Original Article

Stopover refueling rate underlies protandry and seasonal variation in migration timing of songbirds

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We used plasma metabolite analysis to assess refueling rates of songbirds at stopover sites in New York and test hypotheses that males refuel faster than females during spring (in 2 species), migrants refuel faster during spring than autumn (in 5 species), and adults refuel faster than juveniles during autumn (in 4 species). Model selection based on Akaike's information criterion indicated that males had higher refueling rates than females during spring in both species tested. Spring migrants had higher refueling rates than autumn migrants in 4 of the 5 species we examined. Juvenile and adult refueling rates during autumn did not differ in any species. Our results indicate that variation in stopover refueling rate can operate as a mechanism for protandry in spring and faster migration during spring than autumn. We found no evidence that juvenile refueling performance during autumn was poorer than that of adults. *Key words:* differential migration, lipids, protandry, refueling, stopover. *[Behav Ecol]*

INTRODUCTION

Migrating birds spend more time and energy at stopover sites than they do in flight (Wikelski et al. 2003; Bowlin et al. 2005). Refueling rate is a major determinant of stopover duration, and therefore, factors that affect refueling have great potential to influence migration speed and success (Lindström and Alerstam 1992; Moore et al. 2003; Schaub et al. 2008). Food abundance, intra- and interspecific competition, arrival condition, and predation risk are primary factors that affect refueling rate, but behavioral and physiological differences among individual birds may also play a role. Sex and age are thought to be among the most important attributes of birds influencing their refueling (Carpenter, Hixon, Russell, et al. 1993; Woodrey 2000; Moore et al. 2003). Differences in refueling are also expected to occur between spring migration to breeding areas and autumn migration to wintering areas (Berthold 2001).

Greater benefits of early arrival to breeding areas for males than females favor the earlier arrival of males (i.e., protandry; Morbey and Ydenberg 2001; Kokko et al. 2006; Morbey et al. 2012). Protandry can be accomplished by males wintering closer to breeding areas, departing from wintering areas earlier, and/or migrating faster than females (Gwinner 1990; Dierschke et al. 2005; Coppack and Pulido 2009; Maggini and Bairlein 2012). As a corollary of faster migration, males may refuel more rapidly during stopovers and/or depart stopover sites with more fuel in order to bypass poor-quality stopover sites (Dierschke et al. 2005); yet, most studies have failed to detect a sex difference in refueling rate (Carpenter, Hixon, Temeles, et al. 1993; Otahal 1995; Morris and Glasgow 2001; Morris et al. 2003; Dierschke et al. 2005; Smith et al. 2007; MacDade et al. 2011; but see Wang et al. 1998).

Migratory season is likely to affect refueling rate because, during spring, individuals of both sexes benefit from arriving on breeding grounds earlier than others and with large fat stores (Sandberg and Moore 1996; Kokko 1999; Smith and Moore 2003; Tøttrup and Thorup 2008). Greater pressure for time minimization during spring is reflected in the faster pace of northbound than southbound migration (Fransson 1995; Stutchbury et al. 2008; Karlsson et al. 2012; Schmaljohann et al. 2012). Thus, stopover refueling rate, a major (but not the only) contributor to overall migration speed, should be more rapid during spring than autumn. Comparisons of spring and autumn refueling rates, however, have so far been inconsistent. Morris and Glasgow (2001) found that migrants gained mass faster during spring than autumn, Dunn (2001, 2002) found the opposite pattern, and others found no difference (Bonter et al. 2007).

Juvenile birds make their first migration during autumn. Compared with adults, they generally exhibit poor foraging skills, social subordinance, poor food selection, and inefficient nutrient assimilation (reviewed by Woodrey 2000), factors that may explain why they sometimes carry less fat (Woodrey 2000; Moore et al. 2003) and stay longer at stopover sites (Ellegren 1991; Morris et al. 1996; Rguibi-Idrissi et al. 2003; Mackenzie 2010). However, most studies find no evidence that juveniles refuel more slowly than adults (Woodrey and Moore 1997; Wang et al. 1998; Morris and Glasgow 2001; Jones et al. 2002; Morris et al. 2003; Carlisle et al. 2005; Leist 2007; Smith and McWilliams 2010).

In birds, plasma concentrations of triglyceride and β-OH-butyrate quickly rise and decline, respectively, in response to subtle gains in body mass (Jenni-Eiermann and Jenni 1994;

Zajac et al. 2006) and provide sensitive metrics of individual refueling rate (e.g., Guglielmo et al. 2002, 2005; Cerasale and Guglielmo 2010). Here, we use plasma concentrations of triglyceride and β-OH-butyrate in multiple species of migratory songbirds to test the hypotheses that males refuel faster than females during spring, adults refuel faster than juveniles during autumn, and both males and females refuel more rapidly during spring than autumn.

