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LANDSCAPE-SCALE PHYSIOLOGY: SITE DIFFERENCES IN REFUELING RATES INDICATED BY PLASMA METABOLITE ANALYSIS IN FREE-LIVING MIGRATORY SANDPIPERS

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ABSTRACT.—Variation in plasma metabolite profiles can provide information on physiological state and relative rates of mass change in free-living birds caught only once, and this technique could be used to compare rates of mass change (fattening) among populations or sites in relation to habitat quality. We compared plasma metabolite levels, as an index of relative refueling rates, in migratory Arctic-nesting Western Sandpipers (*Calidris mauri*), on a landscape scale at as many as nine sites over two years and during three different migratory stages within the Georgia Basin–Puget Sound region of British Columbia and Washington. There was significant intersite variation in plasma triglyceride levels in both years, but only for the northward migration. By contrast, there was little evidence of intrasite variability (i.e. metabolite profiles of birds using the same site were consistent between years and migratory stages), though we documented intrasite variation resulting from birds' use of different microhabitats at the same site. Plasma glycerol levels did not vary systematically among sites, though they varied among years; on average, birds had higher glycerol levels during northward migration than during southward migration. For the northward migration only, there was a positive relationship between plasma triglyceride levels and total macrofaunal prey abundance among sites. Birds using smaller sites with a lower index of mudflat exposure (mean number of kilometer-hours of mudflat exposed in a 24-h period, reflecting the opportunity to forage at each site) tended to have lower triglyceride levels. Received 7 April 2005, accepted 9 September 2005.

Key words: *Calidris mauri*, fattening rate, geographic variation, plasma metabolites, Western Sandpipers.

Fisiología a Escala de Paisaje: Diferencias entre Sitios en las Tasas de Reabastecimiento Indicadas por Análisis de Metabolitos del Plasma en Chorlos Migratorios Silvestres

RESUMEN.—La variación en los perfiles de metabolitos del plasma puede brindar información sobre el estado fisiológico y sobre las tasas relativas de cambio de peso en las aves silvestres que son capturadas sólo una vez. Esta técnica puede ser usada para comparar las tasas de cambio de peso entre poblaciones o sitios con relación a

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la calidad del hábitat. Comparamos los niveles de metabolitos del plasma, como un índice relativo de las tasas de reabastecimiento, en el chorlo migratorio que nidifica en el ártico *Calidris mauri*. Este estudio se realizó a una escala de paisaje en nueve sitios a lo largo de dos años y durante tres etapas migratorias diferentes en la Cuenca de Georgia y la región de Puget Sound de Columbia Británica y Washington. Hubo una variación significativa entre sitios en los niveles de triglicéridos del plasma en ambos años, pero sólo para la migración hacia el norte. De modo contrastante, hubo poca evidencia de variabilidad interna en los sitios (i.e. los perfiles de metabolitos de las aves que usaron el mismo sitio fueron similares entre años y etapas migratorias), por lo que documentamos la existencia de variación interna en los sitios como resultado del uso por parte de las aves de diferentes micro-ambientes en el mismo sitio. Los niveles de glicerol en el plasma no variaron de modo sistemático entre los sitios, aunque variaron entre años. En promedio, las aves presentaron niveles de glicerol mayores durante las migraciones hacia el norte que durante las migraciones hacia el sur. Solamente para la migración hacia el norte, hubo una relación positiva entre los niveles de triglicéridos en el plasma y la abundancia total de presas de macro fauna en los sitios. Las aves que usaron sitios más pequeños con un índice más bajo de exposición de las marismas (número medio de horas-kilómetro de marismas expuestas en un período de 24 horas que refleja la oportunidad de forrajear en cada sitio) tendieron a presentar niveles más bajos de triglicéridos.

IN MIGRATORY BIRDS, fuel deposition (mass gain) and use (mass loss) involve synthesis of metabolic byproducts and precursor molecules that are transported via the plasma, and several studies have shown that changes in these plasma metabolites can be used to estimate physiological state and rates of mass change (e.g. Jenni-Eiermann and Jenni 1994, 1996; Williams et al. 1999, Gannes 2001). This provides a potentially powerful approach to estimating the dynamics of mass change in free-living migratory birds caught only once, an approach that is superior to static estimates of "condition" based on single body-mass measurements at time of capture (Williams et al. 1999, Schaub and Jenni 2001, Guglielmo et al. 2005). Several studies have suggested that plasma metabolite analysis might not only provide information on rates of mass change in individual birds, but that it could also be used to compare rates of mass change among populations or sites in relation to habitat quality. Consistent with this notion, Guglielmo et al. (2002) reported a difference in triglyceride levels in Western Sandpipers (*Calidris mauri*) using two stopover sites, Boundary Bay and Sidney Island, British Columbia (35 km apart). Similarly, Guglielmo et al. (2005) reported differences in plasma metabolites in migratory passerines using two sites of different quality (based on recapture data) <30 km apart. However, there has been only one study testing

this idea on a larger geographic scale (migratory passerines; Schaub and Jenni 2001) and, to our knowledge, no studies have related site-specific variation in plasma metabolite levels directly to variation in other measures of habitat quality, such as food availability.

