution of Migration* (ed. R. Greenberg and P. P. Marra), pp. 67-78. Baltimore, MD: The Johns Hopkins University Press.
McWilliams, S. R., Guglielmo, C., Pierce, B. and Klaassen, M. (2004). Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. *J. Avian Biol.* **35**, 377-393.
Munro, D. and Thomas, D. W. (2004). The role of polyunsaturated fatty acids in the expression of torpor by mammals: a review. *Zoology* **107**, 29-48.
Norberg, U. M. and Rayner, J. M. V. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philos. Trans. R. Soc. B* **316**, 335-427.
O'Shea, T. J. (1976). Fat content in migratory central Arizona Brazilian free-tailed bats, *Tadarida brasiliensis* (Molossidae). *Southwest. Nat.* **21**, 326.
O'Shea, T. J. and Vaughan, T. A. (1980). Ecological observations on an East African bat community. *Mammalia* **44**, 485-496.
Pennycuick, C. J. (1998). Towards an optimal strategy for bird flight research. *J. Avian Biol.* **29**, 449-457.
Pierce, B. J., McWilliams, S. R., O'Connor, T. P., Place, A. R. and Guglielmo, C. G. (2005). Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. *J. Exp. Biol.* **208**, 1277-1285.
Piersma, T. (1998). Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight? *J. Avian Biol.* **29**, 511-520.
Piersma, T. and Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* **18**, 228-233.
Piersma, T. and Gill, R. E., Jr (1998). Guts don't fly: small digestive organs in obese bar-tailed godwits. *Auk* **115**, 196-203.
Piersma, T. and van Gils, J. A. (2011). *The Flexible Phenotype: a Body-Centered Integration of Ecology, Physiology, and Behaviour*. New York: Oxford University Press.
Piersma, T., Gudmundsson, G. A. and Lilliendahl, K. (1999). Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. *Physiol. Biochem. Zool.* **72**, 405-415.
Portugal, S. J., Thorpe, S. K. S., Green, J. A., Myatt, J. P. and Butler, P. J. (2009). Testing the use/disuse hypothesis: pectoral and leg muscle changes in captive barnacle geese *Branta leucopsis* during wing moult. *J. Exp. Biol.* **212**, 2403-2410.
Portugal, S. J., Green, J. A., White, C. R., Guillemette, M. and Butler, P. J. (2012). Wild geese do not increase flight behaviour prior to migration. *Biol. Lett.* **8**, 469-472.
Price, E. R. (2010). Dietary lipid composition and avian migratory flight performance: development of a theoretical framework for avian fat storage. *Comp. Biochem. Physiol.* **157A**, 297-309.
Price, E. R. and Guglielmo, C. G. (2009). The effect of muscle phospholipid fatty acid composition on exercise performance: a direct test in the migratory white-throated sparrow (*Zonotrichia albicollis*). *Am. J. Physiol.* **297**, R775-R782.
Price, E. R., Krokfors, A. and Guglielmo, C. G. (2008). Selective mobilization of fatty acids from adipose tissue in migratory birds. *J. Exp. Biol.* **211**, 29-34.
Price, E. R., Staples, J. F., Milligan, C. L. and Guglielmo, C. G. (2011). Carnitine palmitoyl transferase activity and whole muscle oxidation rates vary with fatty acid substrate in avian flight muscles. *J. Comp. Physiol. B* **181**, 565-573.
R Development Core Team (2009). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available at http://www.R-project.org.
Raclot, T. (2003). Selective mobilization of fatty acids from adipose tissue triacylglycerols. *Prog. Lipid Res.* **42**, 257-288.
Raclot, T. and Groscolas, R. (1993). Differential mobilization of white adipose tissue fatty acids according to chain length, unsaturation, and positional isomerism. *J. Lipid Res.* **34**, 1515-1526.
Rayner, J. M. V. (1988). The evolution of vertebrate flight. *Biol. J. Linn. Soc. Lond.* **34**, 269-287.
Reimer, J. P., Baerwald, E. F. and Barclay, R. M. R. (2010). Diet of hoary (*Lasiurus cinereus*) and silver-haired (*Lasionycteris noctivagans*) bats while migrating through southwestern Alberta in late summer and autumn. *Am. Midl. Nat.* **164**, 230-237.
