

## SEASONAL, AGE, AND SEX-RELATED VARIATION IN FATTY-ACID COMPOSITION OF DEPOT FAT IN RELATION TO MIGRATION IN WESTERN SANDPIPERS

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**ABSTRACT.**—The composition of depot fat in Western Sandpipers (*Calidris mauri*) was dominated by C<sub>16</sub> and C<sub>18</sub> fatty acids (palmitate, 16:0; palmitoleate, 16:1; stearate, 18:0; oleate, 18:1), which together comprised 80 to 90% of all fatty acids analyzed. Significant seasonal variation occurred in the relative proportion of specific fatty acids and the ratio of saturated to unsaturated fatty acids in relation to the migratory cycle of the sandpipers. In adults of both sexes, the proportion of 16:0 and 18:0 fatty acids decreased from winter (December) to pre-migration (March) to spring migration (May), whereas the proportion of 16:1 and 18:1 fatty acids increased over these same periods. Consequently, total unsaturated fatty acids increased by 34% between the winter and premigratory phase, and by a further 22% between premigration and spring migration. Therefore, biochemical modification of adipose tissue in Western Sandpipers begins during the premigratory period in preparation for long-distance flight but continues as migration progresses, perhaps reflecting a training component of physiological modulation for migration. Juveniles showed similar changes to adults in the composition of their depot fat during the “pre-migratory” period, even though most juveniles do not undergo hyperphagia or rapid fattening at this wintering site. Potential prey species collected from a wintering site in Panama had a higher proportion of saturated fatty acids, less 16:1 and 18:1, and more 18:0 fatty acid compared with those from a migratory stopover site in British Columbia, paralleling the seasonal changes in depot fatty-acid composition observed in Western Sandpipers. However, the fact that Western Sandpipers show an increase in level of unsaturation and in the amounts of 16:1 and 18:1 fatty acids in their fat stores on the wintering ground prior to migration suggests that seasonal variation in fatty-acid composition is not entirely diet dependent. Received 27 August 1998, accepted 7 May 1999.

MIGRATORY BIRDS are known to vary not only the quantity of stored fat, but also the quality or composition of their fat as a possible adaptation for enhanced lipid utilization during long-distance flights (Caldwell 1973, Blem 1990). For example, in the Wood Thrush (*Hylocichla mustelina*), levels of the fatty acid oleate (18:1) increase prior to migration, resulting in an overall increase in the degree of unsaturation of lipid stores (Conway et al. 1994). Similarly, Mallards (*Anas platyrhynchos*) deposit increased amounts of oleate (18:1) during fattening prior to pairing and spring migration (Heitmeyer and Fredrickson 1990). Blem (1990) noted that in migrant passerines, the ratio of 18:1 to 18:2 (linoleate) generally was greater than one, whereas in nonmigratory species it was less than one. These studies suggest a pattern of decreasing proportions of essential fatty

acids (linoleate and linolenate) and replacement by fatty acids such as oleate, possibly of endogenous origin, in preparation for migration. Although high levels of oleate tend to be associated with migratory activity, this does not apply to all migrants. West and Meng (1968) reported an increase in linoleate and a decrease in palmitate (16:0) and oleate (18:1) from summer to the spring migration period in Willow Ptarmigan (*Lagopus lagopus*). Variation among species in fatty-acid composition might be due to differences in the fatty-acid composition of their foods (Blem 1976).

Consistent observations of increasing unsaturation of depot fat during migration have led to speculation on the functional advantage of specific fatty-acid profiles (see Blem 1976). Numerous studies have suggested that unsaturated fatty acids are utilized preferentially relative to saturated fatty acids, with a possible specific preference for monounsaturated fatty acids (Mougios et al. 1995, Raclot and Groscolas

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1995, Leyton et al. 1997). The fact that the depot fat of migratory birds exhibits higher levels of oleic acid (18:1) than does fat of nonmigrants, and generally is more unsaturated (Blem 1976), may be of adaptive significance for lipid utilization during sustained migratory flights.

Although many studies have reported on seasonal variation in fatty-acid composition of depot fat in relation to migration, most of them concerned passerines. Indeed, only one previous report has addressed variation in fatty-acid profiles for long-distance migrant shorebirds (i.e. Napolitano and Ackman 1990). In addition, few studies have examined differences among age and sex classes in fatty-acid composition during migration, or possible differences between spring (i.e. before reproduction) and fall (i.e. after reproduction) migration, when migratory activity is similar but environmental conditions may be very different.

