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EXTRINSIC AND INTRINSIC SOURCES OF VARIATION IN PLASMA LIPID METABOLITES OF FREE-LIVING WESTERN SANDPIPERS (*CALIDRIS MAURI*)

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ABSTRACT.—Plasma lipid metabolites may be useful indicators of mass changes in migratory birds. To test utility of plasma metabolites in field studies, we examined effects of several extrinsic (bleed time, time of day, location) and intrinsic (body mass, sex, age, migratory state) factors on plasma concentrations of triglycerides (TRIG), glycerol (GLYC), and B-OH-butyrate (BUTY) in free-living Western Sandpipers (*Calidris mauri*). TRIG and GLYC decreased rapidly following capture (2–20 min), whereas BUTY did not change. GLYC and BUTY were negatively correlated to body mass. TRIG was positively correlated to body mass in migrant females, but not consistently in migrant males, or in females captured on the wintering grounds. Taking into account other sources of variation, the two measures of lipid utilization (GLYC and BUTY) varied little through the year. TRIG showed the greatest potential for use in field studies. TRIG was lowest during winter, when birds were leanest, and highest during spring and fall migration, when sandpipers were gaining mass rapidly at stopovers. TRIG differed between sandpipers refuelling a two stopover sites separated by 35 km, demonstrating that populations of birds can have characteristic lipid metabolite profiles that may reflect local differences in fattening rate. *Received 12 February 2001, accepted 12 December 2001.*

RESUMEN.—Los metabolitos lipídicos del plasma pueden ser buenos indicadores de cambios en la masa corporal de las aves migratorias. Para poner a prueba su utilidad en estudios de campo, examinamos los efectos de varios factores extrínsecos (tiempo de toma de la muestra de sangre, hora del día y lugar) e intrínsecos (masa corporal, sexo, edad, estado migratorio) sobre las concentraciones plasmáticas de triglicéridos (TRIG), glicerol (GLIC) y B-OH-butirato (BUTI) en individuos silvestres de *Calidris mauri*. TRIG y GLIC disminuyeron rápidamente luego de la captura (2–20 min), mientras que BUTI no cambió. GLIC y BUTI estuvieron correlacionados negativamente con la masa corporal. TRIG se correlacionó positivamente con la masa corporal en hembras migrantes, pero esta relación no fue consistente en machos migrantes ni en hembras capturadas en las áreas de invernada. Teniendo en cuenta otras fuentes de variación, las dos medidas de utilización de lípidos (GLIC y BUTI) variaron poco a través del año. TRIG mostró el máximo potencial para uso en estudios de campo. TRIG fue más bajo durante el invierno cuando las aves estaban más livianas y más alto durante la primavera y el otoño cuando éstas estaban aumentando rápidamente su masa en las áreas de escala migratoria. TRIG mostró diferencias entre aves que se estaban reaprovisionando en dos sitios de escala separados por 35 km, demostrando que las poblaciones pueden tener perfiles de metabolitos lipídicos característicos que podrían reflejar diferencias locales en las tasas de engorde.

VARIATION IN physiological state due to anabolism, catabolism, or disease can result in changes in circulating levels of hormones, metabolic fuels, electrolytes, albumin, nitrogenous wastes, and immune system components (lymphocytes, immunoglobulins). Deciphering this information carried in the blood is essential to medical and veterinary diagnostics (Newsholme and Leech 1985, Duncan et al. 1994, Kaneko et al. 1997, Lehmann 1998). The availability and low cost of analytical kits makes the analysis of blood samples to assess the physiological state (condition) of animals in nature very attractive (Mori and George 1978; Jenni-Eiermann and Jenni 1991, 1996; Andersson and

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Gustafsson 1995; Brown 1996; Jenni and Jenni-Eiermann 1996; Dawson and Bertolotti 1997; Gannes 1999). Nevertheless, reliable interpretation of blood assays requires an understanding of how factors like stress, body mass, age, and sex affect baseline metabolite levels. Whereas these methods have been validated in humans and domestic animals, knowledge of the many possible sources of variation in wild animals remains rudimentary.