MATERIALS AND METHODS

Data collection and study species

We studied migratory birds during spring and autumn in the New York metropolitan area from 2007 to 2008 to assess the quality of urban forests as stopover sites. The study sites included Inwood Park, Prospect Park, Bronx Park, and Marshlands Conservancy, which contain deciduous woodlands that attract large numbers of migrating songbirds and provide similar quality stopover habitat (Seewagen et al. 2011). From our data set, we selected those species and individuals for which we were able to confidently determine sex and/or age and that had sufficient sample sizes during both spring and autumn.

Birds were captured in mist nets from approximately sunrise until 11:00 EST, from 1 to 31 May and 8 September to 20 October. These sampling periods generally coincide with the peaks of spring and autumn songbird migration through the study area (Fowle and Kerlinger 2001). Nets were checked every 8 min or less to minimize changes in plasma metabolite levels between capture and blood sample collection (Guglielmo et al. 2005). We assumed birds were in the net since the net was last checked and measured "bleed time" conservatively as the time elapsed between the previous net check and blood sampling. Up to 10% of total blood volume was collected by brachial veinipuncture with a 26-gauge needle into heparinized capillary tubes. Samples were later centrifuged for 5 min and plasma was transferred to 0.6-mL cryogenic tubes. Plasma was stored at –80 °C for up to 3 months until analysis. After blood sampling, birds were banded with a US Geological Survey aluminum leg band, measured (unflattened wing length to 1 mm), weighed to 0.1 g on a digital balance, assigned to a sex and/or age category when possible, and then released. Sex was determined only during spring and only for species with clear plumage dimorphism. Birds were aged only during autumn, when there are juveniles (hatch-year birds) in the population and age differences in stopover biology are expected to be most apparent. Distinctions between juvenile and adult (after-hatch-year) birds were based on plumage characteristics and/or degree of skull ossification (Pyle 1997).

We examined sex differences in refueling in the common yellowthroat (*Geothlypis trichas*) and yellow-rumped warbler (*Setophaga coronata*). Both species exhibit differential spring migration, with males preceding females by about 4–7 days (Francis and Cooke 1986; Kissner et al. 2003; Morris et al. 2003). Juvenile and adult refueling rates in autumn were compared in Swainson's thrush (*Catharus ustulatus*), common yellowthroat, yellow-rumped warbler, and white-throated sparrow (*Zonotrichia albicollis*). We tested for seasonal differences in Swainson's thrush, ovenbird (*Seiurus aurocapilla*), northern waterthrush (*Parkesia noveboracensis*), common yellowthroat, and yellow-rumped warbler.

Laboratory analyses

Plasma samples were diluted 3-fold with 0.9% NaCl to increase volume. Following Guglielmo et al. (2002), triglyceride was measured as the difference between free and triacylglycerol-bound glycerol concentrations as determined by endpoint assay (Sigma Trinder reagent A and B) and β-OH-butyrate was measured directly by kinetic endpoint assay (kit E0907979, R-Biopharm) using a microplate spectrophotometer (Biotec Powerwave X340). If plasma sample volume was limited, only triglyceride was measured because it is generally considered to be a stronger indicator of refueling rate (Guglielmo et al. 2002, 2005; Cerasale and Guglielmo 2006a, 2006b). For each assay, samples were analyzed in duplicate and values were averaged (all coefficients of variation < 15%).

Statistical analyses

Factors contributing to variation in triglyceride and β-OH-butyrate concentrations ($\log_{10}$[metabolite] + 1 transformed) were determined by model selection based on Akaike's information criterion (AIC; Symonds and Moussalli 2011). Three sets of species-specific analyses were conducted to evaluate effects of sex, season, or age (Table 1). In addition to the main effect of interest (i.e., sex, season, or age coded as 1/0 dummy variables), analyses included size-corrected body mass, capture date (day of year; for sex and age analyses only), capture time, and bleed time as covariates; site (coded as up to 2 dummy variables); and year (coded as a single dummy variable). Size-corrected body mass (hereafter "body mass") was calculated for each species using a scaled mass index and the mean wing length of the study population (Eq. 2 in Peig and Green 2009).

We constructed multiple regression models (PROC REG in SAS v. 9.2) for all possible combinations of intercept and independent variables. AIC adjusted for small sample sizes (AICc) was calculated for each model based on the residual sum of squares from least squares regression. Regression models were then ranked by AICc values to find the global best model. We also summed Akaike weights (Σw_i) across all models to provide a relative measure of the importance of each parameter.