In this paper, we report on changes in fatty-acid profiles of depot fat throughout the migratory cycle of the Western Sandpiper (*Calidris mauri*). In particular, we focus on age and sex differences, changes during the transition from winter to the premigration period, and comparison of spring and fall migrating birds. We also compare the fatty-acid composition of the birds' depot fat with that of their main prey items. Unlike many migratory passerines, Western Sandpipers do not appear to undergo marked shifts in diet among seasons (Wilson 1994), with the possible exception of the breeding period (see Discussion). In previous studies, diet shifts (e.g. from insects to high-fat diets of plant origin) have confounded the interpretation of dietary effects versus specific physiological modulation of fatty-acid composition relative to migratory activity.

#### METHODS

*Study animals.*—Nonmigrating (December 1995) and premigratory (March 1996) Western Sandpipers were collected by mist netting on the wintering grounds at Playa el Agallito, Panama (8°N, 79°W). We collected 15 adult females and 15 juvenile females in each sample period (no males were collected because of permit restrictions). Migrating Western Sandpipers were collected at Boundary Bay, British Columbia (49°N, 123°W), during May of 1995 and 1996 (15 males and 15 females in each sample period; first-year birds could not be distinguished from adults at this time) and during July 1996 (15 birds of each age and sex class [i.e. adult female, adult male, juvenile

female, juvenile male]). In all cases, birds were anesthetized by intramuscular injection with a 1:1 solution of ketamine-rompun (1  $\mu$ L per g body mass) and sacrificed by exsanguination. Samples of furcular fat, subcutaneous fat, and intraperitoneal fat were removed and stored at  $-20^{\circ}$ C until analysis. Capture and collection of birds was carried out under permits issued by Environment Canada, INRENARE (Panama), and Simon Fraser University's Animal Care Committee, following CCAC guidelines.

Samples of sandpiper prey were collected at the wintering site in Panama in March 1996 (i.e. premigration period) and at the fall migration staging area at Boundary Bay in July 1996. Samples were frozen in liquid nitrogen and subsequently analyzed for fatty-acid composition using the same methods as for depot fat of sandpipers (see below); in addition, we determined total fat content (%) using Soxhlet extraction (Dobush et al. 1985).

*Fatty-acid composition analysis.*—Ten mg of adipose tissue were sonicated in methanol, and the lipids were extracted by a modification of the procedure of Blich and Dyer (1959). Chloroform, methanol, and water (1:2:0.8 by volume) were added to the adipose tissue homogenate. The resulting single-phase mixture was allowed to stand for 10 min before addition of water and chloroform to result in a new proportion of chloroform:methanol:water of 2:2:1.8. Phase separation was achieved by slow-speed centrifugation (1,000  $\times$  g), and the chloroform fraction was evaporated under a stream of nitrogen. Lipids were reconstituted in a small amount of hexane and spotted onto silica gel G 60 thin-layer chromatography plates (Sigma-Aldich, Oakville, Canada) that were then developed in a solvent system of hexane:diethyl ether:acetic acid (60:40:1). The triglyceride spot (as identified by its  $R_f$ ) was removed and extracted with chloroform. After evaporation of the solvent, triglycerides were transmethylated overnight at  $50^{\circ}$ C with 100  $\mu$ L of toluene and 1 mL of 1% sulfuric acid in methanol. Methyl esters were then extracted with hexane after addition of 1 mL of water and concentrated under nitrogen. Methyl esters were separated by gas chromatography on a Hewlett-Packard 5890 gas chromatograph fitted with a flame ionization detector using a 30 m  $\times$  0.25 mm  $\times$  0.25 m HP-INNOWAX column (VWR-Canlab, Mississauga, Canada). Fatty acids were identified based on the retention times of their methyl esters as compared to fatty acid methyl ester standards (Sigma-Aldich).