We used plasma metabolite analysis to investigate variation in estimated refueling rates in Arctic-nesting Western Sandpipers during three different migration stages, on a landscape scale at multiple sites over two years, within the Georgia Basin–Puget Sound region of British Columbia and Washington (part of the Pacific Northwest flyway used by migrating Western Sandpipers). We predicted that plasma triglyceride levels would be more informative than variation in plasma glycerol and B-OH-butyrate (B-OH) levels in detecting site differences. This is because elevated plasma triglyceride levels are indicative of fat deposition (e.g. Robinson 1970, de Graw et al. 1979, Ramenofsky 1990), whereas elevated glycerol and B-OH levels indicate mass loss or fasting (Cherel et al. 1988), and most birds captured at stopover sites during migration are actively feeding and gaining mass, not fasting. In addition, in a previous study of migratory Western Sandpipers, Guglielmo et al. (2002) showed that plasma triglyceride levels were more variable in relation to migratory stage than plasma glycerol, B-OH, or free fatty acid levels. Given

substantial variation in macrofaunal invertebrate prey density among different sites in this region (Seaman 2003), we further predicted that, if mass-corrected plasma triglyceride levels reflected site-specific differences in refueling rate, we would find (1) significant intersite differences in plasma triglyceride concentrations at geographic scales, but (2) relatively constant plasma triglyceride concentrations within sites across years and for different migration stages. Finally, we investigated whether site-related variation in plasma triglyceride levels correlates with variation in other biological and physical site characteristics (prey abundance and site size).

METHODS

Study sites and sample collection.—Western Sandpipers were captured with mist nets at multiple sites in 2001 and 2002 during northward spring migration (April–May; hereafter “northward”), and during southward fall migration (hereafter “southward”) for both adults (July) and juveniles (August). We treated southward adult and juvenile migration as two separate migration stages because this allowed for a third independent test for site differences and,

in addition, avoids confounding age and time-of-year effects. We maintained a regular mist-netting effort throughout all three migration stages in both years at Boundary Bay, British Columbia (Fraser River Delta), which served as our reference site. In each migration stage, at least one and up to three additional sites were sampled concurrently and compared with the reference site (Fig. 1). Location, physical characteristics, and sampling periods for each site are detailed in Table 1 (see Seaman [2003] for more details). Not all sites were sampled during all migration stages and years, in part because of differential bird use (see below); only sites sampled concurrently were compared in subsequent analyses.

Banding and all animal handling were done under U.S. Fish and Wildlife Service, Washington Department of Fish and Wildlife, Environment Canada, and Simon Fraser University Animal Care permits. Mist nets were constantly in view during banding, and time to blood sampling was recorded from when birds first hit the net. All birds were weighed, measured, and blood-sampled ($\leq 300 \mu\text{L}$) via brachial venipuncture. Blood was kept cool (on ice) and centrifuged at 5,000 rpm for 3 min within 2 h of sampling; plasma was either stored at -20°C immediately

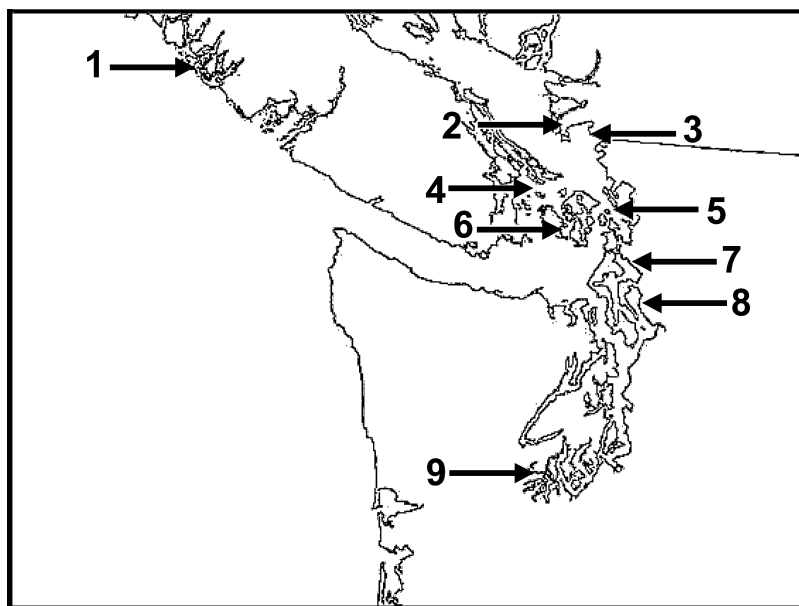


FIG. 1. Map of the Georgia Basin–Puget Sound stopover region indicating the nine stopover sites where samples were obtained: (1) Doug Banks, (2) Robert's Bank, (3) Boundary Bay, (4) Sidney Island, (5) Alice Bay, (6) False Bay, (7) Jensen Access, (8) English Boom, and (9) Totten Inlet.

TABLE 1. Location, mean capture date (1 = 1 Jan), sample size, and sampling sex ratio (% Male) of western sandpipers caught at migratory stopover sites (BC = British Columbia, WA = Washington) during northward (N), southward adult (SA), and southward juvenile (SJ) migrations in 2001 and 2002.

