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FLYWAY-SCALE VARIATION IN PLASMA TRIGLYCERIDE LEVELS AS AN INDEX OF REFUELING RATE IN SPRING-MIGRATING WESTERN SANDPIPERS (*CALIDRIS MAURI*)

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Abstract.—We combined radiotelemetry, plasma metabolite analyses, and macro-invertebrate prey sampling to investigate variation in putative fattening rates (estimated as plasma triglyceride levels) at the flyway scale in Western Sandpipers (*Calidris mauri*) migrating between Punta Banda, Mexico (31°N), and Hartney Bay, Alaska (60°N), a distance of 4,240 km. Birds were caught at a wintering site (San Francisco Bay) and eight stopover sites along this Pacific Flyway. Body mass was higher in females than in males at six sites, but variation was not correlated with latitude for either sex, and the relationship of change in mass by date within sites was uninformative with regard to possible latitudinal variation in fattening rates. At San Francisco Bay, triglyceride levels were higher in the spring than in the winter. Mean plasma triglyceride varied among stopover sites, and there was a significant linear trend of increasing triglyceride levels with latitude as birds migrated north. At San Francisco Bay, length of stay was negatively related to triglyceride levels. However, plasma triglyceride levels at wintering or initial stopover sites (San Francisco and Punta Banda) did not predict individual variation in subsequent rates of travel during migration. We found no significant relationship between triglyceride levels and prey biomass at different stopover sites, which suggests that the latitudinal pattern is not explained by latitudinal changes in food availability. Rather, we suggest that differences in physiology of migratory birds at southern versus northern stopover sites or behavioral differences may allow birds to sustain higher fattening rates closer to the breeding grounds. *Received 24 February 2006, accepted 22 July 2006.*

Key words: *Calidris mauri*, migration, plasma triglycerides, radiotelemetry, stopover, Western Sandpiper.

Variación a la Escala de Corredores de Vuelo en los Niveles de Triglicéridos Plasmáticos como un Índice de la Tasa de Reabastecimiento durante la Migración de Primavera en *Calidris mauri*

Resumen.—Combinamos radiotelemetría, análisis de metabolitos del plasma y muestreos de macroinvertebrados utilizados como presas, para investigar la variación en las tasas aparentes de engorde (medidas como niveles de triglicéridos en el plasma) a la escala de corredores de vuelo en individuos de la especie *Calidris mauri* que se encontraban migrando entre Punta Banda, México (31°N) y la bahía de Hartney, Alaska (60°N), una distancia de 4240 km. Las aves fueron capturadas en un

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sitio de invernada (la bahía de San Francisco) y en ocho sitios de escala migratoria a lo largo de este corredor de vuelo del Pacífico. La masa corporal fue mayor en las hembras que en los machos en seis sitios, pero la variación no se correlacionó con la latitud en ningún sexo, y la relación de cambio en la masa con el tiempo dentro de los sitios no fue informativa con respecto a una posible variación latitudinal en las tasas de engorde. En la bahía de San Francisco, los niveles de triglicéridos fueron mayores en la primavera que en el invierno. El promedio de triglicéridos en el plasma varió entre sitios de escala migratoria, y existió una tendencia lineal significativa de incrementos en los niveles de triglicéridos con la latitud conforme las aves migraban hacia el norte. En la bahía de San Francisco, la extensión del tiempo de permanencia se relacionó negativamente con los niveles de triglicéridos. Sin embargo, los niveles de triglicéridos en el plasma en los sitios de invernada o durante las primeras escalas migratorias (San Francisco y Punta Banda) no permitieron predecir la variación individual en las tasas subsecuentes de viaje durante la migración. No encontramos una relación significativa entre los niveles de triglicéridos y la biomasa de presas en los distintos sitios de escala, lo que sugiere que el patrón latitudinal no se explica por cambios latitudinales en la abundancia de alimento. En cambio, sugerimos que las diferencias en fisiología de las aves migratorias entre los sitios de escala migratoria ubicados al norte y al sur, o diferencias en el comportamiento, podrían permitir que las aves presenten tasas de engorde mayores cuando se acercan a las áreas de cría.

As a group, shorebirds in North America have experienced declines in populations over the past several decades (Morrison 2001, Morrison and Hicklin 2001). Most migratory bird species use intermediate resting and feeding sites between wintering and breeding areas, and understanding the stopover ecology of shorebirds at these sites is critical in determining the effects of factors such as habitat change on population dynamics (Skagen 1997, Warnock and Bishop 1998). Advances in radiotransmitter and stable-isotope technologies have resulted in considerable information being obtained for timing of migration, links between wintering and breeding areas, migration routes, and length of stay (LOS) at stopover sites (e.g., Davis et al. 1996, Marra et al. 1998, Farmer and Wiens 1999). One of the best-studied examples for shorebirds in North America is the Western Sandpiper (*Calidris mauri*; hereafter "sandpiper"), for which migratory patterns have been described in detail for the region between San Francisco, California, and western Alaska (Iverson et al. 1996, Bishop and Warnock 1998, Warnock and Bishop 1998, Bishop et al. 2005). Despite this work, factors that determine site use and LOS and the potential benefit that birds obtain at specific sites (e.g., in terms of fattening rates) remain poorly understood. As an example, simple measures of body condition (e.g., body mass or size-corrected body mass) are poor predictors of LOS at a stopover site or migration times among sites (e.g., Holmgren et al. 1993, Skagen and Knopf 1993, Warnock and Bishop 1998).