*Statistical analysis.*—Chromatographic peaks from the gas chromatograph were integrated and the proportion of each fatty acid determined by dividing the area of its peak by the sum of the area of all identified peaks. Only the 11 most abundant fatty acids were considered. A multiple analysis of variance (MANOVA) was used to test for significant differences in fatty-acid composition among seasons. If the overall MANOVA was significant, the proportions of the

four most abundant fatty acids (16:0, 16:1, 18:0, 18:1) were compared among seasons using the general linear models procedure of SAS (1990). In addition, two indices of the degree of unsaturation were calculated: UNSAT and MONO. UNSAT is the sum of the quantities of all analyzed unsaturated fatty acids divided by the sum of the quantities of all analyzed fatty acids (as a proportion). MONO is the sum of the relative quantities of the two most common mono-unsaturated fatty acids (16:1 and 18:1) divided by the sum of the quantities of all analyzed fatty acids. MONO is therefore an index of the quantity of mono-unsaturated fatty acids stored in adipose tissue, whereas UNSAT is an index of total unsaturation of the fat depot. Seasonal variation in these indices, as well as age and sex effects, were also examined using the general linear models procedure of SAS (1990). If mass had a significant effect on the level of a fatty acid, MONO, or UNSAT, it was included as a variable in the model. Where age or sex effects were not significant in a given season, data was pooled across age and sex classes. We adjusted *P*-values for multiple comparisons by the Bonferroni method as appropriate (Rice 1989).

## RESULTS

*Fatty-acid composition.*—The depot fat of all Western Sandpipers was dominated by  $C_{16}$  and  $C_{18}$  fatty acids (Figs. 1A to D). The four most abundant fatty acids were palmitate (16:0), palmitoleate (16:1), stearate (18:0), and oleate (18:1), which together comprised 80 to 90% of all fatty acids analyzed. Moderate quantities of myristic acid (14:0), cis-eicosenoic acid (20:1), and behenic acid (22:0) were also identified, and together these fatty acids made up 10 to 14% of the fatty acids analyzed. Linoleic (18:2) and linolenic (18:3) acid were detected in smaller quantities (1 to 4%) and varied little throughout the year.  $C_{20}$  and  $C_{22}$  polyunsaturated fatty acids and fatty acids of chain length greater than 22 carbons occurred in very small quantities (<1%) and were not included in the analysis. Most of the seasonal variation in fatty-acid composition was due to fluctuations in the proportions of  $C_{16}$  and  $C_{18}$  saturated and mono-unsaturated fatty acids (Fig. 2).

*Seasonal variation in the most abundant fatty acids.*—Seasonal variation in all 11 analyzed fatty acids and in the four most common ones (16:0, 16:1, 18:0, 18:1) was highly significant (MANOVA, all *P*s < 0.001; Table 1, Fig. 2). None of the analyzed fatty acids varied significantly with body mass. Levels of palmitic acid (16:0) were

significantly higher during winter and fall migration than during premigration and spring migration ( $P < 0.05$ ). Stearic acid (18:0) levels were highest during winter, intermediate during premigration, and lowest during spring and fall migration ( $P < 0.05$ ). Palmitoleate (16:1) levels were lowest during winter, intermediate during premigration and spring migration, and highest during fall migration ( $P < 0.05$ ), whereas oleate (18:1) levels were highest during spring migration, slightly lower during fall migration, intermediate during premigration, and lowest during winter ( $P < 0.05$ ). In addition, levels of palmitic acid in spring were higher in 1995 than in 1996 ( $P < 0.05$ ), whereas levels of stearic acid and oleic acid were lower in 1995 than in 1996 ( $P < 0.05$  in both cases; Fig. 1C). The largest variations occurred in the  $C_{18}$  fatty acids stearate and oleate. Levels of stearate (18:0) ranged from 8.9% of all analyzed fatty acids in adult males during fall migration to 17.9% in adult females during winter. Levels of oleate (18:1) ranged from 18% during winter to 29.7% during spring migration.

*Seasonal variation in indices of unsaturation.*—Both MONO and UNSAT were correlated with body mass ( $P = 0.03$  and  $P = 0.02$ , respectively), so subsequent analyses of these variables were corrected for mass. UNSAT increased significantly from winter to premigration ( $P < 0.001$ ) and increased further from premigration to spring migration ( $P < 0.001$ ; Table 2). Total unsaturation was lower during fall migration than during spring migration in adults ( $P < 0.01$ ), but fall juveniles did not differ from spring adults ( $P > 0.05$ ; Table 2). Seasonal variation in MONO followed the same pattern (Table 2), with a stepwise increase in degree of monounsaturations from winter to premigration ( $P < 0.001$ ) and again to spring migration ( $P < 0.001$ ), whereas the degree of monounsaturations was reduced during fall migration in adults ( $P < 0.001$ ).