Site	Coordinates	Stage	Year	Date	<i>n</i>	% Male
Boundary Bay, BC	49°04'N, 122°58'W	N	2001	118.7	31	53.3
Jensen Access, WA	48°20'N, 122°26'W	N	2001	122.2	12	45.5
Totten Inlet, WA	47°06'N, 123°05'W	N	2001	123.4	21	57.9
Boundary Bay, BC	49°04'N, 122°58'W	N	2002	120.2	44	51.2
Jensen Access, WA	48°20'N, 122°26'W	N	2002	127.2	28	59.3
Robert's Bank, BC	49°03'N, 123°08'W	N	2002	120.8	48	57.5
Totten Inlet, WA	47°06'N, 123°05'W	N	2002	120.6	27	53.9
Boundary Bay, BC	49°04'N, 122°58'W	SA	2001	189.6	29	38.5
English Boom, WA	48°16'N, 122°26'W	SA	2001	190.6	19	44.4
Alice Bay, WA	48°33'N, 122°29'W	SA	2002	200.3	13	46.5
Boundary Bay, BC	49°04'N, 122°58'W	SA	2002	191.1	13	8.3
English Boom, WA	48°16'N, 122°26'W	SA	2002	191.6	31	38.7
Robert's Bank, BC	49°03'N, 123°08'W	SA	2002	199.8	13	75.0
Boundary Bay, BC	49°04'N, 122°58'W	SJ	2001	230.0	13	38.5
Sidney Island, BC	48°37'N, 123°26'W	SJ	2001	223.0	19	42.1
Boundary Bay, BC	49°04'N, 122°58'W	SJ	2002	223.9	28	46.4
Doug Banks, WA	49°07'N, 125°53'W	SJ	2002	229.9	20	57.9
False Bay, WA	48°29'N, 123°04'W	SJ	2002	218.9	7	42.9
Robert's Bank, BC	49°03'N, 123°08'W	SJ	2002	223.5	53	46.2

or frozen and transported in liquid nitrogen for ≤ 2 weeks and then stored at -20°C until assayed. We determined bird sex by culmen length (male < 24.2 mm, female > 24.8 mm, intermediate values = unknown; Page and Fearis 1971) and aged by plumage characteristics (according to Prater et al. 1977). Macrofaunal prey density (number of organisms per core) was measured throughout the sampling period, using sediment cores collected at the same location as the capture effort. Cores ($n = 12\text{--}44$ per site) were taken to a depth of 40 mm using a 60-cc syringe, following Sutherland et al. (2000). Cores were frozen at -20°C within 2 h of sampling or at -80°C in liquid nitrogen for ≤ 2 weeks and then stored at -20°C until analyzed. In the laboratory, each sediment core was thawed and sieved to obtain the macrofaunal (500- μm) fraction; this was stored at room temperature in 85% ethanol and analyzed for prey type and quantity. Site size was estimated using a mudflat exposure index: mean number of kilometer-hours of mudflat exposed in a 24-h period, which represents the opportunity to forage provided by each site (calculated using the distance to shore and the tidal cycle for each site; R. Ydenberg and V. Klein pers. comm.).

Metabolite assays.—Free glycerol and triglyceride were assayed via sequential color endpoint assay (Trinder reagent A and B, respectively, Sigma-Aldrich Canada, Oakville, Ontario), using 5 μL of plasma with 240 and 60 μL of reagents A and B, respectively, with a reading taken at 540 nm after 10 min of incubation at 37°C after the addition of each reagent. Triglyceride concentration (mmol L^{-1}) was calculated by subtracting free glycerol from total triglyceride. B-OH-butyrate (B-OH) was measured by kinetic endpoint assay (kit E0907979, R-7 Biopharm, Marshall, Michigan) at room temperature ($22\text{--}25^{\circ}\text{C}$) following Guglielmo et al. (2005). Assays were run in 400- μL flat-bottom 96-well microplates (NUNC, Denmark) and read with a microplate spectrophotometer (Biotec 340EL or Powerwave X 340). For triglyceride and glycerol, each plate was run with a standard curve based on a serial dilution of 2.54 mmol glycerol (Sigma-Aldrich), and a 19-day-old hen-plasma pool was used to calculate interassay coefficients of variation (CVs). Interassay CVs were 6.6% and 3.7% ($n = 15$), and intra-assay CVs were 3.2% and 3.9% ($n = 6$) for glycerol and triglyceride, respectively. For

the B-OH assay, intra-assay CV was 4.3% and interassay CV was 17% (using a goose plasma pool). On the basis of previous studies, we prioritized assays for triglyceride and glycerol and, because of small sample volumes, not all samples could be assayed for B-OH. Therefore, for B-OH, we restricted our analyses to sites during northward and southward juvenile migration where $n > 5$ per site.

Statistical analysis.—All three metabolites were non-normally distributed, so we transformed the data using $\log_{10}(\text{metabolite}) + 1$. We first investigated potential covariates of plasma metabolite levels: body mass, time between capture and blood sampling (bleed time), and time of day (see below). We then used analysis of covariance to compare metabolite levels among sites, within migratory stages, initially including all three factors as covariates but then backward-eliminating any covariates that did not contribute significantly to the model. For analysis of body mass, we used tarsus length as a covariate; that is, we analyzed size-corrected body mass. Metabolite and body-mass values are therefore reported as least-squares means, and multiple pairwise comparisons were tested, controlling for experiment-wise error ($\alpha = 0.05$) using Bonferroni adjustment. All statistical analyses were performed using SAS, version 6.0 (SAS Institute, Cary, North Carolina).

RESULTS

Effects of bleed time, body mass, time of day, sex, and age.—Bleed time, defined as number of minutes between capture and blood sampling, averaged 16.8 ± 11.4 min, and 95% of the bleed times were < 36 min. All three metabolites varied with bleed time, though all relationships were weak, judging from the slope of the relationships. Plasma triglyceride was negatively related to bleed time ($F = 9.87$, $df = 1$ and 467 , $P < 0.002$, $b = -0.002$; i.e. plasma triglyceride levels decreased by $\sim 3\%$ between 0 and 10 min). By contrast, plasma glycerol ($F = 6.40$, $df = 1$ and 467 , $P < 0.05$, $b = 0.0006$) and plasma B-OH ($F = 7.15$, $df = 1$ and 227 , $P < 0.01$, $b = 0.002$) were positively related to bleed time (representing a $\sim 4\%$ and $\sim 8\%$ increase, respectively, between 0 and 10 min). Plasma levels of all three metabolites were also dependent on body mass. Plasma triglyceride was positively related to mass ($F = 76.6$, $df = 1$ and 467 , $P < 0.001$, $b = 0.017$), whereas

plasma glycerol ($F = 14.1$, $df = 1$ and 467 , $P < 0.001$, $b = -0.004$) and B-OH ($F = 20.1$, $df = 1$ and 227 , $P < 0.001$, $b = -0.007$) were negatively related to mass.