Numerous studies have shown that changes in certain plasma metabolites, especially triglycerides, can be used to estimate physiological state and rates of mass change in birds (e.g., Jenni-Eiermann and Jenni 1994, Jenni-Eiermann and Jenni 1996, Williams et al. 1999, Acevedo Seaman et al. 2006). During fat deposition, triglycerides increase in the plasma, owing to transport of lipids to peripheral adipose tissue (Ramenofsky 1990). Thus, evaluating plasma triglyceride levels allows for an estimate of the dynamics of mass change, or fattening, 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). More recently, several studies have suggested that this approach could also be used to compare rates of mass change or fattening among different populations or different migratory sites (Schaub and Jenni 2001, Ydenberg et al. 2002), though mainly at relatively small geographic scales (Guglielmo et al. 2005, Acevedo Seaman et al. 2006). Only one previous study has investigated site differences in metabolite levels at a broad geographic or latitudinal scale: Schaub and Jenni (2001) compared an index of fattening rate, based on plasma metabolite analysis, in migratory passerines at 14 stopover sites from northern Europe to sub-Saharan Africa. They found no effect of latitude, and only a weak effect of distance to major ecological barriers, on variation in fattening rates.

We combined radiotelemetry, plasma metabolite analyses, and macroinvertebrate prey sampling to investigate variation in putative fattening rates (estimated as plasma triglyceride levels) at the flyway scale in sandpipers. Specifically, we compared plasma triglyceride and glycerol levels in sandpipers wintering at San Francisco Bay, California, with those in birds caught at eight migratory Pacific Flyway stopover sites between Punta Banda, Baja California, Mexico (31°N), and Hartney Bay, Alaska (60°N, western Copper River Delta). In addition, we investigated the relationships of plasma triglyceride levels to (1) migratory behavior—LOS at the initial banding site and duration and rate of migration, determined from radiomarked birds; and (2) macrofaunal prey biomass, a measure of habitat quality.

Methods

Radiotelemetry.—We captured sandpipers during daylight hours at Punta Banda and San Francisco Bay by mist netting at shallow, open-ponded areas using taped alarm calls to lure birds. For each bird, we recorded body mass (±0.05 g) and exposed culmen (±0.1 mm). Individuals were sexed as males if their exposed culmen was ≤24.2 mm and as females if their exposed culmen was ≥24.8 mm (Page and Fearis 1971), and we aged first-year birds on the basis of extensive wear of the inner wing coverts as compared with adults (illustrated in Warnock and Warnock 2001). All birds were marked with federal bands. A sample of 88 birds was marked at Punta Banda (n = 47) and San Francisco Bay (n = 41), with 1.0-g radiotransmitters (lifespan six weeks; Holohil Systems, Woodlawn, Ontario) glued to their lower backs (see Warnock and Warnock 1993), and we obtained blood from a subsample (Mexico: n = 33; California: n = 34) of these birds. Radiotransmitter weight was ~3% of body mass, and we affixed transmitters to the birds with cyanoacrylate glue (QuickTite Super Glue, Loctite, Rocky Hill, Connecticut).

Radiotelemetry monitoring methods and number and dates of surveys are given in Table 1 (for sites for which we also obtained blood samples). Our ability to detect transmitters varied by method and equipment: range was <2 km from the ground with a hand-held antenna, 1–4 km from the ground with a truck-mounted antenna (3–7 km from a 120-m hill), and <10 km from an airplane (we used radiotransmitters at all major stopover areas to test aerial telemetry equipment). Trucks equipped with dual-Yagi,

Table 1. Telemetry methods (A = aerial, G = ground) and number of surveys for monitoring of migratory Western Sandpipers, March–May 2004. Information is reported only for locations included in the present study.

General location	Method	Surveys	Monitoring dates
Punta Banda, Mexico	G	26	March 31; April 1–16, 18–25, 29; May 1
Elkhorn Slough, California	A	14	April 7, 11, 13, 21, 23, 25, 27, 29, 30; May 1, 4, 5, 7, 10
	G	16	April 6, 8, 10, 11, 13, 14, 17, 18, 21–25; May 3, 4, 6
San Francisco, California	A	14	April 7, 11, 13, 21, 23, 25, 27, 29, 30; May 1, 4, 5, 7, 10
	G	18	April 4–6, 8–10, 12, 13–22, 24, 26, 28; May 2, 3, 6, 11, 12
Bandon Marsh, Oregon	G	10	April 22–26, 28, 29, 30; May 1, 4
Bottle Beach and Bowerman, Washington	A	12	April 19, 21, 23, 25, 27, 29; May 1, 3, 5, 7, 9, 11
	G	15	April 20, 22, 24, 26, 28, 30; May 2–8, 10
Boundary Bay, British Columbia	G	10	April 26–30; May 1–3, 5, 6
Hartney Bay, Alaska	A	25	April 29; May 1–2, 4–25

null-peak telemetry systems were used at San Francisco Bay; hand-held, three-element Yagi antennas were used at remaining ground monitoring sites; and aerial monitoring was conducted from planes equipped with exterior, dual-mounted antennas. Flights were conducted at altitudes of 300–1,500 m, with timing of flights varying by area. When a bird was located at a site, we monitored its presence until it had not been detected for at least two days or the bird had been relocated at another site. Monitoring at a site ended either when all radiomarked birds had departed or when minimal migratory activity was observed. For analysis of metabolite levels in relation to LOS, we used data from a subsample of radiomarked birds in San Francisco Bay (n = 33) and Punta Banda (n = 34) from which blood samples were taken. Length of stay was the number of days from capture and transmitter attachment to the last detection at the initial banding site. We assumed that a detected bird remained on the site the entire day (i.e., LOS ≥ 1 day) and that it remained on site from the first to last detection day. For birds arriving or departing on days when we were unable to monitor, we estimated the arrival or departure date by taking the midpoint between dates we monitored. Although we know that there were differences in probability of detection between San Francisco and Punta Banda (N. Warnock unpubl. data), this will not bias the within-site analysis we report below. For analysis of metabolite levels in relation to migration schedule, we calculated the number of days and rate of travel (km day^{-1}) for birds banded at San Francisco Bay and Punta Banda that were relocated either at a more northerly stopover site nearest the initial banding location (Mexico: n = 18; California: n = 18) or at Hartney Bay (Mexico: n = 8; California: n = 16).