The possibility of different capture dates between males and females during spring or between juveniles and adults during autumn, coupled with a capture date effect on plasma

Table 1

Sample sizes for comparisons of triglyceride and β-OH-butyrate between sexes in spring (M = males, F = females), between seasons (S = spring, A = autumn), and between ages in autumn (J = juveniles, A = adults) in 6 passerine species

Species	Triglyceride			β-OH-butyrate		
	Sex (M/F)	Season (S/A)	Age (J/A)	Sex (M/F)	Season (S/A)	Age (J/A)
Swainson's thrush	—	61/53	18/17	—	61/53	18/17
Common yellowthroat	37/14	79/77	22/28	—	58/73	18/26
Northern waterthrush	—	90/51	—	—	81/51	—
Ovenbird	—	126/74	—	—	109/71	—
Yellow-rumped warbler	15/17	38/43	20/35	9/11	27/40	17/33
White-throated sparrow	—	—	26/34	—	—	26/31

Reliable sex information was available in spring for 2 species and reliable age information was available in autumn for 4 species.

metabolite levels, has the potential to confound any sex or age effect. Therefore, we first tested whether capture date differed between sexes (or ages) and years, and whether there was a sex (or age) by year interaction using general linear models (PROC GLM). If the interaction was not significant ($P > 0.05$), it was removed from the model before assessing the main effects. If capture dates differed between years, we standardized capture date within years by subtracting the year-specific, mean capture date. We then tested for interactions between sex (or age) and capture day (or standardized capture day) in analyses of triglyceride and β-OH-butyrate concentrations using general linear models. All independent variables were included in statistical models when testing for interactions.

RESULTS

Sex differences

In yellow-rumped warbler during spring, males had higher plasma triglyceride levels and lower β-OH-butyrate levels than females (Figure 1), sex was included in the global best models (Tables 2 and 3), and sex had high parameter weights (Tables 4 and 5). In common yellowthroat during spring, males had higher triglyceride levels than females (Figure 1), sex was included in the global best model (Table 2), and sex had a high parameter weight (Table 4). In models with sex,
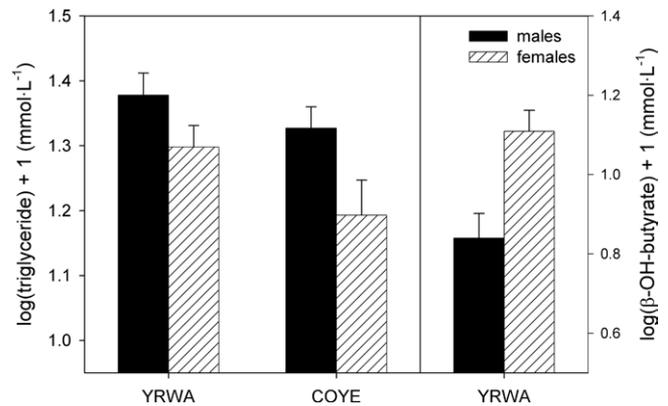

Figure 1
Comparison of triglyceride levels [log(x) + 1] and β-OH-butyrate levels [log(x) + 1] between male and female common yellowthroats (COYE) and yellow-rumped warblers (YRWA) in the spring. Shown in each pair of bars are the least square means (±SE) from a general linear model including parameters in the global best model (Tables 2 and 3).

body mass and time of day also influenced plasma triglyceride levels (Tables 2 and 4); birds that were heavier and captured later in the day had higher plasma triglyceride.

Table 2
Parameter coefficients for the global best multiple regression models of triglyceride levels

Species	Intercept	Sex	Season	Year	Bronx	Inwood	Marshlands	Prospect	Mass	Day	Time	Bleed time	k	w_i
Models with sex														
Common yellowthroat		0.137	—			−0.110		—	—	0.117		0.075	−0.021	6	0.194
Yellow-rumped warbler		0.092	—		—					0.100				3	0.049
Models with season														
Swainson's thrush	0.684	—				0.065	—		0.015	—	0.055		5	0.109
Common yellowthroat	0.444	—	0.122	0.067			—		0.046	—	0.069		6	0.157
Northern waterthrush	0.480	—	0.094			—	—		0.031	—	0.029		5	0.261
Ovenbird	0.343	—	0.124	0.097					0.030	—	0.039	−0.006	7	0.170
Yellow-rumped warbler	0.894	—	0.321	0.107	−0.154		—	—		—	0.046		6	0.291
Models with age														
Swainson's thrush	1.158	—	—		—	—	—				0.049		3	0.096
Common yellowthroat	0.959	—	—			−0.108				0.009	0.075		5	0.079
Yellow-rumped warbler	0.953	—	—		—			—			0.065		3	0.092
White-throated sparrow	0.996	—	—	0.109	—			—	0.007		0.041	−0.022	6	0.094

Separate analyses were done for each combination of species and main effect of interest (sex, season, or age). Size-corrected body mass, day of year (standardized capture date was used in age models for common yellowthroat, yellow-rumped warbler, and white-throated sparrow), time of day, and bleed time were included as covariates; site and year were included as factors. Age is not listed because it was not retained in any global best model. Also presented are the number of parameters (k) and the Akaike weight (w_i) for each global best model. All factors were coded as dummy variables [sex_1 = male, sex_0 = female; $season_1$ = spring, $season_0$ = autumn; $year_1$ = 2007, $year_0$ = 2008; $Bronx_1$ = Bronx, $Bronx_0$ = all other sites; $Inwood_1$ = Inwood, $Inwood_0$ = all other sites; $Marshlands_1$ = Marshlands, $Marshlands_0$ = all other sites (coding Prospect would be redundant)]. Dashes indicate parameters that were excluded (or were missing) from an analysis. Blank cells indicate that the parameter was not retained in the global best model.