We found systematic variation only with time of day for plasma triglyceride during northward migration ($F = 12.2$, $df = 11$ and 207 , $P < 0.001$; Fig. 2). Birds sampled between 05.00–08.00 had lower plasma triglyceride levels than birds sampled later in the day ($P < 0.025$). This difference between migration stages was not the result of temporal variation in time of sunrise: there was no difference in sunrise during northward and southward juvenile migration (0610 vs. 0607 hours PST; $P > 0.25$), and sunrise during southward adult migration was, on average, only 45 min earlier (0530 hours; $P < 0.001$). Although overall variation with time of day was statistically significant for triglyceride during fall adult ($P < 0.001$) and fall juvenile ($P < 0.01$) migration, there was no systematic variation (Fig. 2), and few pairwise comparisons were significant. Variation in glycerol ($P > 0.05$) and B-OH levels ($P > 0.15$) was independent of time of day for all three migration stages (we pooled data over 2-h periods for B-OH analysis because of smaller sample sizes).

There was no sex difference in triglyceride, glycerol, or B-OH levels ($P > 0.25$, controlling for mass and bleed time). Furthermore, during northward migration there was no effect of age

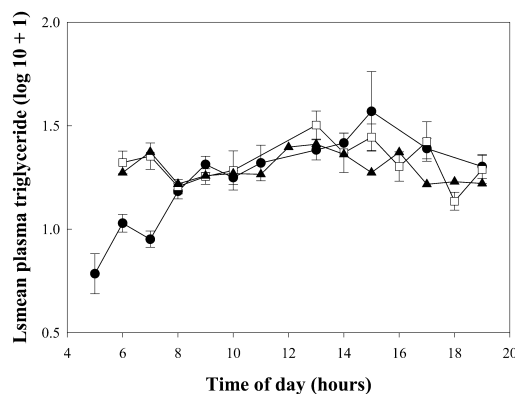


FIG. 2. Variation in plasma triglyceride levels in relation to time of day for northward migration (closed circles), southward adult migration (open squares), and southward juvenile migration (closed triangles). Values are least-square means \pm SE.

(yearling vs. after-second-year birds) on plasma levels of any of the three measured metabolites ($P > 0.30$). Therefore, for all subsequent analyses, we pooled data for age and sex classes and included bleed time and body mass as covariates, but we included time of day as a covariate only for analysis of northward triglyceride data.

Site and year differences in body mass and size.—There was no site or year effect for body size (tarsus length) for any of the three migratory periods ($P > 0.10$ in all cases). In general, site-specific variation in size-corrected body mass was greater during southward migration (for both adults and juveniles) than during northward migration (Fig. 3). Size-corrected body mass was independent of year and site ($P > 0.09$) during northward migration (Fig. 3). During southward adult migration (July), there was a significant year * site interaction term for size-corrected body mass ($F = 19.5$, $df = 1$ and 106 , $P < 0.001$) and, in both years, there was significant intersite variation in size-corrected body mass (2001: $F = 5.94$, $df = 1$ and 45 , $P < 0.02$; 2002: $F = 9.09$, $df = 3$ and 60 , $P < 0.001$). In 2001, birds at English Boom were heavier than those at Boundary Bay but, in 2002, this pattern was reversed ($P < 0.01$ in both cases; Fig. 3). In addition, in 2002, birds at both Alice Bay and Boundary Bay were heavier than those at Robert's Bank ($P < 0.02$). During southward juvenile migration (August), sampling was very unbalanced, so we analyzed each year separately. In 2001, there was a significant site effect for size-corrected body mass, with birds at Sidney Island being lighter than birds at Boundary Bay ($F = 10.15$, $df = 1$ and 31 , $P < 0.01$). Similarly, in 2002, there was a significant site effect ($F = 9.20$, $df = 3$ and 108 , $P < 0.001$): birds using False Bay were lighter than birds at Doug Banks and Robert's Bank ($P < 0.05$) but not Boundary Bay ($P > 0.05$; Fig. 3).

Site and year differences in plasma metabolite levels: Northward migration.—During northward migration, there was a significant year * site interaction term for triglyceride levels ($F = 8.31$, $df = 2$ and 208 , $P > 0.001$), with particularly low triglyceride levels in birds at Jensen Access in 2001 (Fig. 4). Analyzing by year, there was a significant site effect on plasma triglyceride in both 2001 ($F = 8.32$, $df = 2$ and 61 , $P < 0.001$) and 2002 ($F = 9.87$, $df = 3$ and 146 , $P < 0.001$). In 2001, birds at Jensen had lower triglyceride than those at Boundary Bay ($P < 0.001$) or at