Blood sampling and metabolite analysis.—Blood samples were obtained from wintering sandpipers at San Francisco Bay between 12 January and 30 April (n = 58) and from 396 sandpipers at various migratory stopover sites between April and May (see Table 2). Distances between Punta Banda and each site were calculated using ARCVIEW, version 3.2a (ESRI, Redlands, California), with the azimuthal-equidistance, north-polar projection. We treated Elkhorn Slough, California, and San Francisco Bay (~88 km apart), and Bottle Beach and Bowerman, Washington (both in Grays Harbor, but ~10 km

Table 2. Absolute plasma triglyceride and glycerol values (means ± SD, mmol L^{-1}) and body mass (g) by site, with sampling periods (number of sampling days in parentheses), latitude, distance from Ensenada, Mexico, and sample sizes (n).

General location	Sampling period	Latitude (°N)	Distance	n	Triglyceride	Glycerol
Punta Banda, Mexico	March 29–April 4 (6)	31	0 km	33	0.632 ± 0.280	0.634 ± 0.238
Elkhorn Slough, California	April 7–April 23 (3)	36	920 km	11	1.075 ± 0.460	0.830 ± 0.302
San Francisco, California (winter)	January 12–March 3 (15)	37	990 km	59	0.588 ± 0.264	0.649 ± 0.243
San Francisco, California (spring)	April 8–May 3 (16)	37	990 km	121	1.158 ± 0.635	0.739 ± 0.237
Bandon Marsh, Oregon	April 29–May 1 (3)	43	1,685 km	7	1.542 ± 0.871	0.725 ± 0.225
Bottle Beach, Washington	April 24–May 14 (14)	46	2,100 km	63	0.834 ± 0.427	0.773 ± 0.207
Bowerman, Washington	April 24–May 14 (14)	47	2,100 km	27	1.314 ± 0.552	0.818 ± 0.237
Boundary Bay, British Columbia	April 21–May 2 (5)	49	2,340 km	21	1.361 ± 0.463	0.621 ± 0.234
Hartney Bay, Alaska	May 2–May 18 (12)	60	4,240 km	113	1.586 ± 0.549	0.674 ± 0.231

apart) as separate sites, because we had (1) relatively large numbers of plasma samples from each site and (2) invertebrate data for each site. At the time of capture, birds were actively feeding or had recently been feeding, and most birds (>75%) were caught within 2 h before or after high tide. Blood (≤150 μL) was sampled via brachial venipuncture, transported in coolers, and centrifuged at 5,000 rpm with portable centrifuges for 3 min within 2 h of sampling, and the plasma was stored at –20°C until assayed.

We assayed plasma samples for free glycerol and triglyceride only (measured in the same assay), because several studies have shown that plasma triglyceride is the most informative metabolite for predicting fattening rate in free-living birds. Additionally, other metabolites (e.g., uric acid, non-esterified fatty acids, beta-hydroxybutyrate) show little or no systematic variation with site or migratory stage in sandpipers (Guglielmo 1999; Guglielmo et al. 2002, 2005; Seaman 2003; Landys et al. 2005; see Acevedo Seaman et al. [2006] for further discussion). We used a sequential color endpoint assay (Sigma-Aldrich Canada, Oakville, Ontario), using 5 μL of plasma sample with 240 μL and 60 μL of glycerol reagent and triglyceride reagent, 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. Assays were run in 400-μL flat-bottom, 96-well microplates (NUNC, Roskilde, Denmark) and read with a microplate reader (Biotek 340 EL; Biotek Instruments, Winooski, Vermont). Each plate was run with a standard curve based on a serial dilution of a 2.54-mmol glycerol standard (Sigma-Aldrich, St. Louis, Missouri), and a 19-day-old hen plasma pool was used to calculate assay variation (CV%; intra-assay CV%: 3.3% and 3.6% (n = 10); inter-assay CV%: 5.2% and 12.5% (n = 17); for triglyceride and glycerol, respectively).

Invertebrate prey sampling.—We sampled benthic invertebrate biomass at migratory stopover sites as an index to habitat quality. Benthic samples were collected with a 10-cm-diameter clam gun, 10 cm deep. At each site, we quasi-randomly selected three locations on mud flats close to the mist nets at low tide and midtide, and three cores were taken at each location. From these banding sites, cores were taken along a random direction and distance within 25 m of the nets, or parallel to the tideline. Once collected, the samples were placed in a plastic bag labeled with the site name, date of collection, and tide stage. Samples were refrigerated and transported to a 0°C freezer for storage. The total number of samples (193) represented the following sites: Punta Banda (n = 25), Elkhorn Slough (n = 29), San Francisco (n = 44), Bandon Marsh, Oregon (n = 22), Bottle Beach (n = 28), Bowerman Basin (n = 30), and Hartney Bay (n = 15). All samples were washed with saltwater (tap water mixed to 20 ppt with Instant Ocean [Marineland, Moorpark, California]) onto a 0.5-mm sieve. The contents of all remaining material on the sieve were preserved with a solution of 70% ethanol with rose bengal. Invertebrates were sorted into taxonomic groups, enumerated, weighed (±0.001 g), and measured (mm). Macro-invertebrates were identified under stereoscopes with standard invertebrate keys (Kozloff 1996, Smith and Carlton 1975). For this analysis, larger clams (>6 mm) that likely were not consumable prey were excluded, and samples were grouped by site to determine mean biomass (m^2).