Studies with captive birds indicate that plasma lipid metabolites can be used to measure mass change at the individual or population level. Mass gain from morning to mid-day related positively to plasma triglyceride and negatively to B-OH-butyrate levels in Garden Warblers (*Sylvia borin*; Jenni-Eiermann and Jenni 1994). These same relationships were found in captive Western Sandpipers (*Calidris mauri*), and in addition, plasma glycerol was negatively correlated with rate of mass gain (Williams et al. 1999). At the individual level, determining rate and direction of mass change may provide a measure of physiological performance (fitness). At the population level, lipid metabolite profiles could be used to compare rates of mass gain at different sites, and thus provide an index of habitat quality based on animal performance (Jenni and Jenni-Eiermann 1996, Williams et al. 1999). Field studies of day-feeding passerines seem to support the use of lipid metabolites in that manner (Jenni and Jenni-Eiermann 1996, Jenni-Eiermann and Jenni 1996, Schaub and Jenni 2001). It is unclear if the technique can be applied easily to species like shorebirds, whose feeding may be related to both light and tide conditions.

In this study, we examined effects of several extrinsic (bleed time, time of day, site) and intrinsic (body mass, sex, age, migratory state) factors on plasma concentrations of triglycerides (TRIG), glycerol (GLYC), and B-OH-butyrate (BUTY) in Western Sandpipers. This shorebird migrates long distances between Arctic breeding areas and wintering grounds located mainly along the Pacific coast of the Americas (Wilson 1994). At some wintering areas (e.g. Panama), most first-year birds do not migrate north in the spring, whereas, simultaneously, premigratory adults fatten in preparation for their first northward flight (P. D. O'Hara unpubl. data, Guglielmo 1999). We measured metabolites in sandpipers during (1) winter residency, when body mass is low and stable, (2) premigration, when adults gain mass at a low rate (0.09 g day^{-1}; P. D. O'Hara unpubl. data), and (3) migratory stopover, when birds fatten at a high rate (0.4–1 g day^{-1}; Butler et al. 1997). We hypothesized that TRIG, an indicator of mass gain, would be higher at migratory stopover than during winter, and in Panama during premigration, TRIG would be higher in adults than yearlings. We expected GLYC and BUTY would follow a pattern opposite to TRIG. We also compared metabolites at two migratory stopover sites.

METHODS

Sample collection.—Wintering (nonmigratory) Western Sandpipers were sampled between 18 December 1995 and 9 February 1996 at Chitré on the Gulf of Panama (8°N, 79°W). Premigratory birds were sampled at Chitré from 4 to 24 March 1996. Adult migrants were sampled during spring northward (30 April–7 May) and ''fall'' southward migration (16–25 July), 1996 on the mudflats (25,000 ha) at Boundary Bay and Roberts Bank, British Columbia, Canada (49°10′N, 123°05′W). Juveniles were sampled on southward migration in 1996 at Boundary Bay (19–29 August) and at Sidney Island (8–28 August), a small stop-over site located 35 km southwest of Boundary Bay in the southern Strait of Georgia (Lissimore et al. 1999). Due to permit limitations, only females were studied in Panama.

We captured sandpipers with mist nets (Avinet, Dryden, New York) under permits from the Canadian Wildlife Service and INRENARE (Panama). Nets were in constant view, and blood sampling was timed from the moment of capture. In Panama, birds were caught on the falling tide returning from roosting and feeding at inland salt and shrimp ponds. At Boundary Bay and Sidney Island, birds were caught near the end of a high–low–high tide cycle to ensure that they had access to feeding areas for an extended period (6–10 h). About one-half of the samples were taken from the jugular vein of birds collected for body composition analysis (Guglielmo 1999). Approximately 140 birds were sampled from the brachial vein (26 gauge needle, 200–300 μL) with heparinized capillary tubes (VWR Scientific, Buffalo Grove, Illinois) at Chitré, Boundary Bay, and Sidney Island. Whole blood was transferred to heparinized Eppendorf tubes (rinsed with 1,000 IU / mL porcine sodium heparin; SIGMA, USA), and stored above ice in a small cooler. Blood was centrifuged at 6,000 rpm (2,000 × g) for 10 min. Plasma was stored at −20°C until analysis. Animal handling protocols were approved by the Simon Fraser University Animal Care

Table 1. Body mass (grams) and plasma lipid metabolite concentrations (mmol L^{-1}) of adult (A) and juvenile (J) Western Sandpipers sampled at a variety of migratory stages and locations (within each sex, masses with shared superscripts are not significantly different; see text for metabolite statistics).