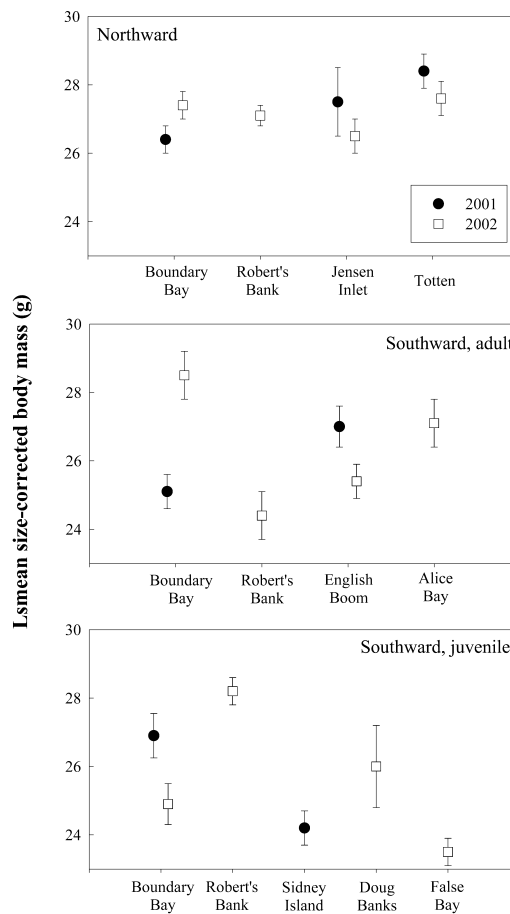


FIG. 3. Intersite variation in size-corrected body mass (grams) for northward migration (top), southward adult migration (middle), and southward juvenile migration (bottom). Values are least-square means \pm SE.

Totten Inlet ($P = 0.05$). In 2002, birds at Totten had lower triglyceride than those at all other sites ($P < 0.01$). If data from Jensen in 2001 is excluded, there is no significant year * site interaction ($P > 0.15$) or year effect ($P > 0.05$), but site differences remained significant ($F = 9.69$, $df = 3$ and 196 , $P < 0.001$): birds using Totten Inlet had lower triglyceride levels than birds using any of the other three sites ($P < 0.001$ in all cases).

For glycerol, the year * site interaction was not significant ($F = 2.08$, $df = 2$ and 208 , $P > 0.10$), so this was dropped from the model. There was no overall site effect for glycerol ($F = 1.42$, $df = 3$ and 208 , $P > 0.20$), but there was a significant year effect ($F = 9.65$, $df = 1$ and 208 , $P < 0.01$; Fig. 5).

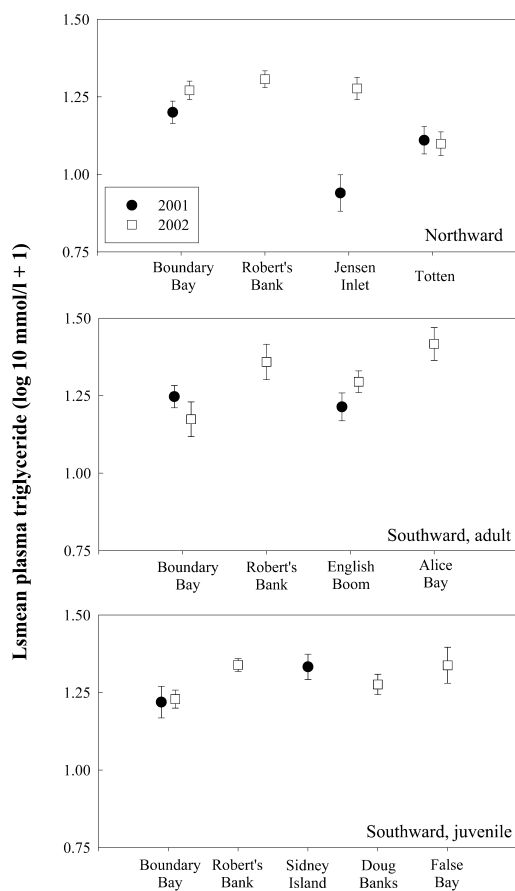


FIG. 4. Intersite variation in plasma triglyceride levels for northward migration (top), southward adult migration (middle), and southward juvenile migration (bottom). Values are least-square means \pm SE.

Plasma glycerol levels were higher in 2002 than in 2001 (0.789 ± 0.017 vs. 0.683 ± 0.028). Similarly, for B-OH in 2002, there was no significant site difference ($F = 2.59$, $df = 3$ and 36 , $P > 0.05$) among the four sampled sites (Boundary, Roberts Bank, Jensen Access, and Totten Inlet).

Site and year differences in plasma metabolite levels: Adult southward migration.—During southward adult migration (July), year \times site interaction was not significant for triglyceride ($F = 2.83$, $df = 1$ and 114 , $P > 0.05$). When the interaction term was dropped from the model, there was no significant year effect ($F = 0.15$, $df = 1$ and 114 , $P > 0.70$), and the site effect was only marginally significant ($F = 2.68$, $df = 3$ and 114 , $P = 0.05$; Fig. 4),

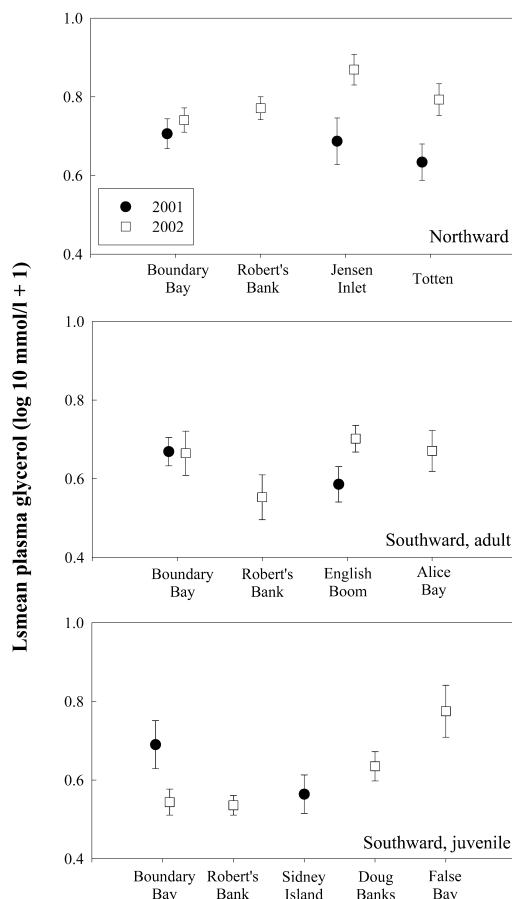


FIG. 5. Intersite variation in plasma free glycerol levels for northward migration (top), southward adult migration (middle), and southward juvenile migration (bottom). Values are least-square means \pm SE.