Statistical analysis.—Statistical analyses were performed using STATA (Computing Resource Center, Santa Monica, California) and SAS (SAS Institute, Cary, North Carolina). Significance was set at $P \leq 0.05$, unless otherwise indicated. Triglyceride and glycerol data were transformed using $\log_{10}$(metabolite) + 1 to normalize these data. In preliminary analyses, we investigated the relationship between plasma metabolites and body mass, handling time, age, sex, time of day, and time in relation to the nearest high tide. Handling time was the time between birds hitting the nets and blood being drawn (median = 18 min, 95% < 45 min; birds with handling time >60 min were excluded from analysis). We controlled for any significant factors by including them as covariates in the model to analyze site differences in relation to latitude. Given that we have documented significant sex effects on stopover ecology in other studies (Warnock and Bishop 1998, Bishop et al. 2005, 2006), we included sex as a variable in our triglyceride analyses of LOS of our radiomarked birds, though Guglielmo et al. (2002) and Acevedo Seaman et al. (2006) reported no sex or age differences in triglyceride levels (and see below).

Results

Variation in body mass.—Body mass of sandpipers varied markedly among sites ($F = 27.9$, df = 8 and 439, $P < 0.001$) and with sex ($F = 18.1$, df = 1 and 439, $P < 0.001$, controlling for tarsus length because females are structurally larger than males; site*sex interaction not significant, $P > 0.80$). In general, body mass was higher in females than in males, and this sex difference was significant ($P < 0.05$, controlling for tarsus length) among birds caught at San Francisco during the winter and in Mexico, Oregon, Bottle Beach, and Alaska, but not at other sites (Fig. 1). Among stopover sites, variation in body mass was not explained by latitude for either males ($F = 0.24$, df = 1 and 7, $P > 0.80$) or females ($F = 0.09$, df = 1 and 7, $P > 0.90$). We estimated the change in body mass with date (as a potential measure of fattening rate; but see below) for three stopover sites where we had robust sample sizes with sampling spread over >16 days. Body mass varied significantly with date in San Francisco during spring ($F = 7.10$, df = 1 and 119, $P < 0.001$, $b = 0.381 \pm 0.054$) and at the Washington sites ($F = 2.10$, df = 1 and 89, $P < 0.05$, $b = 0.090 \pm 0.042$), but not at Hartney Bay ($P > 0.80$, controlling for tarsus length in each case).

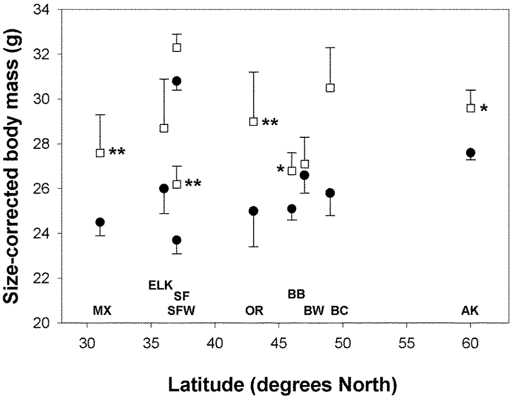

Fig. 1. Variation in size-corrected body mass (least square means ± SE, controlling for tarsus length) for male (filled circles) and female (open squares) Western Sandpipers at different sites along the Pacific Flyway and in relation to latitude; significant sex differences in mass within site are indicated as * $P < 0.05$, ** $P < 0.01$. Sites are at Punta Banda, Mexico (MX); Elkhorn Slough, California (ELK); San Francisco Bay, California in winter (SFW) and spring (SF); Bandon Marsh, Oregon (OR); Bottle Beach, Washington (BB); Bowerman Basin, Washington (BW); Boundary Bay, British Columbia (BC); and Hartney Bay, Alaska (AK).

Effect of body mass, handling time, age, sex, time of day, and date on metabolite levels.—Plasma triglyceride levels were positively related to body mass ($F = 8.58$, df = 1 and 453, $P < 0.001$, $b = 0.024$) and negatively related to handling time between capture and blood sampling ($F = 4.17$, df = 1 and 453, $P < 0.001$, $b = -0.004$). By contrast, plasma glycerol levels were independent of both mass and handling time ($P > 0.25$). There was no effect of age or sex on either metabolite for all data pooled or by site ($P > 0.05$ in all cases). Mean time of blood sampling varied with latitude ($F = 33.2$, df = 7 and 395, $P < 0.001$); birds were caught relatively late in the day in Oregon and Alaska (mean = 1603 and 1427 hours PST, respectively), whereas birds at Bottle Beach and in British Columbia were caught relatively early (0821 and 0819 hours PST, respectively); other sites were sampled between 1000 and 1400 hours PST. Univariate analysis of pooled data for stopover sites showed that residual plasma triglyceride, controlling for body mass and handling time, was positively related to capture hour ($b = 0.004$, $P < 0.005$), whereas glycerol was independent of time of day ($b = -0.001$, $P > 0.10$). By contrast, plasma triglyceride levels were independent of the time at which birds were caught in relation to nearest high tide ($F = 0.07$, df = 1 and 453, $P > 0.70$, controlling for body mass and handling time). We therefore pooled data by age and sex (including 13 birds of unknown sex) and controlled for body mass, handling time, and time of day in subsequent analyses.

Controlling for mass, handling time, and time of day, we investigated whether plasma metabolite levels were related to date within sites where blood samples were collected over a period of >16 days. Residual plasma triglyceride was positively related to date for San Francisco in the winter ($F = 17.5$, df = 1 and 58, $P < 0.001$; Fig. 2A), San Francisco in the spring ($F = 14.99$, df = 1 and 120, $P = 0.001$; Fig. 2B), and Washington ($F = 7.44$, df = 1 and 89, $P = 0.01$, pooling the two sites that were sampled over the same period; Fig. 2C), but not for Alaska ($P > 0.90$).