		Mass		Triglyceride		Glycerol		B-OH-Butyrate	
Stage (Site)	Age / sex	Mean	SE / N	Mean	SE / N	Mean	SE / N	Mean	SE / N
Winter (Panama)	A / ♀	^{c,d}24.6	0.3 / 19	0.78	0.06 / 14	0.34	0.03 / 14	0.73	0.09 / 13
	J / ♀	^{d}23.3	0.3 / 16	0.70	0.09 / 14	0.42	0.05 / 14	1.08	0.16 / 9
Premigration (Panama)	A / ♀	^{a,b}27.9	0.8 / 15	1.61	0.18 / 15	0.30	0.02 / 15	0.51	0.10 / 13
	J / ♀	^{d}22.5	0.2 / 18	1.87	0.40 / 17	0.40	0.04 / 17	0.92	0.14 / 13
Spring (Boundary Bay)	A / ♀	^{a}29.2	0.4 / 14	2.12	0.24 / 14	0.29	0.02 / 14	0.82	0.13 / 13
	A / ♂	†25.6	0.6 / 14	1.75	0.21 / 14	0.33	0.03 / 14	—	—
Fall (Boundary Bay)	A / ♀	^{a}29.0	0.9 / 11	2.47	0.27 / 11	0.26	0.03 / 11	0.50	0.14 / 11
	A / ♂	†25.0	0.7 / 8	1.79	0.22 / 8	0.27	0.02 / 8	—	—
	J / ♀	^{a}29.4	0.5 / 30	3.22	0.40 / 27	0.46	0.04 / 27	0.60	0.10 / 12
	J / ♂	†26.4	0.5 / 27	2.80	0.24 / 26	0.38	0.03 / 26	0.57	0.11 / 14
Fall (Sidney Island)	J / ♀	^{b,c}26.1	0.8 / 17	3.81	0.33 / 15	0.49	0.04 / 15	—	—
	J / ♂	‡22.7	0.5 / 16	2.97	0.40 / 14	0.46	0.02 / 14	—	—

Committee and conformed with Canadian Committee for Animal Care guidelines.

Metabolite assays.—Lipid metabolites were assayed on a microplate spectrophotometer (Biotec 340EL). Assays were run in 400 μL flat-bottom, 96 well polystyrene microplates (NUNC, Roskilde, Denmark). Total triglycerides (triglyceride plus free glycerol) were measured by endpoint assay (WAKO Diagnostics, Richmond, Virginia; 5 μL plasma, 300 μL reagent). Lipemic samples were diluted with 0.9% NaCl to bring them within the range of the standard curve (0–3.4 mmol L^{-1} triolein equivalents). Free glycerol was measured by endpoint assay (SIGMA, Trinder reagent A; 5 μL plasma, 300 μL reagent). In some cases, free glycerol and total triglyceride were measured sequentially by endpoint assay (SIGMA Trinder reagent A and B; 4 μL plasma, 240 μL reagent A, 60 μL reagent B). Measurements by the WAKO and SIGMA kits were equivalent (C. G. Guglielmo unpubl. data). B-OH-butyrate was measured by kinetic assay (SIGMA). Five microliters of standard or plasma was pipetted to form a droplet at one side of a well, and 2 μL of B-OH-butyrate dehydrogenase reagent was pipetted along the opposite side. Sample and standard blanks received no enzyme. The reaction was initiated by adding 250 μL of reagent buffer, and absorbance at 340 nm was monitored for 10 min at room temperature (20°C). Metabolite concentrations were calculated from standard curves made with commercially available reagents (SIGMA, WAKO). Samples were run in duplicate or triplicate, and intra-assay coefficients of variation (CV) were 4.1, 5.2, and 12.3% for total triglyceride, glycerol and B-OH-butyrate, respectively. Inter-assay CVs in the same order were 6.2, 8.1, and 18.1%.