Roberts, T. J., Weber, J.-M., Hoppeler, H., Weibel, E. R. and Taylor, C. R. (1996). Design of the oxygen and substrate pathways. II. Defining the upper limits of carbohydrate and fat oxidation. *J. Exp. Biol.* **199**, 1651-1658.
Ruf, T. and Arnold, W. (2008). Effects of polyunsaturated fatty acids on hibernation and torpor: a review and hypothesis. *Am. J. Physiol.* **294**, R1044-R1052.
Ruf, T., Valencak, T., Tataruch, F. and Arnold, W. (2006). Running speed in mammals increases with muscle n-6 polyunsaturated fatty acid content. *PLoS ONE* **1**, e65.
Schalk, G. and Brigham, R. M. (1995). Prey selection by insectivorous bats: are essential fatty acids important? *Can. J. Zool.* **73**, 1855-1859.
Shump, K. A., Jr and Shump, A. U. (1982). *Lasiurus cinereus*. *Mamm. Species* **185**, 1-5.
Speakman, J. R. and Thomas, D. W. (2003). Physiological ecology and energetics of bats. In *Bat Ecology* (ed. T. H. Kunz and M. B. Fenton), pp. 430-490. Chicago, IL: University of Chicago Press.
Voigt, C. C., Sörgel, K. and Dechmann, D. K. N. (2010a). Refueling while flying: foraging bats combust food rapidly and directly to power flight. *Ecology* **91**, 2908-2917.
Voigt, C. C., Schuller, B.-M., Greif, S. and Siemers, B. M. (2010b). Perch-hunting in insectivorous *Rhinolophus* bats is related to the high energy costs of manoeuvring in flight. *J. Comp. Physiol. B* **180**, 1079-1088.
Voigt, C. C., Sörgel, K., Šuba, J., Keišs, O. and Pētersons, G. (2012). The insectivorous bat *Pipistrellus nathusii* uses a mixed-fuel strategy to power autumn migration. *Proc. Biol. Sci.* **279**, 3772-3778.
Weber, J.-M., Roberts, T. J., Vock, R., Weibel, E. R. and Taylor, C. R. (1996a). Design of the oxygen and substrate pathways. III. Partitioning energy provision from carbohydrates. *J. Exp. Biol.* **199**, 1659-1666.
Weber, J.-M., Brichon, G., Zwingelstein, G., McClelland, G., Saucedo, C., Weibel, E. R. and Taylor, C. R. (1996b). Design of the oxygen and substrate pathways. IV. Partitioning energy provision from fatty acids. *J. Exp. Biol.* **199**, 1667-1674.
Weibel, E. R., Taylor, C. R., Weber, J.-M., Vock, R., Roberts, T. J. and Hoppeler, H. (1996). Design of the oxygen and substrate pathways. VII. Different structural limits for oxygen and substrate supply to muscle mitochondria. *J. Exp. Biol.* **199**, 1699-1709.
Welch, K. C., Jr, Herrera M, L. G. and Suarez, R. K. (2008). Dietary sugar as a direct fuel for flight in the nectarivorous bat *Glossophaga soricina*. *J. Exp. Biol.* **211**, 310-316.
Wikelski, M., Tarlow, E. M., Raim, A., Diehl, R. H., Larkin, R. P. and Visser, G. H. (2003). Avian metabolism: costs of migration in free-flying songbirds. *Nature* **423**, 704.
Williams, D. F. and Findley, J. S. (1979). Sexual size dimorphism in vespertilionid bats. *Am. Midl. Nat.* **102**, 113-126.
Willis, C. K. R. and Brigham, R. M. (2005). Physiological and ecological aspects of roost selection by reproductive female hoary bats (*Lasiurus cinereus*). *J. Mammal.* **86**, 85-94.
Willis, C. K. R., Brigham, R. M. and Geiser, F. (2006). Deep, prolonged torpor by pregnant, free-ranging bats. *Naturwissenschaften* **93**, 80-83.
Wojciechowski, M. S. and Pinshow, B. (2009). Heterothermy in small, migrating passerine birds during stopover: use of hypothermia at rest accelerates fuel accumulation. *J. Exp. Biol.* **212**, 3068-3075.