with none of the pairwise comparisons between sites being significant. For glycerol, there was no year \times site interaction ($F = 1.78$, $df = 1$ and 114 , $P > 0.15$), and there was no site or year effect ($P > 0.10$ in both cases) when the interaction term was dropped from the model (Fig. 5).

Site and year differences in plasma metabolite levels: Juvenile southward migration.—Because sampling during southward juvenile migration was very unbalanced, we analyzed each year separately. Triglyceride levels were independent of site in 2001 ($P > 0.10$). However, there was significant intersite variation in 2002 ($F = 3.17$, $df = 3$ and 108 , $P < 0.05$; Fig. 4): juveniles at Robert's Bank had higher triglyceride levels than those

at Boundary Bay ($P < 0.05$), with no differences among other sites. Similarly, for glycerol, there was no site effect in 2001 ($P > 0.10$), but there was significant intersite variation in 2002 ($F = 4.87$, $df = 3$ and 108 , $P < 0.01$): juveniles at False Bay had higher values than those at both Boundary Bay ($P < 0.05$) and Robert's Bank ($P < 0.01$; Fig. 5). In 2002, there was no significant effect of site on B-OH levels for three sites, where $n \geq 5$ birds (Boundary Bay, Robert's Bank and Doug Banks: $F = 1.47$, $df = 2$ and 37 , $P > 0.20$).

Within-site variability in metabolites.—We sampled birds at Boundary Bay during each migratory stage in both years, and at Robert's Bank during each migratory stage in 2002, allowing us to look at consistency of metabolite levels within sites. Within Boundary Bay, there was a significant year * stage interaction for triglyceride ($F = 3.26$, $df = 2$ and 155 , $P = 0.04$), but only one of the paired contrasts was significant: triglyceride levels were lower ($P = 0.04$) during northward migration in 2001 compared with northward migration in 2002 (Fig. 4). For glycerol, the year * stage interaction was not significant ($P > 0.20$), and when this term was dropped from the model there was no year effect ($P > 0.50$). There was a significant stage effect, with higher glycerol levels during northward migration ($F = 12.09$, $df = 2$ and 155 , $P < 0.001$; Fig. 5). Within Robert's Bank, there was no effect of migratory stage for triglyceride levels ($F = 2.10$, $df = 2$ and 113 , $P > 0.10$) but, as for Boundary Bay, birds had higher glycerol levels during northward migration ($F = 23.8$, $df = 2$ and 155 , $P < 0.001$; Fig. 5). For three other sites sampled in both years during the same migratory stage, triglyceride levels did not differ among years at English Boom ($P > 0.25$) or Totten Inlet ($P > 0.80$) but at Jensen Access birds had lower triglyceride levels in 2001 compared with 2002 ($F = 29.6$, $df = 1$ and 39 , $P < 0.001$; Fig. 4). Plasma glycerol levels were significantly higher in 2002 compared with 2001 at all three sites ($P < 0.05$ in all cases), though the same pattern was not seen for Boundary Bay (Fig. 5).

Relationship between metabolites, macrofaunal prey density, and site size.—During northward migration, there was a positive relationship between mean total macrofaunal prey abundance and mean triglyceride level based on site averages ($r_s = 0.829$, $P = 0.04$; Fig. 6A) when data for Jensen in 2001 were excluded (for rationale, see below). By contrast, there was no significant

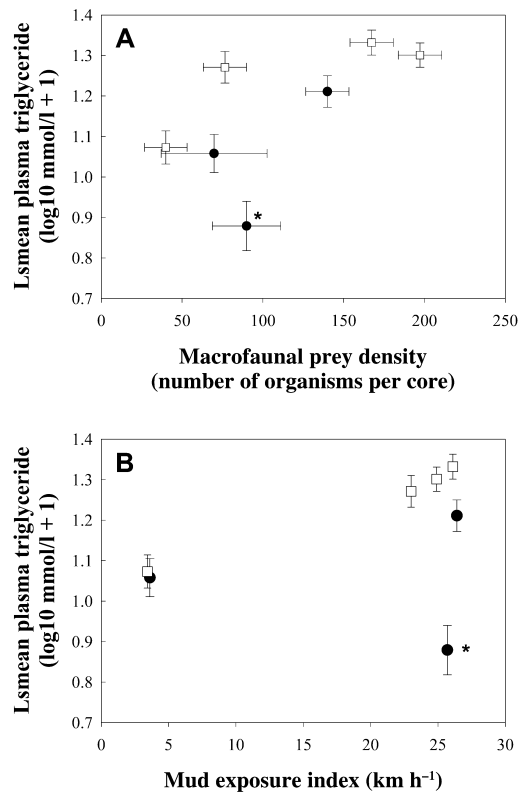


FIG. 6. Relationship between plasma triglyceride levels in free-living Western Sandpipers and total macrofaunal prey abundance (top) and foraging opportunity, as indicated by the mud-flat exposure index (bottom), during northward migration in 2001 (closed symbol) and 2002 (open symbol). Values are least-square means \pm SE. Asterisk indicates Jensen Access, which was excluded from analysis (see text).

relationship between mean triglyceride levels and prey abundance during southward migration ($P > 0.3$), or between glycerol and prey abundance for any migratory stage ($P > 0.30$). For sites sampled in 2001 and 2002 during northward migration, birds using larger sites also tended to have higher triglyceride levels than those using small sites (Fig. 6B).