Table 3

Parameter coefficients for the global best multiple regression models of butyrate levels

Species	Intercept	Sex	Season	Year	Bronx	Inwood	Marshlands	Prospect	Mass	Day	Time	Bleed time	k	w_i
Models with sex														
Yellow-rumped warbler	1.109	−0.269	—		—								3	0.098
Models with season														
Swainson's thrush	0.799	—	0.133	−0.099			—				−0.040		5	0.131
Common yellowthroat	1.114	—			−0.210	−0.180	—			—	−0.049	0.014	6	0.246
Northern waterthrush	1.553	—			−0.070	—	—		−0.033	—	−0.030	0.014	6	0.231
Ovenbird	1.235	—			−0.135				−0.014	—	−0.057	0.017	6	0.113
Yellow-rumped warbler	1.191	—	−0.235				—	—		—			3	0.103
Models with age														
Swainson's thrush	0.868	—	—	−0.418	—	—	—						3	0.180
Common yellowthroat	1.151	—	—		—	−0.212							3	0.065
Yellow-rumped warbler	0.510	—	—		—	0.213		—	0.034			0.010	5	0.063
White-throated sparrow	1.065	—	—	−0.134	—		—				−0.088	0.036	5	0.149

Separate analyses were done for each combination of species and main effect of interest (sex, season, or age). Size-corrected body mass, day of year, time of day (standardized capture date was used in age models for common yellowthroat, yellow-rumped warbler, and white-throated sparrow), and bleed time were included as covariates; site and year were included as factors. Age is not listed because it was not retained in any global best model. Also presented are the number of parameters (k) and the Akaike weight (w_i) for each global best model. All factors were coded as dummy variables [sex_1 = male, sex_0 = female; $season_1$ = spring, $season_0$ = autumn; $year_1$ = 2007, $year_0$ = 2008; $Bronx_1$ = Bronx, $Bronx_0$ = all other sites; $Inwood_1$ = Inwood, $Inwood_0$ = all other sites; $Marshlands_1$ = Marshlands, $Marshlands_0$ = all other sites (coding Prospect would be redundant)]. Dashes indicate parameters that were excluded (or were missing) from an analysis. Blank cells indicate that the parameter was not retained in the global best model.

In yellow-rumped warbler, capture date was similar between years ($F_{1,29} = 0.16$, $P > 0.5$) and differed between the sexes ($F_{1,29} = 5.32$, $P = 0.028$). The difference between sexes was similar between years ($F_{1,28} = 0.0$, $P > 0.5$); on average, males were captured 4.7 days earlier than females. There was no interaction between sex and capture date in the analysis of triglyceride ($F_{1,21} = 1.36$, $P = 0.26$) or β-OH-butyrate ($F_{1,9} = 0.03$, $P > 0.5$). In common yellowthroat, capture date was similar between years ($F_{1,75} = 0.26$, $P > 0.5$), differed between the sexes ($F_{1,75} = 15.9$, $P = 0.0002$), and the difference between sexes was similar between years ($F_{1,74} = 0.35$, $P > 0.5$). Males were captured an average of 5.6 days earlier than females. There was no interaction between sex and capture date in the analysis of triglyceride levels ($F_{1,41} = 0.18$, $P > 0.5$).

Seasonal differences

In 4 of 5 species, plasma triglyceride levels were higher during spring than autumn (Figure 2), season was included in the global best models (Table 2), and season had high parameter weights (Table 4). In Swainson's thrush, season was not important for explaining variation in triglyceride (Tables 2 and 4), but seasonal differences were in the predicted direction (Figure 2). In models with season, body mass and time of day also influenced triglyceride (Tables 2 and 4). In most cases, birds that were heavier and captured later in the day had higher plasma triglyceride levels.