*Sex- and age-specific differences.*—The proportion of monounsaturated fatty acids was greater in females than in males during spring migration, although this effect was only marginally significant ( $P = 0.06$ ); the same trend was observed for unsaturated fatty acids ( $P = 0.07$ ). Of the four most abundant fatty acids, only one sex-specific difference was detected: palmitoleate levels were elevated in adult females com-

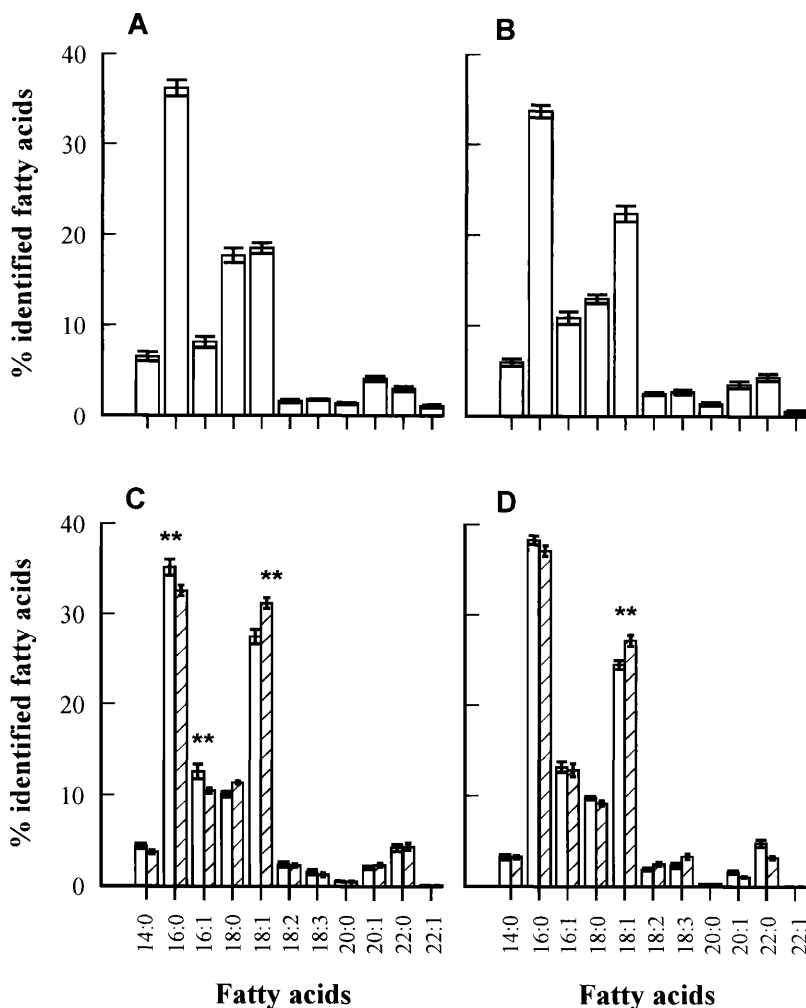


FIG. 1. Seasonal variation in fatty-acid profiles of depot fat in Western Sandpipers during (A) the wintering period (December); (B) premigration (March); (C) spring migration (May) in 1995 (open bars) and 1996 (hatched bars); and (D) fall migration (July) for adults (open bars) and juveniles (hatched bars). Values are percentages of all identified fatty acids  $\pm$  SE.

pared with adult males during spring migration ( $P < 0.05$ ).

Age-related differences in fatty-acid composition were detected during fall migration (cf. winter and premigration). MONO and UNSAT were higher in juveniles than adults during fall migration ( $P = 0.004$  and  $P < 0.001$ , respectively; Table 2). Juveniles in fall exhibited a degree of unsaturation of depot fat that was similar to that of adults during spring migration, whereas fall adults had reduced levels of monounsaturation and total unsaturation that were similar to those during premigration. Juveniles had a significantly higher proportion of

18:1 in fall than did adults ( $P < 0.05$ ), but no other fatty acids varied with age (Fig. 1).

*Fatty-acid composition of depot fat from different body regions.*—We detected no differences in the composition of fatty acids in furcular fat, subcutaneous fat, and intraperitoneal fat (Table 3).