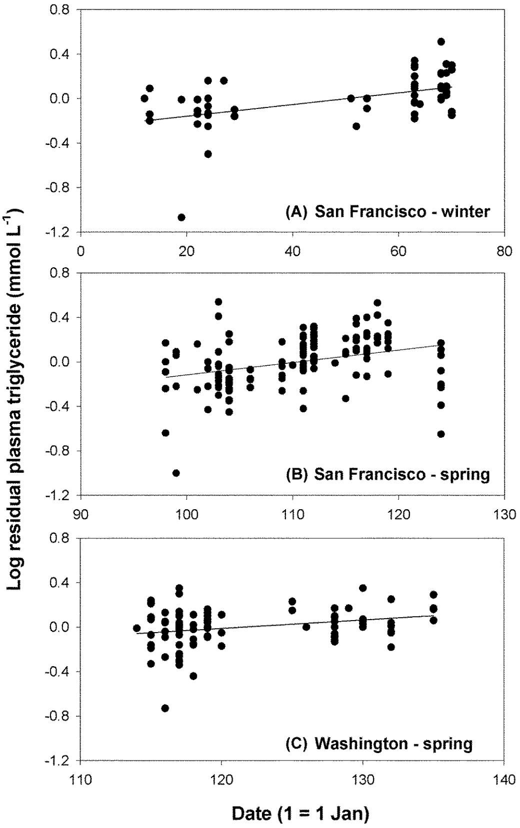

FIG. 2. Variation in residual plasma triglyceride in Western Sandpipers in relation to 2004 capture date for (A) San Francisco winter sample, (B) San Francisco spring sample, and (C) Washington spring sample.

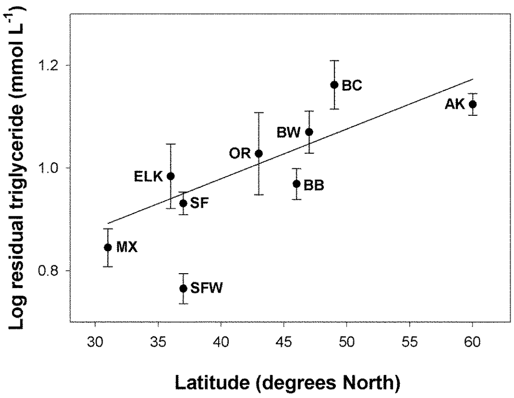

FIG. 3. Relationship between plasma triglyceride levels and latitude in Western Sandpipers for 2004 spring stopover sites. The fitted line excludes San Francisco (winter). See Figure 1 caption for explanation of abbreviations.

Variation in plasma metabolite levels with latitude.—Using data from all birds, we found a highly significant effect of site on mean plasma triglyceride levels ($F = 18.9$, df = 8 and 453, $P < 0.001$, controlling for body mass, handling time, and time of day; time relative to nearest high tide was not significant in this model, $P > 0.05$). Plasma triglyceride levels were higher in the spring at San Francisco than in the winter ($F = 7.50$, df = 1 and 178, $P < 0.01$). In spring-migrating birds, there was a significant linear trend for increasing residual triglyceride levels with latitude at stopover sites as birds migrated north ($F = 2.77$, df = 1 and 7, $P = 0.035$; Fig. 3). The lowest mean plasma triglyceride levels occurred in Punta Banda (the earliest spring stopover site), the second lowest in San Francisco, and plasma triglyceride levels then increased through Oregon, Washington, British Columbia, and Alaska. Glycerol levels also varied with site ($F = 3.59$, df = 8 and 453, $P < 0.001$), but only 1 of the 36 pairwise comparisons between sites was significant (based on Bonferroni-adjusted P values), and this variation was not systematically related to latitude.

Variation in metabolite levels and migratory behavior.—We analyzed the relationship between LOS and plasma metabolite levels for birds that were blood-sampled at the time when transmitters were attached at San Francisco Bay ($n = 33$) and Punta Banda ($n = 34$). Initially, we included body mass, handling time, and time of day as covariates in the model, but these variables were not significant ($P > 0.20$), so they were eliminated from the model. With the reduced model for birds at San Francisco, both plasma triglyceride level ($F = 4.13$, df = 1 and 32, $P = 0.05$) and sex ($F = 2.90$, df = 2 and 32, $P = 0.07$) explained a significant amount of the variation in LOS (the interaction term sex*triglyceride level was not significant, $P > 0.70$). In other words, LOS was negatively related to plasma triglyceride level, but for a given triglyceride level, females had a greater LOS than males (Fig. 4). Including capture date in the model, the relationship between LOS and triglyceride level was still marginally significant ($F = 3.59$, df = 1 and 32, $P = 0.069$), but the effect of sex was not significant ($P > 0.20$). Using this same model with birds radiomarked at Punta Banda, there was no significant relationship between

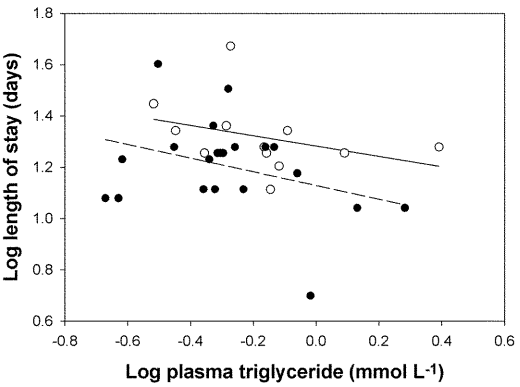

FIG. 4. Relationship between length of stay and plasma triglyceride levels for male (closed circles, solid line) and female (open circles, dashed line) Western Sandpipers caught at San Francisco, April 2004.

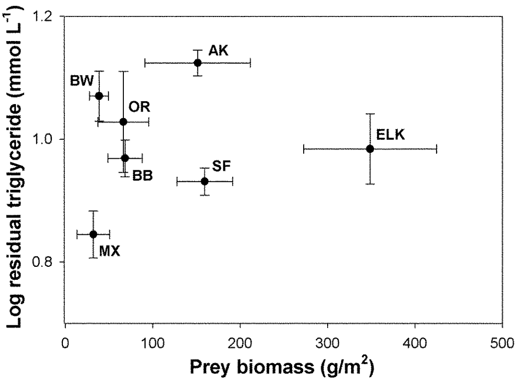

FIG. 5. Relationship between mean site-specific plasma triglyceride levels in Western Sandpipers and prey biomass (m^2) at spring stopover sites in 2004. See Figure 1 caption for explanation of abbreviations.