In yellow-rumped warbler, plasma β-OH-butyrate levels were lower during spring than autumn (Figure 2), season

appeared in the global best model (Table 3), and season had a high parameter weight (Table 5). In common yellowthroat, northern waterthrush, and ovenbird, season was not important in explaining plasma β-OH-butyrate levels (Tables 3 and 5), although the differences were in the predicted direction (Figure 2). In Swainson's thrush, plasma β-OH-butyrate levels were higher during spring than autumn (Figure 2), season appeared in the global best model (Table 3), and season had a high parameter weight (Table 5). Body mass, time of day, and bleed time also influenced β-OH-butyrate in models with season (Tables 3 and 5). In most cases, birds that were heavier, captured later in the day, or had shorter bleed times had lower plasma β-OH-butyrate levels.

Age differences

Age in autumn did not affect plasma triglyceride or β-OH-butyrate levels in any of the species we studied (Tables 2–5). Capture time, and to a lesser extent, capture date were the most frequently retained variables in the global best models of triglyceride and had high parameter weights (Tables 2 and 4). Birds that were captured later in the day and later in the autumn had higher plasma triglyceride levels. In the analysis of β-OH-butyrate, no single variable was consistently retained in the best global model.

Overall, interactions between age and capture date in autumn were not important in explaining plasma metabolite levels, but there were age differences in capture date that needed to be considered. In Swainson's thrush, juveniles

Table 4

Parameter weights (Σw_i) for all multiple regression models of triglyceride levels for each combination of species and main effect of interest (sex, season, or age)

Species	Sex	Season	Age	Year	Bronx	Inwood	Marshlands	Prospect	Mass	Day	Time	Bleed time
Models with sex												
Common yellowthroat	**0.705**	—	—	0.219	**0.639**		—	—	**0.996**	0.250	**0.996**	**0.814**
Yellow-rumped warbler	**0.635**	—	—	0.202	—	0.249	0.212		**0.999**	0.211	0.541	0.415
Models with season												
Swainson's thrush	—	0.531	—	0.277	0.271	**0.510**	—		**0.988**	—	**1.000**	0.257
Common yellowthroat	—	**0.980**	—	**0.715**	0.361	0.385	—		**0.937**	—	**1.000**	0.422
Northern waterthrush	—	**0.988**	—	0.413	0.306	—	—		**1.000**	—	**0.853**	0.279
Ovenbird	—	**1.000**	—	**0.998**	0.381	0.292	0.266		**1.000**	—	**0.997**	**0.561**
Yellow-rumped warbler	—	**1.000**	—	**0.785**	**0.933**		—	—	0.429	—	**0.889**	0.236
Models with age												
Swainson's thrush	—	—	0.216	0.400	—	—	—		0.454	0.418	**0.748**	0.350
Common yellowthroat	—	—	0.413	0.233	—	**0.590**	0.231		0.509	**0.992**	**0.981**	0.231
Yellow-rumped warbler	—	—	0.307	0.403	—	0.280		—	0.368	0.290	**0.914**	0.233
White-throated sparrow	—	—	0.399	**0.873**	—	0.360	—		**0.539**	0.269	**0.803**	**0.847**

Size-corrected body mass, day of year, time of day (standardized capture date was used in age models for common yellowthroat, yellow-rumped warbler, and white-throated sparrow), and bleed time were included as covariates; site and year were included as factors. Bolded values represent parameters in the global best model (Table 2). All factors were coded as dummy variables [sex$_1$ = male, sex$_0$ = female; season$_1$ = spring, season$_0$ = autumn; year$_1$ = 2007, year$_0$ = 2008; Bronx$_1$ = Bronx, Bronx$_0$ = all other sites; Inwood$_1$ = Inwood, Inwood$_0$ = all other sites; Marshlands$_1$ = Marshlands, Marshlands$_0$ = all other sites (coding Prospect would be redundant)]. Dashes indicate parameters that were excluded (or were missing) from an analysis. Blank cells indicate that the parameter was not retained in the global best model.

and adults were captured at different times in the 2 years (age × day interaction: $F_{1,33} = 8.90$, $P = 0.0053$), with juveniles captured earlier in the season than adults in 2008 but not in 2007. In 2007, triglyceride levels did not depend on any interaction between age and capture date ($F_{1,14} = 0.33$, $P > 0.5$), but β-OH-butyrate levels depended on an interaction between age and capture date ($F_{1,14} = 9.67$, $P = 0.008$). β-OH-butyrate levels declined with capture date in juveniles, but increased with capture date in adults. However, on visual inspection of the data, only a few individuals were driving the difference between juveniles and adults at the beginning and end of the capture season. In 2008, there was no interaction between age and capture date in the analysis of triglyceride ($F_{1,7} = 4.41$, $P = 0.074$) or β-OH-butyrate ($F_{1,7} = 1.65$, $P = 0.24$).