Statistical analysis.—TRIG (mmol L^{-1}) was calculated by subtracting free glycerol from total triglyceride. BUTY measurements were not made on males except for fall migrating juveniles at Boundary Bay, and Sidney Island samples were not assayed for this metabolite. Metabolite concentrations and bleed-times (time from net to blood collection) were log$_{10}$ transformed to make the data approximately normal within each sample group (migratory stage, sex, age). Median bleed-time was 5 min, but we used data for bleed-times up to 20 min. Body masses were compared among ages and migratory stages using one factor ANOVA followed by a Ryan-Einot-Gabriel-Welsch multiple range test. Before testing for variation in metabolite levels due to sex, age, or season, we tested for the effects of bleed-time, body mass, and time of day using linear regression techniques. Type III analysis of covariance (ANCOVA) was used to test for sex (spring, fall Boundary Bay, fall Sidney Island) and age (winter, premigration, fall) differences where possible, and data were combined if no age or sex effects were detected. Differences among migratory stages and stopover sites were tested with ANCOVA, and comparisons were made only if interactions were not significant. Tests were considered significant at $\alpha = 0.05$. For multiple comparisons, differences are only discussed if significant after experiment-wise error was controlled at $\alpha = 0.05$ by Bonferroni adjustment.

Results

Body masses and plasma metabolite concentrations by sex, age, and migratory stage are shown in Table 1. Females are structurally larger than males (Wilson 1994); however, within each sex, body mass varied significantly among ages and migratory stages (females $F = 24.9$, df $= 7$ and 132, $P = 0.0001$; males $F = 7.9$, df $= 3$ and 61, $P = 0.0002$). Females of both ages were lighter during winter than during migration periods, and only adult birds increased body mass during premigration. As found previously (Lissimore et al. 1999), birds of both sexes were lighter at Sidney Island than at Boundary Bay.

Triglycerides.—TRIG decreased with bleed-time ($\log_{10}\mathrm{trig} = -0.31[\log_{10}\mathrm{bleed\text{-}time}] + 0.51$; $F = 18.0$, df = 1 and 189, $R^2 = 0.08$, $P = 0.0001$), and bleed-time was therefore entered as a covariate in all subsequent models. TRIG was positively related to body mass (grams) in all migrating birds combined ($\log_{10}\mathrm{TRIG} = -0.26[\log_{10}\mathrm{bleed\text{-}time}] + 0.017[\mathrm{mass}] + 0.10$; $F = 10.0$, df = 1 and 126, $R^2 = 0.19$, $P = 0.002$), but not in wintering or premigrant birds ($F = 2.2$, df = 1 and 57, $R^2 = 0.05$, $P = 0.14$; see below). Controlling for bleed-time and body mass, there was no relationship between TRIG and time of day (0800–2100) in any stage, age or sex category ($P > 0.15$ in all cases). There was a consistent positive relationship between TRIG and body mass in females ($P < 0.02$), but not males ($P > 0.14$) except possibly for males at Sidney Island ($P = 0.06$). Thus, males and females were analyzed separately because the effect of body mass differed between them.

Within females, there were no differences in residual TRIG (controlling for bleed-time and body mass) between adults and juveniles at any stage ($P > 0.23$), and there were no age by mass interactions ($P > 0.29$). Migratory stage and body mass interacted significantly in females ($P = 0.01$), because there was no relationship between body mass and TRIG in Panama samples (Fig. 1). Within the Panama samples, premigratory birds of both ages had higher TRIG than winter birds ($P = 0.0001$; Fig. 1). In migrants, residual TRIG varied among stages ($F = 17.3$, df = 2 and 62, $P = 0.0001$), and was higher in the fall at Sidney Island than during spring or fall at Boundary Bay ($P = 0.0001$), which did not differ ($P > 0.48$; Fig. 1).