LOS and any of the measured variables for this site ($P > 0.20$ in all cases). Plasma triglyceride levels in birds at Punta Banda or San Francisco did not explain variation in the number of days of travel ($P > 0.50$), or rate of travel (km day^{-1}, $P > 0.30$), between these initial banding sites and either the first stopover site where birds were relocated or between the initial banding site and Hartney Bay (body mass, handling time, and time of day were not significant; $P > 0.20$ in these analyses).

Variation in metabolite levels and prey abundance.—We found no significant relationship between plasma triglyceride levels and mean total prey biomass for the seven stopover sites for which we had data ($F = 0.84$, df = 1 and 6, $P > 0.40$; Fig. 5). However, it is noticeable that Elkhorn Slough, which has a high residual plasma triglyceride value for its latitude (see Fig. 3), also had a very high prey biomass.

DISCUSSION

We have shown, first, that plasma triglyceride levels in sandpipers increased between winter and migration at San Francisco Bay, which indicates an increase in fattening rates, as would be predicted if birds are switching from a nonmigratory to a premigratory or migratory hyperphagic state (Ramenofsky 1990, Zwarts 1990). Second, we found that there was a strong positive relationship between plasma triglyceride levels and latitude through the spring as birds moved from Mexico to Alaska. We interpret this as evidence that birds are fattening more rapidly at stopover sites as they move farther north and get closer to the breeding grounds. Third, we have shown a systematic relationship between plasma triglyceride levels and, thus, putative fattening rate, and LOS, a behavioral component of migration in individual birds. However, plasma triglyceride levels at wintering or initial stopover sites (San Francisco and Punta Banda) did not predict individual variation in subsequent rates of travel during migration. Finally, we found no relationship between plasma triglyceride levels and prey biomass (cf. Acevedo Seaman et al. 2006), which suggests that the latitudinal change in fattening rates may not be driven by changes in food availability *per se*.

Body mass itself was highly variable at different stopover sites, but this variation was not systematically related to latitude, and analysis of mass by capture date was also not useful in elucidating patterns of fattening with latitude (see also Williams et al. 1999, Schaub and Jenni 2001, Guglielmo et al. 2005). Slopes of mass by capture date indicated a putative high rate of fattening at San Francisco Bay (0.4 g day^{-1}) but low (0.09 g day^{-1}) or no increase in mass at more northerly sites (Washington and Alaska). However, these analyses are clearly confounded in terms of predicting actual fattening rates, by the short LOS and rapid turnover of birds at stopover sites. If birds arrived, fattened rapidly, departed, and then were replaced by newly arriving birds of lower body mass, body mass would not change with capture date at the population level, even though individual birds were fattening. Interestingly, the slope of mass with date for San Francisco in the present study was very similar to that reported for the same site by Warnock and Bishop (1998; 0.4 g day^{-1}). It is possible that the greater mean LOS of birds at San Francisco (7.8 ± 7.1 days; N. Warnock et al. unpubl. data) compared with other sites (mean: 1–3 days) means that the method of analysis of mass by date provides a more accurate estimate of actual fattening rate at this site.

Body mass of sandpipers was higher at San Francisco Bay than at any of the stopover sites farther north. Higher body mass, combined with the greater LOS, confirms previous suggestions (e.g., Warnock and Bishop 1998) that San Francisco Bay is a staging area for sandpipers (*sensu* Skagen and Knopf 1993). By contrast, most other sites where LOS is generally short (<3 days) are better classified as true stopover sites (Warnock and Bishop 1998). Interestingly, the second-highest mean body masses in 2004 were recorded at Hartney Bay, the site sampled closest to the breeding grounds in the present study. It is possible that this reflects the advantages of arriving on the breeding grounds with higher body mass or nutrient reserves (e.g., Smith and Moore 2003). However, once sandpipers depart Hartney Bay, they still have a 1,000-km journey to their breeding grounds west of the Copper River Delta, where there appears to be fewer coastal stopover sites available for subsequent refueling (Bishop and Warnock 1998) and where the probability of encountering frozen landscape and scarce food resources increases. Thus, the higher departure masses at this site—achieved via high fattening rates (see below)—may be essential to allow birds to complete this final stage of migration successfully and in good condition to commence breeding.

We found a negative relationship between plasma triglyceride levels (and therefore putative fattening rate) and LOS at San Francisco Bay, but not with other measures of the bird's subsequent migration schedule. This suggests that birds fatten more slowly if they remain at this site longer and that they fatten more rapidly when they are closer to departure. Landys et al. (2005) reported a similar result in migrating Bar-tailed Godwits (*Limosa lapponica*); plasma triglyceride levels were higher in advanced-refueling birds than in initial-refueling birds, which suggests that mass gains were especially pronounced during the later stages of stopover (again with no differences for glycerol or beta-hydroxybutyrate). Landys et al. (2005) suggested that fattening rates early in stopover might be constrained by reduced or atrophied digestive system in newly arriving birds (e.g., Landys-Ciannelli et al. 2003, Karasov et al. 2004). However, this pattern could also be explained by a behaviorally mediated strategy whereby birds maintain a lower body or fat mass to reduce predation risk (Kullberg et al. 2000, Gentle and Gosler 2001) until just before departure. At the Delaware Bay stopover, later-arriving Red Knots (*C. canutus*) also gain weight more rapidly than earlier-arriving birds (Robinson et al. 2003).