In common yellowthroat, capture date was similar between years ($F_{1,47} = 3.84$, $P = 0.056$), similar between ages ($F_{1,47} = 1.99$, $P = 0.16$), and there was no age × year interaction ($F_{1,46} = 0.19$, $P > 0.5$). Because capture dates were close to differing between years, we used standardized capture date in the AIC-based model selection (Tables 2–5). There was no interaction between age and capture date in the analysis of triglyceride ($F_{1,39} = 0.09$, $P > 0.05$) or β-OH-butyrate ($F_{1,33} = 2.47$, $P = 0.13$).

In yellow-rumped warbler, capture date differed between years ($F_{1,52} = 157.3$, $P < 0.0001$), was similar between ages ($F_{1,52} = 0.09$, $P > 0.5$), and there was no age × year interaction ($F_{1,51} = 1.30$, $P = 0.26$). Therefore, we used standardized capture date in the AIC-based model selection (Tables 2–5).

There were no interactions between age and capture date in the analysis of triglyceride ($F_{1,46} = 0.05$, $P > 0.5$) or β-OH-butyrate ($F_{1,41} = 0.22$, $P > 0.5$).

In white-throated sparrow, juveniles and adults were captured at different times in the 2 years (age × day interaction: $F_{1,56} = 5.04$, $P = 0.029$), with juveniles captured earlier in autumn than adults in 2008 but not in 2007. Because capture dates were about 10 days earlier in 2008 than in 2007, we used standardized capture date in the AIC-based model selection (Tables 2–5). In 2007, triglyceride levels depended on an interaction between age and capture date ($F_{1,33} = 6.14$, $P = 0.019$), but β-OH-butyrate levels did not ($F_{1,31} = 1.84$, $P = 0.076$). This interaction was driven by a few, very early adults with no corresponding early juveniles. On visual inspection of the data and over a comparable capture period, the relationship between triglyceride levels and capture date was similar in juveniles and adults. In 2008, there was no interaction between age and capture date in the analysis of triglyceride ($F_{1,11} = 0.08$, $P > 0.5$) or β-OH-butyrate ($F_{1,10} = 0.91$, $P = 0.36$).

DISCUSSION

Our results indicate that variation in refueling rate may be a proximate mechanism contributing to sex-related and seasonal differences in migration speeds of birds. More rapid refueling by males than females is consistent with the earlier arrival of males at breeding grounds. The seasonal

Table 5

Parameter weights (Σw_i) for all multiple regression models of butyrate levels for each combination of species and main effect of interest (sex, season, or age)

Species	Sex	Season	Age	Year	Bronx	Inwood	Marshlands	Prospect	Mass	Day	Time	Bleed time
Models with sex (spring)												
Yellow-rumped warbler	**0.856**	—	—	0.247	—	0.148	0.320		0.333	0.424	0.171	0.168
Models with season												
Swainson's thrush	—	**0.921**	—	**0.833**	0.335	0.466	—		0.366	—	**0.859**	0.265
Common yellowthroat	—	0.390	—	0.431	**0.999**	**0.989**	—		0.263	—	**0.983**	**0.963**
Northern waterthrush		0.297	—	0.260	**0.626**	—	—		**0.998**	—	**0.765**	**0.929**
Ovenbird		0.333	—	0.322	0.307	0.479	**0.974**		**0.735**	—	**0.999**	**0.998**
Yellow-rumped warbler	—	**1.000**	—	0.365	0.249		—	—	0.424	—	0.394	0.445
Models with age (autumn)												
Swainson's thrush	—	—	0.216	**1.000**	—	—	—		0.268	0.421	0.211	0.253
Common yellowthroat	—	—	0.407	0.248	—	**0.701**	0.262		0.335	0.294	0.388	0.229
Yellow-rumped warbler	—	—	0.243	0.419	—	**0.998**		—	**0.597**	0.233	0.289	**0.604**
White-throated sparrow	—	—	0.244	**0.668**	—	0.235	—		0.339	0.407	**0.971**	**0.951**

Size-corrected body mass, day of year (standardized capture date was used in age models for common yellowthroat, yellow-rumped warbler, and white-throated sparrow), time of day, and bleed time were included as covariates; site and year were included as factors. Bolded values represent parameters in the global best model (Table 3). All factors were coded as dummy variables [sex_1 = male, sex_0 = female; $season_1$ = spring, $season_0$ = autumn; $year_1$ = 2007, $year_0$ = 2008; $Bronx_1$ = Bronx, $Bronx_0$ = all other sites; $Inwood_1$ = Inwood, $Inwood_0$ = all other sites; $Marshlands_1$ = Marshlands, $Marshlands_0$ = all other sites (coding Prospect would be redundant)]. Dashes indicate parameters that were excluded (or were missing) from an analysis. Blank cells indicate that the parameter was not retained in the global best model.

differences we observed are supportive of the hypothesis that stronger selection for early arrival at breeding grounds relative to wintering grounds drives faster refueling during spring than autumn. We found no evidence, however, to support the commonly held idea that juvenile refueling performance is inferior to that of adults during autumn stopovers.