DISCUSSION

Plasma triglyceride levels of Western Sandpipers varied among different migratory stopover sites in both years, and this variation was much more pronounced during northward

than during southward migration. By contrast, there was little evidence of intrasite variability (i.e. metabolite profiles of birds using the same stopover site were relatively consistent between years or migratory stages). The only exception to this was for Jensen Access: birds using this site in 2001 had significantly lower triglyceride levels than birds using other sites in 2001 or birds that used the same site in 2002. This lack of consistency in metabolite profiles at this site most likely reflected changes in within-site habitat use and, in fact, supports the hypothesis that metabolite analysis can detect differences in habitat quality (e.g. when it is known that birds are actually feeding in microhabitats of different quality within the same site). During northward migration, Western Sandpipers at Jensen Access used (and therefore were caught in) a 200-m stretch of marshy hummocks. They were observed pecking and probing directly into the sides of the hummocks, which contained a terrestrial prey base of >25% insects and mites (Seaman 2003). During southward migration in 2001 and 2002, this hummock marsh was much more completely vegetated and was not available to foraging shorebirds, so birds foraged and were caught on the firmer sandy flats along the edge of the marsh. The sandy flats had a benthic invertebrate fauna, composed mainly of polychaete and nematode worms, more typical of all other sites sampled in our study (Seaman 2003). This demonstrates the dynamic nature of habitat use even within stopover sites and the importance of microhabitats; but at the physiological level, this was reflected in differences in plasma triglyceride levels. In contrast to data for triglyceride, plasma glycerol levels did not vary systematically among sites, though they varied among years and, on average, birds had higher glycerol levels during northward than during southward migration. For the northward migration only, there was a positive relationship between plasma triglyceride levels and total macrofaunal prey abundance among sites, which provides direct support for the idea that triglycerides reflect fattening rate—which, in turn, reflects habitat quality. In addition, there was some evidence that birds using smaller sites, with a lower index of mudflat exposure and less opportunity to forage, tended to have lower triglyceride levels.

Ideally, we should have sampled multiple sites concurrently, but this was impossible, in part for logistical reasons but also because of temporal

variation in the use of different stopover sites by the migrating Western Sandpipers. Clearly, if there is an increasing or decreasing trend in metabolites with date within a migration stage, it is possible that site effects are an artifact of this temporal effect, rather than an actual difference between sites. At Robert's Bank and Boundary Bay, which were sampled continuously, there was a positive relationship between triglyceride levels and capture date: late-caught birds had higher triglyceride levels than early-caught birds (data not shown) during northward and southward adult migration. However, during northward migration, our mean capture date was earlier at Boundary Bay, where birds had higher triglyceride levels, than at Jensen Access and Totten Inlet. Thus, variation in capture date does not provide an explanation for the site differences we found (also see Schaub and Jenni 2001). Another potential confounding factor is a bias in age- or sex-ratios of birds using different sites, if metabolite profiles vary with age or sex or both. However, we found no effect of age or sex on plasma metabolite levels, which confirms results of an earlier study on this species with substantial sample sizes (Guglielmo et al. 2002). Finally, variation in the time of day of blood sampling might have affected our comparison among sites. We caught birds around high tide, thus controlling for any effects of tidal cycle, and most birds sampled in our study were actively feeding around mist nets at time of capture. Timing of the tidal cycle did vary between sites, but we found a significant time-of-day effect only for plasma triglyceride, and then only during the northward migration. This suggests that during northward migration, feeding opportunities and refueling rates may be constrained in the early hours of the morning (0500–0700 hours), though our results for intersite variability were robust when controlling for this effect.

Variation in plasma triglyceride levels were more informative than variation in glycerol and B-OH levels for detecting site differences; the latter two metabolites showed no consistent relationship with site. This is what we would have predicted on the basis of our physiological understanding of these metabolites. Elevated plasma triglyceride levels are indicative of fat deposition (i.e. transport of lipids of dietary origin, or of lipids synthesized in the liver, to peripheral adipose tissue; e.g. Robinson 1970, de Graw et al. 1979, Ramenofsky 1990). Several studies have

demonstrated a positive relationship between plasma triglyceride levels and mass change in captive passerines and shorebirds (Jenni-Eiermann and Jenni 1994, Williams et al. 1999, Jenni and Schilch 2001; but see Jenni-Eiermann et al. 2002). By contrast, glycerol and B-OH indicate lipid catabolism and are elevated during mass loss (Jenni-Eiermann and Jenni 1994, Williams et al. 1999, Jenni and Schilch 2001). Glycerol and free fatty acids are released into the plasma during lipolysis of triglycerides, and B-OH (a ketone body) is synthesized from fatty acids and replaces glucose as the principal fuel for respiration in some tissues (Ramenofsky 1990). Thus, glycerol and B-OH should increase in the plasma during mass loss and should characterize fasting individuals (Cherel et al. 1988). However, Jenni-Eiermann and Jenni (1991) found no difference in glycerol levels of migrating passerines between actively foraging and fasting individuals, though elevated glycerol levels were detected in recently flown (and fasting) individuals. Similarly, Gannes (2001) detected elevated levels of glycerol in recently arrived passerine migrants, which suggests that a nonforaging factor (i.e. exercise) may affect realized glycerol levels. Furthermore, Guglielmo et al. (2005) found high glycerol levels in birds when triglycerides were very high, possibly as a result of high rates of fatty-acid uptake by adipocytes. It is clear from these studies that interpreting elevated glycerol levels in free-living birds is more complicated than interpreting elevated triglyceride levels. Given that we captured actively feeding birds near the peak of a rising tide, and that we captured birds over several days at each site, it is unlikely that we sampled a high proportion of birds that were fasting or recently arrived (or both). In other words, plasma triglyceride was the most physiologically relevant and informative metabolite in the present study, because most of the birds we sampled were not fasting and were gaining mass through fat deposition. In previous studies of migratory Western Sandpipers (Guglielmo et al. 2002) and passerines (Jenni-Eiermann and Jenni 1996), plasma triglyceride has similarly been shown to better discriminate between migratory stages than glycerol, free fatty acids, or B-OH.