*Fatty-acid composition of prey species.*—Fatty-acid composition of sandpiper prey varied markedly between the wintering site in Panama and the migratory staging area at Boundary Bay (Table 4). For example, the  $C_{18}$  fatty acid stearate (18:0) varied from 4 to 29%, and oleic acid (18:1) varied from 4 to 31%, among prey taxa. On average, prey collected at the fall mi-

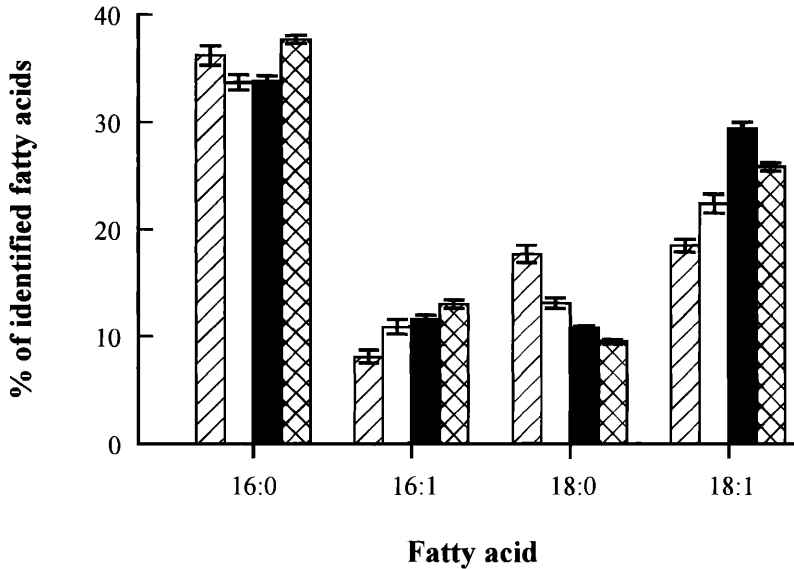


FIG. 2. Seasonal variation in the four most common fatty acids (16:0, 16:1, 18:0 and 18:1) in Western Sandpipers, for winter (hatched bars), premigration (open bars), spring migration (solid bars) and fall migration (crosshatched bars). Values are percentages of all identified fatty acids  $\pm$  SE.

gratory stopover site had a lower proportion of saturated fatty acids than at the wintering site (58 vs. 69%), and they contained more oleic acid (20 vs. 11%) and less stearic acid (6 vs. 15%). In general, seasonal differences in fatty-acid composition of prey paralleled those of the sandpipers' depot fat (Fig. 3). Total fat content of

prey was low ( $\bar{x}$  = 2.2%), being highest in *Uca* sp. (3.6%).

#### DISCUSSION

The overall fatty-acid profile of depot fat in Western Sandpipers is similar to that of most

TABLE 1. Seasonal variation in the relative proportion ( $\pm$ SE) of the four most abundant fatty acids in depot fat of Western Sandpipers. Data from 1995 and 1996 are pooled for spring migration ( $n$  = 30). Within columns, values with the same superscript are not significantly different ( $P$  > 0.05).