Our findings suggest that protandry can be achieved, in part, by sex differences in en route refueling. Males had higher triglyceride levels (in common yellowthroat and yellow-rumped warbler) and lower β-OH-butyrate levels (in yellow-rumped warbler) than females. Males were also captured earlier in the spring than females, which is consistent with protandry in stopover timing. However, sex differences in plasma metabolite levels did not appear to have been caused by an effect of capture date. Earlier onset of spring migration, wintering further north, longer flight bouts, and faster flight speed are other mechanisms by which male migrants can arrive on breeding grounds before females (Gwinner 1990; Coppack and Pulido 2009; Maggini and Bairlein 2012), and these need not be mutually exclusive. Coppack and Pulido (2009) suggested that it was unlikely that males migrate faster than females, and instead emphasized differential wintering latitude and onset of spring migration as the most important mechanisms underlying protandry in birds. In part, their conclusion was influenced by a study of captive common redstarts (*Phoenicurus phoenicurus*) in which food intake and body mass changes did not differ between sexes. Perhaps sex differences are expressed more readily in natural environments, or perhaps species differ in how they achieve protandry. Sex-specific decisions about stopover departure timing with

respect to fuel load and time of day may also contribute to protandry. For example, by departing with larger fuel loads than females, males can increase their flight range, reduce the number of subsequent stopovers, and thus migrate at a faster overall pace (Dierschke et al. 2005). Not only may these factors contribute to sex differences in migration speed, they may also oppose sex-specific patterns in refueling rate. We, therefore, recommend future research to establish the causal relationship between refueling rate and migration speed.

More rapid refueling may be required of spring migrants to satisfy the energy demands of flying faster, making shorter stopovers, and ultimately completing migration in less time than during autumn (Stutchbury et al. 2008; Karlsson et al. 2012; Schmaljohann et al. 2012). Yet, comparisons of spring and autumn refueling rates of songbirds have been highly inconsistent (Dunn 2001, 2002; Morris and Glasgow 2001; Bonter et al. 2007). For example, Schaub and Jenni (2001) measured plasma triglyceride and β-OH-butyrate at 10 stopover sites in Europe and Africa. In their study, songbirds refueled faster during spring than autumn at 5 of the sites, whereas the opposite occurred at the other 5 sites. In our study, sites were fairly similar in quality (Seewagen et al. 2011), and overall, migrants refueled faster during spring than autumn. This suggests that the expression of seasonal differences in refueling by migrant birds may depend on stopover habitat quality. The position of a stopover site relative to an ecological barrier could also conceivably lead to seasonal differences in refueling if birds headed in one direction have to prepare to cross the barrier, whereas birds headed in the opposite direction do not. Bauchinger and Klaassen (2005) proposed that
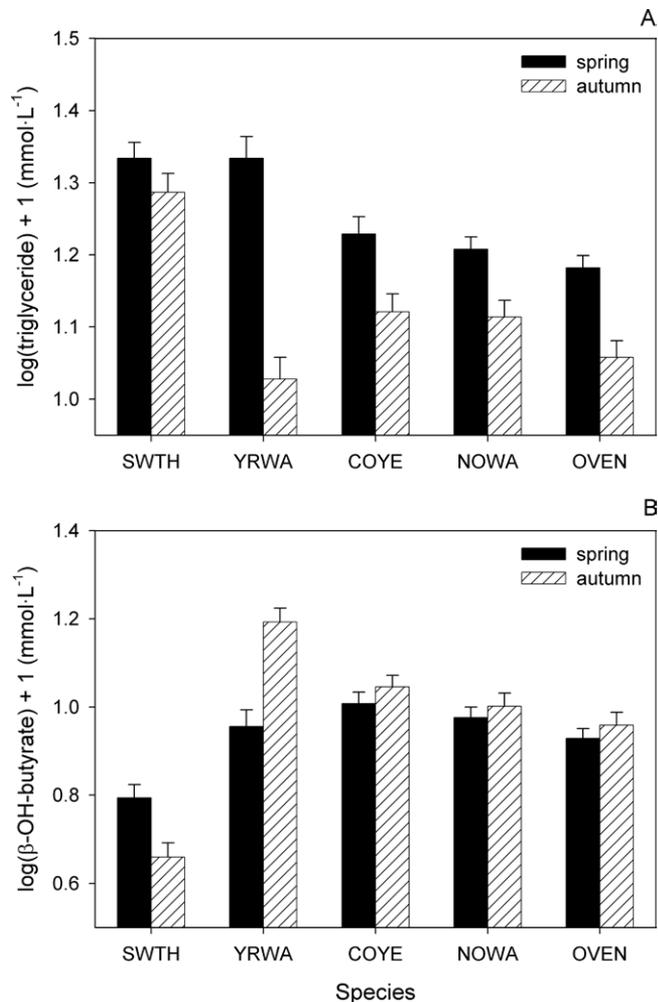
Figure 2