Assuming that variation in plasma metabolites reflects differences in refueling rates, these data provide very different information than measurements of body mass. In general, body mass was more highly variable among sites during

southward than during northward migration, whereas the opposite was true for triglyceride levels and, therefore, for predicted fattening rates. For example, whereas size-corrected body mass varied within both Boundary Bay and Robert's Bank between migration stages, metabolite data suggest that birds of different mass were refueling at the same rate across migration stages. This highlights the fact that size-corrected body mass is a static measure that perhaps indicates which birds are using a particular site, whereas triglyceride levels reflect physiological state (i.e. how birds perform once they arrive at a particular site; Williams et al. 1999, Guglielmo et al. 2005).

The present study clearly demonstrates intersite variation in plasma triglyceride levels, indicative of different refueling rates, in migratory Western Sandpipers within the Georgia Basin–Puget Sound region, which spans 300 km between the two most distant sites and approximately two degrees of latitude. Only one previous study has investigated site differences in metabolite levels on a broad geographic scale (but see Ydenberg et al. [2002] and Guglielmo et al. [2005] for site differences on a more local scale, <35 km apart). Schaub and Jenni (2001) compared an index of fattening rate, based on a principal component analysis of triglyceride and B-OH levels, in migratory passerines at 14 stop-over sites from northern Europe to sub-Saharan Africa (note: they reported plasma triglyceride levels as total plasma glycerol, including both free glycerol and triglyceride). Schaub and Jenni (2001) focused on the effects of latitude and distance to major ecological barriers, which did not explain or only weakly explained variation in fattening rates. They found that the largest variation in fattening rates was between sites, and that variation in fattening index between days within sites was much smaller than variation among sites, which supports our finding of significant intersite variability but low intrasite variability. However, in their study, the extent of site variability did not differ between spring (northward) and autumn (southward) migration (Schaub and Jenni 2001).

We found greater intersite variation in plasma triglyceride levels during northward than during southward migration, and this consistently stronger signal in site differences in northward migration may indicate inherent differences in migration strategy or physiology

between northward and southward migration. Northward migration tends to be shorter and more synchronous than southward migration in Western Sandpipers (Wilson 1994); for example, 10- to 15-fold more individuals are counted on any given day during northward than during southward migration (based on data for the Fraser River Delta; Butler 1994). Birds heading north will more often encounter inclement weather, maintenance of body reserves may be more important for activities after arrival on the breeding grounds (e.g. for territoriality in males) than on arrival on the wintering grounds (Smith and Moore 2003), and there may be strong selection for early arrival on the breeding grounds (Ruthrauff 2002). In addition, we found evidence for constraints on refueling rates during the early part of the day during northward migration. Thus, during northward migration, birds may be operating closer to their maximum metabolic capacity, with strong selection to maximize refueling rates, which would accentuate site differences. There is also some evidence that length of stay at stopover sites is greater during southward than during northward migration in Western Sandpipers (D. Lank pers. comm.). Thus, during southward migration, birds may refuel for a longer time at fattening rates below site-specific maximum rates, which leads to fewer detectable site differences in plasma metabolite profiles.

To our knowledge, the present study is the first to attempt to correlate site differences in metabolite levels with the prey bases at those sites (but see Guglielmo et al. [2005], who correlated metabolites with site-specific rates of mass change, which presumably reflected differences in prey availability). We found a positive relationship between plasma triglyceride levels and total macrofaunal prey abundance among sites (excluding data for Jensen in 2001, which we consider to be anomalous; see above). We recognize that macrofaunal prey density is a gross measure of total food availability and does not consider prey selectivity, the importance of which in this system has been demonstrated (Sutherland et al. 2000). Furthermore, our analysis had relatively low power, because of small sample sizes and large intersite variation in prey abundance (see also Sewell and Elner 2001), and does not account for error inherent in both variables (B. Smith pers. comm.). However, to our knowledge, this is the first study to report

a positive correlation between plasma metabolite levels, as an index of refueling, and any measure of food availability in free-living birds. We also found evidence that birds using smaller sites during northward migration (i.e. sites with lower opportunity for feeding, judging from the index of mudflat exposure) tended to have lower triglyceride levels than birds using larger sites. This pattern is opposite to that reported by Ydenberg et al. (2002), who showed that, during southward migration, birds using a smaller site (Sidney Island) had higher mass-specific plasma triglyceride levels than birds using a larger site (Boundary Bay). Thus, the present study does not support a general trend of small sites supporting high fattening rates for lighter individuals, which perhaps suggests that site-specific fattening rates are dependent on other site characteristics than site size *per se* (e.g. an interaction between site size and prey density).

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