Numerous theoretical studies of optimal migration have included predictions about fattening rates and fuel loads (e.g., Alerstam and Lindström 1990, Lindström 1991, Houston 1998), including some components of site-specific variation (e.g., Gudmundsson et al. 1991), but these studies do not appear to have addressed systematic variation in fattening rate in relation to latitude or distance from the breeding grounds. With time-selected migration, stopover decisions should be affected by actual fat status and the prospects for rapid fattening at future potential sites (Alerstam and Lindström 1990, Lindström 1991). Therefore, if an increase in fattening rate with latitude is predictable, as suggested by the present study, this could have important consequences for various aspects of migration, such as departure fuel loads and "giving up" densities during foraging. For example, higher refueling rates are predicted to decrease stopover duration and departure fat load, and our data support the idea of a reduction of stopover duration. By contrast, with energy-selected migration, stopover decisions should be independent of fattening rate (Alerstam and Lindström 1990). Although we obtained data for only one year, the latitudinal cline in plasma triglyceride is similar to that reported by Guglielmo et al. (2002) for birds sampled in Panama and British Columbia. The cause of this relationship is unknown, though our data suggest that this does not simply reflect a latitudinal increase in prey availability at stopover sites. Jenni and Schaub (2003) also suggested that migrant passerines show a large behavioral flexibility in foraging, such that fattening rate may not be tightly coupled to food availability.

Similarly, because fattening represents a rate per unit of time, higher fattening rates cannot simply be attributable to birds having more time to feed per day (e.g., more hours of daylight at northern sites: ~13 h at San Francisco vs. ~17 h in Alaska) or having a greater LOS. In any case, there is no evidence for significant differences in LOS for stopover sites at different latitudes; LOS averages 2–3 days at all sites between northern California and Alaska (Iverson et al. 1996, Warnock and Bishop 1998), as was the case in 2004 (N. Warnock et al. unpubl. data). Higher fattening rates could be achieved by either behavioral or physiological mechanisms. Behaviorally, birds could feed more intensively per unit of time (e.g., they could spend more time actively feeding and spend less time in vigilance behavior or in social interactions). Physiologically, comparing nonmigrating with migrating birds, it is known that there is a significant up-regulation of the digestive system, morphologically (Guglielmo and Williams 2003), as well as histologically and enzymatically (Stein et al. 2005). In sandpipers, some of these physiological adjustments occur after the onset of migratory flight and may reflect a "training effect" of migration itself (Guglielmo and Williams 2003). Thus, it is possible that within the migratory state there are differences in physiology of birds at southern versus northern stopovers that allow birds to sustain higher fattening rates as they get closer to the breeding grounds.

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Literature Cited

Acevedo Seaman, D. A., C. G. Guglielmo, R. W. Elner, and T. D. Williams. 2006. Landscape-scale physiology: Site differences in refueling rates indicated by plasma metabolite analysis in free-living migratory sandpipers. Auk 123:563–574.

Alerstam, T., and Å. Lindström. 1990. Optimal bird migration: The relative importance of time, energy and safety. Pages 331–351 *in* Bird Migration: Physiology and Ecophysiology (E. Gwinner, Ed.). Springer-Verlag, Berlin.

Bishop, M. A., and N. Warnock. 1998. Migration of Western Sandpipers: Links between their Alaskan stopover area and breeding grounds. Wilson Bulletin 110:457–462.

Bishop, M. A., N. Warnock, and J. Y. Takekawa. 2005. Differential spring migration of male and female Western Sandpipers at interior and coastal stopover sites. Ardea 92:185–196.

Bishop, M. A., N. Warnock, and J. Y. Takekawa. 2006. Spring migration patterns in Western Sandpipers *Calidris mauri*. Pages 545–550 *in* Waterbirds Around the World (G. C. Boere, C. A. Galbraith, and D. A. Stroud, Eds.). The Stationery Office, Edinburgh, United Kingdom.

Davis, L. S., P. D. Boersma, and G. S. Court. 1996. Satellite telemetry of the winter migration of Adélie Penguins (*Pygoscelis adeliae*). Polar Biology 16:221–225.

Farmer, A. H., and J. A. Wiens. 1999. Models and reality: Time–energy trade-offs in Pectoral Sandpiper (*Calidris melanotos*) migration. Ecology 80:2566–2580.

Gentle, L. K., and A. G. Gosler. 2001. Fat reserves and perceived predation risk in the Great Tit, *Parus major*. Proceedings of the Royal Society of London, Series B 268:487–491.

Gudmundsson, G.A., Å Lindström, and T. Alerstam. 1991. Optimal fat loads and long-distance flights by migrating Knots *Calidris canutus*, Sanderlings *C. alba*, and Turnstones *Arenaria interpres*. Ibis 133:140–152.

Guglielmo, C. G. 1999. Physiological and biochemical modulation for long-distance migration: The functional significance of intra-specific variation in the Western Sandpiper. Ph.D. dissertation, Simon Fraser University, Burnaby, British Columbia.

Guglielmo, C. G., D. J. Cerasale, and C. Eldermire. 2005. A field validation of plasma metabolite profiling to assess refueling performance of migratory birds. Physiological and Biochemical Zoology 78:116–125.

Guglielmo, C., P. D. O'Hara, and T. D. Williams. 2002. Extrinsic and intrinsic sources of variation in plasma lipid metabolites of free-living Western Sandpipers (*Calidris mauri*). Auk 119:437–445.

Guglielmo, C. G., and T. D. Williams. 2003. Phenotypic flexibility of body composition in relation to migratory state, age and sex in the Western Sandpiper (*Calidris mauri*). Physiological and Biochemical Zoology 76: 84–98.

Holmgren, N., H. Ellegren, and J. Pettersson. 1993. Stopover length, body mass and fuel deposition rate in autumn migrating adult Dunlins *Calidris alpina*: Evaluating the effects of moulting status and age. Ardea 81:9–20.

Houston, A. I. 1998. Models of optimal avian migration: State, time and predation. Journal of Avian Biology 29:395–404.