In males, ages classes could not be combined because juveniles had higher TRIG than adults at Boundary Bay (evident in Table 1; $P = 0.03$). TRIG did not differ between spring and fall migrating adult males ($P = 0.68$). Male juveniles stopping at Sidney Island could not be compared directly to Boundary Bay juveniles because of a significant site by mass interaction ($P = 0.035$). However, juvenile males and females could be combined at Sidney Island with no sex by mass interaction ($P = 0.58$). Doing so confirmed our previous finding in females alone; TRIG was higher at Sidney Island than at Boundary Bay ($P = 0.0001$).

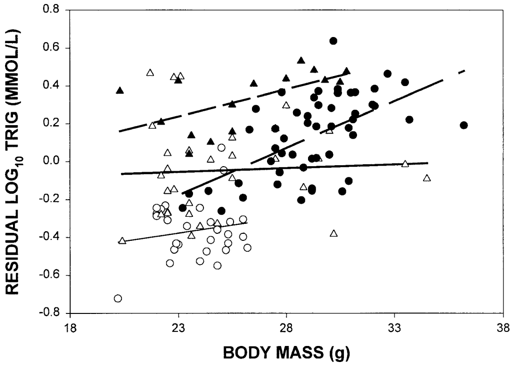

FIG. 1. Plasma triglyceride concentrations of female Western Sandpipers at various sites and seasons plotted against body mass. In nonmigrating birds sampled in Panama during the winter (open circles, thin solid line) and premigratory periods (open triangles, thick solid line), triglyceride levels were not related to body mass, but were higher in premigrants ($P = 0.0001$). Triglyceride was significantly related to body mass in spring and fall migrants sampled at Boundary Bay (closed circles, long dashed line), and fall migrants sampled at Sidney Island (closed triangles, short dashed line). Controlling for body mass, triglyceride concentrations were higher at Sidney Island than Boundary Bay ($P = 0.0001$).

Glycerol.—There was a negative relationship between GLYC and bleed-time ($\log_{10}\mathrm{GLYC} = -0.22[\log_{10}\mathrm{bleed\text{-}time}] - 0.28$; $F = 26.4$, df = 1 and 187, $R^2 = 0.12$, $P = 0.0001$), and a consistent negative relationship between GLYC and body mass controlling for bleed-time ($\log_{10}\mathrm{GLYC} = -0.24[\log_{10}\mathrm{bleed\text{-}time}] - 0.011[\mathrm{mass}] + 0.017$; $F = 9.8$, df = 1 and 186, $P = 0.002$). Residual GLYC declined significantly with time of day in fall migrants ($F = 3.1$, df = 1 and 70, $R^2 = 0.12$, $P = 0.003$), and tended to do so in all other migratory stages. Thus, we controlled for bleed-time, mass, and time of day in all analyses.

There were no sex effects ($P > 0.11$) or sex by mass interactions ($P > 0.21$) at any migratory stage, or for the data set as a whole. There were also no effects of age ($P > 0.32$) or age by mass interactions ($P > 0.26$). Compared to the other sites, Boundary Bay birds were generally caught later in the day, when time of day appeared to have the strongest negative effect on GLYC. That caused a significant stage by time

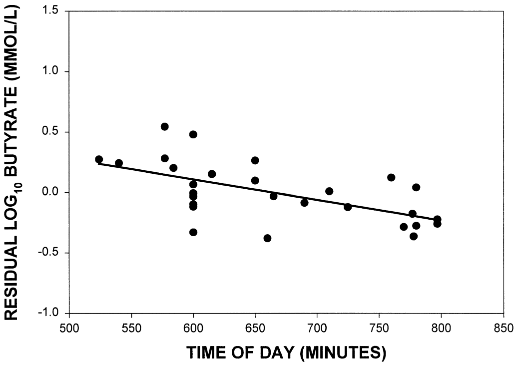

FIG. 2. Residual plasma B-OH-butyrate concentration (controlling for body mass) in wintering and premigrant female Western Sandpipers versus time of day ($R^2 = 0.36$, $P = 0.0008$). Time is minutes from midnight (sunrise approximately 0630 = 390 min; 0900 = 540 min; 1300 = 780 min).

of day interaction ($P = 0.009$). Analysing sites separately, we found no difference in GLYC between spring and fall migrants ($P = 0.91$), or between winter and premigrant birds ($P = 0.70$). Considering only the overlap in sampling times between Sidney Island and Boundary Bay (before 1600) eliminated the site by time of day interaction ($P = 0.49$), and indicated no difference in residual GLYC between the two sites ($P = 0.58$).