| Sex/age                 | Fatty acid                  |                              |                             |                              |
|-------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|
|                         | 16:0                        | 16:1                         | 18:0                        | 18:1                         |
| <b>Winter</b>           |                             |                              |                             |                              |
| Adult female            | 36.0 $\pm$ 1.1 <sup>A</sup> | 8.2 $\pm$ 0.8 <sup>A</sup>   | 17.9 $\pm$ 1.0 <sup>A</sup> | 18.0 $\pm$ 0.7 <sup>A</sup>  |
| Juvenile female         | 36.6 $\pm$ 1.9 <sup>A</sup> | 8.0 $\pm$ 0.9 <sup>A</sup>   | 17.3 $\pm$ 1.6 <sup>A</sup> | 19.3 $\pm$ 1.2 <sup>AB</sup> |
| <b>Premigration</b>     |                             |                              |                             |                              |
| Adult female            | 33.4 $\pm$ 0.9 <sup>B</sup> | 10.3 $\pm$ 2.0 <sup>AB</sup> | 12.6 $\pm$ 0.7 <sup>B</sup> | 23.5 $\pm$ 1.3 <sup>B</sup>  |
| Juvenile female         | 33.9 $\pm$ 1.1 <sup>B</sup> | 11.6 $\pm$ 1.1 <sup>B</sup>  | 13.5 $\pm$ 0.7 <sup>B</sup> | 21.3 $\pm$ 1.1 <sup>B</sup>  |
| <b>Spring migration</b> |                             |                              |                             |                              |
| Adult male              | 34.8 $\pm$ 0.7 <sup>B</sup> | 10.2 $\pm$ 0.5 <sup>A</sup>  | 10.9 $\pm$ 0.3 <sup>C</sup> | 29.7 $\pm$ 0.6 <sup>C</sup>  |
| Adult female            | 32.7 $\pm$ 0.7 <sup>B</sup> | 12.7 $\pm$ 0.6 <sup>B</sup>  | 10.8 $\pm$ 0.3 <sup>C</sup> | 29.5 $\pm$ 1.0 <sup>C</sup>  |
| <b>Fall migration</b>   |                             |                              |                             |                              |
| Adult male              | 38.3 $\pm$ 0.7 <sup>A</sup> | 13.6 $\pm$ 0.7 <sup>B</sup>  | 10.0 $\pm$ 0.3 <sup>C</sup> | 24.5 $\pm$ 0.6 <sup>B</sup>  |
| Juvenile male           | 36.7 $\pm$ 0.9 <sup>A</sup> | 14.3 $\pm$ 1.0 <sup>B</sup>  | 8.9 $\pm$ 0.4 <sup>C</sup>  | 26.5 $\pm$ 0.9 <sup>C</sup>  |
| Adult female            | 38.3 $\pm$ 0.5 <sup>A</sup> | 12.7 $\pm$ 0.9 <sup>B</sup>  | 9.7 $\pm$ 0.3 <sup>C</sup>  | 24.6 $\pm$ 0.8 <sup>B</sup>  |
| Juvenile female         | 37.4 $\pm$ 0.9 <sup>A</sup> | 11.5 $\pm$ 0.7 <sup>B</sup>  | 9.5 $\pm$ 0.3 <sup>C</sup>  | 27.9 $\pm$ 0.9 <sup>C</sup>  |

TABLE 2. Seasonal variation in the proportion ( $\pm$ SE) of major monounsaturated fatty acids and unsaturated fatty acids in the depot fat of Western Sandpipers. See text for details of indices of fatty-acid composition.

| Age                                | Winter     | Pre-migration | Spring migration | Fall migration |
|------------------------------------|------------|---------------|------------------|----------------|
| <b>Monounsaturated fatty acids</b> |            |               |                  |                |
| Adult                              | 26 $\pm$ 1 | 34 $\pm$ 1    | 41 $\pm$ 1       | 37 $\pm$ 1     |
| Juvenile                           | 27 $\pm$ 1 | 32 $\pm$ 1    | —                | 40 $\pm$ 1     |
| <b>Unsaturated fatty acids</b>     |            |               |                  |                |
| Adult                              | 35 $\pm$ 1 | 43 $\pm$ 1    | 47 $\pm$ 1       | 43 $\pm$ 1     |
| Juvenile                           | 34 $\pm$ 2 | 42 $\pm$ 1    | —                | 47 $\pm$ 1     |

TABLE 3. Fatty-acid composition of depot fat of Western Sandpipers in three body regions. Values are percentages  $\pm$ SE.

| Fatty acid | Location of depot fat |                |                |
|------------|-----------------------|----------------|----------------|
|            | Furcular              | Subcutaneous   | Peritoneal     |
| 14:0       | 4.2 $\pm$ 0.5         | 5.6 $\pm$ 0.7  | 5.2 $\pm$ 0.8  |
| 16:0       | 35.7 $\pm$ 1.7        | 36.9 $\pm$ 1.9 | 35.1 $\pm$ 1.3 |
| 16:1       | 11.9 $\pm$ 1.8        | 10.5 $\pm$ 1.6 | 12.0 $\pm$ 1.9 |
| 18:0       | 8.3 $\pm$ 0.6         | 9.0 $\pm$ 1.1  | 9.5 $\pm$ 1.0  |
| 18:1       | 30.2 $\pm$ 1.2        | 28.0 $\pm$ 1.7 | 30.0 $\pm$ 1.7 |
| 18:2       | 2.4 $\pm$ 0.4         | 1.8 $\pm$ 0.3  | 2.0 $\pm$ 0.5  |
| 18:3       | 0.6 $\pm$ 0.1         | 0.4 $\pm$ 0.1  | 0.6 $\pm$ 0.2  |
| 20:0       | 0.5 $\pm$ 0.1         | 0.6 $\pm$ 0.2  | 0.4 $\pm$ 0.1  |
| 20:1       | 2.3 $\pm$ 0.7         | 1.7 $\pm$ 0.4  | 1.7 $\pm$ 0.5  |
| 22:0       | 3.9 $\pm$ 0.7         | 5.3 $\pm$ 1.6  | 3.3 $\pm$ 1.8  |
| 22:1       | 0                     | 0.1 $\pm$ 0.05 | 0.1 $\pm$ 0.05 |