Iverson, G. C., S. E. Warnock, R. W. Butler, M. A. Bishop, and N. Warnock. 1996. Spring migration of Western Sandpipers (*Calidris mauri*) along the Pacific Coast of North America: A telemetry study. Condor 98:10–21.

Jenni, L., and M. Schaub. 2003. Behaviour and physiological reactions to environmental variation in bird migration: A review. Pages 155–171 *in* Avian Migration (P. Berthold, E. Gwinner, and E. Sonnenschein, Eds.). Springer-Verlag, Berlin.

Jenni-Eiermann, S., and L. Jenni. 1994. Plasma metabolite levels predict individual body-mass changes in a small long-distance migrant, the Garden Warbler. Auk 111: 888–899.

Jenni-Eiermann, S., and L. Jenni. 1996. Metabolic differences between the post-breeding, moulting and migratory periods in feeding and fasting passerine birds. Functional Ecology 10:62–72.

Karasov, W. H., B. Pinshow, J. M. Starck, and D. Afik. 2004. Anatomical and histological changes in the alimentary tract of migrating Blackcaps (*Sylvia atricapilla*): A comparison among fed, fasted, food-restricted, and refed birds. Physiological and Biochemical Zoology 77:149–160.

Kozloff, E. N. 1996. Marine Invertebrates of the Pacific Northwest. University of Washington Press, Seattle.

Kullberg, C., S. Jakobsson, and T. Fransson. 2000. High migratory fuel loads impair predator evasion in Sedge Warblers. Auk 117:1034–1038.

Landys, M. M., T. Piersma, C. G. Guglielmo, J. Jukema, M. Ramenofsky, and J. C. Wingfield. 2005. Metabolic profile of long-distance migratory flight and stopover in a shorebird. Proceedings of the Royal Society of London, Series B 272:295–302.

Landys-Ciannelli, M. M., T. Piersma, and J. Jukema. 2003. Strategic size changes in internal organs and muscle tissue in the Bar-tailed Godwits during fat storage on a spring stopover site. Functional Ecology 17:151–159.

Lindström, Å. 1991. Maximum fat deposition rates of migrating birds. Ornis Scandinavica 22:12–19.

Marra, P. P., K. A. Hobson, and R. T. Holmes. 1998. Linking winter and summer events in a migratory bird by using stable-carbon isotopes. Science 282:1884–1886.

Morrison, R. I. G. 2001. Trends in shorebird populations in North America using Breeding Bird Survey data. Bird Trends 8:12–15.

Morrison, R. I. G., and P. Hicklin. 2001. Recent trends in shorebird populations in the Atlantic Provinces. Bird Trends 8:16–19.

Page, G., and B. Fearis. 1971. Sexing Western Sandpipers by bill length. Bird-Banding 42: 297–298.

Ramenofsky, M. 1990. Fat storage and fat metabolism in relation to migration. Pages 214–231 *in* Bird Migration: Physiology and Ecophysiology (E. Gwinner, Ed.). Springer-Verlag, Berlin.

Robinson, R. A., P. W. Atkinson, and N. A. Clark. 2003. Arrival and weight gain of Red Knot *Calidris canutus*, Ruddy Turnstone *Arenaria interpres* and Sanderling *Calidris alba* staging in Delaware Bay in spring. BTO Research Report no. 307, British Trust for Ornithology, The Nunnery, Thetford, United Kingdom.

Schaub, M., and L. Jenni. 2001. Variation of fuelling rates among sites, days and individuals in migrating passerine birds. Functional Ecology 15:584–594.

Seaman, D. A. 2003. Landscape physiology: Plasma metabolites, fattening rates and habitat quality in migratory Western Sandpipers. M.Sc. thesis, Simon Fraser University, Burnaby, British Columbia.

Skagen, S. K. 1997. Stopover ecology of transitory populations: The case of migrant shorebirds. Ecological Studies 125:244–269.

Skagen, S. K., and F. L. Knopf. 1993. Toward conservation of midcontinental shorebird migrations. Conservation Biology 7:533–541.

Smith, R. I., and J. T. Carlton, Eds. 1975. Light's Manual: Intertidal Invertebrates of the Central California Coast, 3rd ed. University of California Press, Berkeley.

Smith, R. J., and F. R. Moore. 2003. Arrival fat and reproductive performance in a long-distance passerine migrant. Oecologia 134: 325–331.

Stein, R. W., A. R. Place, T. Lacourse, C. G. Guglielmo, and T. D. Williams. 2005. Digestive organ sizes and enzyme activities of refueling Western Sandpipers (*Calidris mauri*): Contrasting effects of season and age. Physiological and Biochemical Zoology 78:434–446.

Warnock, N., and M. A. Bishop. 1998. Spring stopover ecology of migrant Western Sandpipers. Condor 100:456–467.

Warnock, N., and S. Warnock. 1993. Attachment of radio-transmitters to sandpipers: Review and methods. Wader Study Group Bulletin 70:28–30.

Warnock, N., and S. Warnock. 2001. Sandpipers, phalaropes, and allies. Pages 273–287 *in* The Sibley Guide to Bird Life and Behavior (C. Elphick, J. B. Dunning, Jr., and D. A. Sibley, Eds.). Knopf, New York.

Williams, T. D., C. G. Guglielmo, O. Egeler, and C. J. Martyniuk. 1999. Plasma lipid metabolites provide information on mass change over several days in captive Western Sandpipers. Auk 116:994–1000.

Ydenberg, R. C., R. W. Butler, D. B. Lank, C. G. Guglielmo, M. Lemon, and N. Wolf. 2002. Trade-offs, condition dependence and stopover site selection by migrating sandpipers. Journal of Avian Biology 33:47–55.

Zwarts, L. 1990. Increased prey availability drives premigration hyperphagia in Whimbrels and allows them to leave the Banc d'Arguin, Mauritania, in time. Ardea 78:279–300.

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