B-OH-Butyrate.—BUTY was not significantly related to bleed-time overall ($F = 2.3$, df = 1 and 97, $R^2 = 0.01$, $P = 0.14$), or in any stage ($P > 0.35$), but was consistently negatively related to body mass ($\log_{10}$BUTY $= -0.036$[mass] $+ 0.72$; $F = 15.3$, df = 1 and 97, $R^2 = 0.14$, $P = 0.0002$). Controlling for body mass, BUTY tended to decline with time of day at all stages, but not significantly ($F = 1.8$, df = 1 and 126, $P = 0.18$). However, in Panama, where morning sampling was possible, a decline was readily apparent in the first half of the day (08:00–13:00; $F = 14.5$, df = 1 and 26, $R^2 = 0.36$, $P = 0.0008$; Fig. 2). We controlled for body mass and time of day in subsequent analyses.

Sexes were combined because they did not differ ($P = 0.57$) and there was no sex by mass interaction ($P = 0.30$). There was a significant age by mass interaction in fall migrants ($P = 0.01$), but not among Panama samples ($P > 0.53$). Within Panama samples, BUTY did not differ among winter adults, winter juveniles, and premigration season juveniles ($P > 0.18$), however all three had higher BUTY than premigratory adults (evident in Table 1; $P < 0.05$). To compare Panama to Boundary Bay, we analyzed ages separately. BUTY did not differ among winter, premigration season juveniles and fall migrant juveniles ($F = 1.1$, df = 1 and 44, $P = 0.34$). In adults, fall data could not be included in the analysis because of a significant stage by mass interaction ($P = 0.04$). However, BUTY in premigrant adults was lower than spring migrant ($P = 0.03$) and winter adults ($P = 0.05$), which did not differ ($P = 0.52$).

DISCUSSION

Bleed-time, body mass and time of day can affect plasma lipid metabolite levels to an extent that could alter the interpretation of field data. Those covariates can be controlled statistically, however, so that hypotheses regarding sex, age, season, or site can be tested. Our most important findings were that (1) whereas in captivity TRIG, BUTY, and GLYC were related to rate of mass change (Williams et al. 1999), only TRIG varied in a predictable manner given the expected patterns of mass change in the field, and (2) TRIG can differ significantly between stopover sites, indicating differences in the rate of mass gain (see also Ydenberg et al. 2002).

Effects of covariates.—Lipid metabolite concentrations were influenced by several variables and interactions (Table 2). TRIG and GLYC declined following capture, most likely due to capture stress rather than short-term fasting, given the short bleed-times (5 min) of most of our samples. A similar rapid decline in TRIG after capture has been found in several passerine species (Jenni-Eiermann and Jenni 1991, 1996; Jenni and Jenni-Eiermann 1996). In passerine birds, longer fasts (30–120 min) are characterized by decreased TRIG and increased B-OH-butyrate (Jenni-Eiermann and Jenni 1991, 1996, 1997).

Body mass was a significant covariate for every metabolite. The two measures of lipid utilization (GLYC, BUTY) decreased with increasing body mass, whereas TRIG was positively related to body mass (but not under all conditions). Those relationships could result from (1) baseline metabolite levels changing inherently with body mass or fatness or (2) heavier birds having elevated fat deposition rates. In captive Western Sandpipers, absolute body mass ex-

TABLE 2. Summary of the effects of several covariates and class variables on plasma lipid metabolite concentrations. (0 = no trend, ? = uncertain, Y = yes, N = no).