Comparison of (A) triglyceride levels [log(x) + 1] and (B) β-OH-butyrate levels [log(x) + 1] between spring and autumn in Swainson's thrushes (SWTH), common yellowthroats (COYE), northern waterthrushes (NOWA), ovenbirds (OVEN), and yellow-rumped warblers (YRWA). Shown in each pair of bars are the least square means (±SE) from a general linear model including season and all other parameters in the global best model (Tables 2 and 3).

longer periods of daylight during spring than autumn extend foraging opportunities, and in turn, enable spring migrants to have shorter stopover durations and a faster overall migration speed. In such case, refueling rates of spring and autumn migrants need not differ for spring migrants to still achieve greater net diurnal mass gains. Although longer daily feeding opportunities could contribute to shorter stopovers and faster vernal migration speed, our plasma metabolite data indicate that spring migrants also refuel at higher instantaneous rates than autumn migrants within the same time period relative to sunrise.

Juvenile birds have often been characterized as having low social status, limited competitive ability, and poor foraging skills (Terrill 1987; Burger 1988; Marchetti and Price 1989; Wunderle 1991; Carpenter, Hixon, Russell, et al. 1993; Woodrey 2000; Moore et al. 2003). There is also evidence that the digestive systems of juveniles can differ in size and enzymatic capacity from adults (Hume and Biebach 1996; Guglielmo and Williams 2003; Stein et al. 2005; see also Karasov 1990). However, most age comparisons of stopover mass gains have failed to support predictions of superior

refueling by adults (Morris et al. 1996, 2003; Woodrey and Moore 1997; Wang et al. 1998; Morris and Glasgow 2001; Jones et al. 2002; Carlisle et al. 2005). This includes the only 2 studies of songbirds, to our knowledge, that have measured plasma metabolite levels in this context (Leist 2007; Smith and McWilliams 2010). Plasma triglyceride and β-OH-butyrate levels of the birds we studied also failed to indicate age differences in refueling rate, and accounting for potential capture date effects did not help to illuminate any age differences. It has been suggested that the ability of juvenile birds to refuel as well as adults may indicate that food sources at the stopover site are plentiful (Woodrey 2000; Jones et al. 2002; Moore et al. 2003). This interpretation would be consistent with previous conclusions that the stopover sites we examined offer high-quality refueling conditions (Seewagen and Slayton 2008; Seewagen et al. 2011). However, it is also possible that by the onset of autumn migration, age differences in social dominance and foraging skills have lessened to the extent that juvenile and adult birds do not differ in their abilities to refuel during stopovers (Woodrey 2000; Jones et al. 2002). Indeed, Heise and Moore (2003) found that juvenile gray catbirds (*Dumetella carolinensis*) were initially less effective foragers than adults during the postbreeding period, but their skills improved with age to the point that they were able to forage as well as adults by the time of their departure on autumn migration. Similar observations were made in the savannah sparrow (*Passerculus sandwichensis*; Wheelwright and Templeton 2003). We also note, however, that plasma triglyceride and β-OH-butyrate levels are indicators of instantaneous energy intake, not energy storage. Higher basal metabolic rates (Weathers and Sullivan 1989; Chappell et al. 1999; TR McCabe, CG Guglielmo, unpublished data) or other components of energy expenditure (e.g., foraging effort) of juvenile birds may still result in smaller net daily gains of body mass. Greater energy expenditure and poorer energy storage, rather than lower energy intake, may explain the smaller fat stores (Woodrey 2000; Moore et al. 2003) and longer stopover durations (Ellegren 1991; Morris et al. 1996; Rguibi-Idrissi et al. 2003; Mackenzie 2010) that have been observed in juvenile migrants relative to adults.

The failure of many previous studies to detect expected sex-related and seasonal asymmetries in stopover refueling rates may be partly due to the shortcomings of the recapture and mass–time of capture regression methods traditionally used to measure and compare body mass changes of migrants (discussed by Winker et al. 1992; Dunn 2002; Jones et al. 2002; Guglielmo et al. 2005; Delingat et al. 2009). The regression method is particularly weak for detecting differences in mass change rates among study groups because of wide confidence intervals that result from the extensive individual variation in body mass at any given time of day (Dunn 2002; Jones et al. 2002). In contrast, blood plasma triglyceride and β-OH-butyrate levels provide a sensitive tool with which to compare the refueling rates of different groups of birds (e.g., Guglielmo et al. 2002, 2005; Cerasale and Guglielmo 2010). We encourage future studies that examine the effects of sex, season, and age on refueling more closely, and combine plasma metabolite profiling with measurements of time budgets, foraging behavior, competitive interactions, metabolic rates, and stopover duration.

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