Effect	Triglyceride	Glycerol	B-OH-Butyrate
Covariates			
Bleed-time	Negative	Negative	0
Body mass	Positive/0	Negative	Negative
Time of day	0	Negative	Negative/?
Class Variables			
Sex	?	N	N
Sex × mass	Y	N	N
Age	♀ = N, ♂ = Y	N	Y
Age × mass	N	N	Y/N
Stage	Y	N	Y
Stage × mass	Y	Y	Y

plained, respectively, 64 and 21% of the variation in TRIG and BUTY, regardless of whether birds were gaining or losing mass at the time (Williams et al. 1999). TRIG was also positively related to body mass in captive Red Knots (*Calidris canutus*; L. Jenni pers. comm.). Based on that information, we treated body mass as a confounding factor to be controlled by ANCOVA. Nevertheless, heavier birds could fatten at higher rates if they gain better access to food through social dominance, or have enhanced physiological capacity for fattening (e.g. Carpenter et al. 1993, Piersma et al. 1999). Studies of captive passerines indicate that body mass is not a significant covariate of metabolite concentrations (Jenni-Eiermann and Jenni 1994), yet in the field, metabolite levels in passerines have sometimes been found to be related to body mass (Gannes 1999, Schaub and Jenni 2001). That suggests that heavier birds may fatten at higher rates (Schaub and Jenni 2001), but netting studies suggest the opposite (Schaub and Jenni 2000). Clearly, experimental study of this complex issue is needed.

Metabolite levels can vary with time of day mainly as a function of a bird's feeding behavior. In day-feeding passerines, BUTY or GLYC may be elevated, and TRIG may be low in the morning following the overnight fast (Jenni and Jenni-Eiermann 1996; Jenni-Eiermann and Jenni 1991, 1997; Swain 1992; Gannes 1999). After feeding opportunities become available, metabolites reflecting fasting decrease, and TRIG increases rapidly, often stabilizing within a few hours of dawn (Jenni and Jenni-Eiermann 1996, but see Marsh 1983). Western Sandpipers are thought to feed mostly during daylight, and consistent with an overnight fast, GLYC and BUTY decreased through the day. In contrast, TRIG was unaffected by time of day, possibly due to two factors. First, we had few early morning captures, and may have missed a morning increase. Second, at each site we standardized our netting effort to catch at a similar stage of the tide each day, a factor which may have an overriding influence on feeding behavior. Foraging by forest dwelling passerines is linked with light availability, and one might expect a similar pattern of metabolic changes each day (Jenni and Jenni-Eiermann 1996). Western Sandpiper feeding is probably related to both light and tide height. Our data suggest that sandpipers recover from overnight fasting in a similar way each day, regardless of tide conditions, possibly by finding terrestrial or littoral invertebrates. Rapid fattening, and associated high TRIG, may be more dependent on tide and the availability of high-quality feeding habitat (mudflats).

Sex and age.—Sex was not generally an important factor determining circulating levels of lipid metabolites. Raw TRIG values appear to be 13–27% lower in males than females (Table 1), but some of that difference can probably be attributed to the smaller body size of males (Wilson 1994). Comparison is complicated by the fact that in males, TRIG generally did not depend on body mass, whereas in females the relationship was consistently positive. However, in the one case where the relationship was positive in both males and females (Sidney Island), there was no difference in mass-corrected TRIG, indicating that fat deposition rate was similar in the two sexes.

Bird age appeared to have little effect on lipid metabolite concentrations, except for BUTY during the premigration period, and for TRIG in fall migrant males at Boundary Bay. BUTY was lower in adult birds during premigration, but that can most likely be attributed to the fact that adults were undergoing fattening at that time, rather than to age per se (see below). Juvenile males had higher TRIG than adults at Boundary Bay, but that was not true of females. Thus, based on TRIG of feeding sandpipers, juveniles making their first migration appear to have similar fattening performance to adults.

Seasonal variation.—Plasma levels of metabolites related to lipid utilization (GLYC and BUTY) were relatively low throughout the year, and were similar to concentrations recorded in fed or short-term fasted birds (Jenni-Eiermann and Jenni 1991, Swain 1992, Gannes 1999). Stage by mass interactions were relatively common, making direct comparisons among some stages impossible. GLYC did not differ among seasons. BUTY was low at all stages, as might be seen in fed birds, but was significantly reduced in premigratory adults.

During February and March in Panama, adult sandpipers deposit fat, but at a much slower rate (0.09 g day^{-1}; P. D. O'Hara unpubl. data) than birds at migratory stopovers (0.4–1.0 g day^{-1}; Butler et al. 1997). How premigratory adults adjust their physiology or time and energy budgets during that period to allow for a net gain in body fat is unclear. Low BUTY could indicate reduced lipid use during premigration to spare stored lipids, as has been suggested in other studies (Suarez et al. 1990, Carpenter et al. 1993, Jenni and Jenni-Eiermann 1996, Jenni-Eiermann and Jenni 1996). BUTY influences protein metabolism (Robinson and Williamson 1980, Le Maho et al. 1981), and low BUTY could increase protein use from the diet, allowing ingested lipids to be stored more efficiently. A reduced contribution of lipid to energy metabolism could be responsible for the moderate rate of fat deposition (and lack of change in lean body mass; Guglielmo 1999) during premigration in Panama.

TRIG was the most informative of the lipid metabolites we measured. There was a pronounced seasonal difference in the relationship between TRIG and body mass that made interpretation difficult. However, the changes in concentration of this metabolite followed a pattern one might predict from expected changes in fattening rate through the year. TRIG was lowest, and was unrelated to body mass, during the winter when sandpipers were not depositing fat. TRIG increased during premigration, but contrary to our hypothesis, TRIG did not differ between juveniles and adults at that time. That may relate to the fact that birds were not caught during the height of feeding, or that rate of fattening in adults was too low to be detected. TRIG was highest, and was affected by body mass during spring and fall stopover when sandpipers increase body mass at a high rate (Lindström 1991, Butler et al. 1997). TRIG was higher during migration than during the breeding season in wild passerine species (Bairlein and Totzke 1992, Jenni and Jenni-Eiermann 1996), and TRIG cycled in relation to endogenous mass changes in captive Garden Warblers (Totzke and Bairlein 1998).

Landscape-level variation in stopover quality.—Several studies suggest that plasma metabolites can be used to assess the relative quality of stopover sites for refuelling migrants (Jenni-Eiermann and Jenni 1994, Jenni and Jenni-Eiermann 1996, Williams et al. 1999). Our results, and those of Schaub and Jenni (2001) demonstrate, in a natural situation, that birds at different stopover sites can have distinctly different metabolite profiles, indicating differences in fattening rates. The mudflat at Sidney Island is much smaller than Boundary Bay, and consists of a tidal lagoon bordered by forest and dune shrubs which provide excellent attack cover for hunting raptors. Sandpipers of three species (*Calidris mauri*, *C. minutilla*, and *C. pusilla*) consistently weigh less at Sidney Island (Lissimore et al. 1999). Ydenberg et al. (2002) recently explored potential explanations for the use of Sidney Island by sandpipers of low body mass, and proposed a trade-off hypothesis, whereby higher predation risk at Sidney Island is offset by better feeding conditions and more rapid fattening. At some point, predation risk, due to increased body mass (wing loading), outweighs the feeding benefits and birds leave Sidney Island. The TRIG data supported the prediction of the trade-off hypothesis that fattening rates are greater at Sidney Island than Boundary Bay (Ydenberg et al. 2002). Most programs for the conservation of migratory shorebirds (e.g. Western Hemisphere Shorebird Reserve Network, Ramsar Convention on Wetlands) focus on the protection of major stopover sites, such as Boundary Bay. Our results indicate that small, high-quality stopover sites also may be important, especially for individuals with low nutrient stores.

Plasma metabolite analysis offers unique insight into the dynamics of physiological state of wild birds, and should be considered a standard tool for the avian biologist. Future research should be directed at (1) demonstrating in the field that plasma metabolite levels correlate with the rate of mass gain estimated independently (Winker et al. 1992, Gannes 1999, Dunn 2000), and (2) the effects of diet nutrient composition on metabolite to mass change relationships.

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