other vertebrate species (e.g. Palokangas and Vihko 1972, Caldwell 1973, Blem 1976); 16 and 18 carbon fatty acids comprise the majority of all lipids, and longer-chain and polyunsaturated fatty acids occur in relatively smaller proportions. However, the fatty-acid composition of migratory Western Sandpipers differs somewhat from that of migratory passerines (Blem 1976) in that sandpipers have much lower levels of the essential fatty acids linoleate (18:2) and linolenate (18:3). Most likely, this is directly related to differences in diet, because passerines often consume plants or seeds containing high levels of essential fatty acids during migration (Bower and Helms 1969, Morton and Liebman

1974, Conway et al. 1994), whereas Western Sandpipers do not (Wilson 1994). As a result, the unsaturation index is higher in migratory passerines (1.85; calculated from Blem 1976) than in Western Sandpipers (0.84). Fatty-acid composition in Western Sandpipers is similar to that reported for the closely related Semipalmated Sandpiper (*Calidris pusilla*; Napolitano and Ackman 1990). Both species exhibit low proportions of polyunsaturated fatty acids and high proportions of oleic and stearic acids characteristic of carnivorous and insectivorous species.

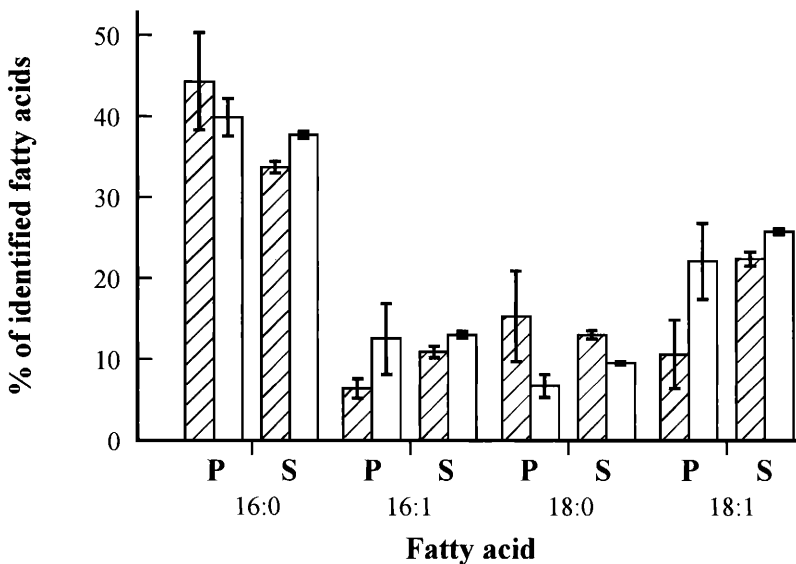


FIG. 3. Comparison of the fatty-acid composition of Western Sandpiper (S) depot fat with that of their prey (P) for the four most common fatty acids (16:0, 16:1, 18:0 and 18:1) during premigration (hatched bars) and fall migration (open bars). Values are percentages of all identified fatty acids  $\pm$  SE.

TABLE 4. Fatty-acid composition of selected potential prey species collected from the Western Sandpiper's wintering ground (Panama, March 1996) and a migratory stopover site (Boundary Bay, British Columbia, July 1996). Values are percentages of analyzed fatty acids.

| Prey                                   | Fatty acid |      |      |      |      |      |      |      |      |      |      |
|--|------------|------|------|------|------|------|------|------|------|------|------|
|  | 14:0       | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 22:0 | 22:1 |
| <b>Wintering ground (premigration)</b> |            |      |      |      |      |      |      |      |      |      |      |
| <i>Amphioxus</i>                       | 4.1        | 48.3 | 5.9  | 6.0  | 5.2  | 18.2 | 5.0  | 0.4  | 0.6  | 1.2  | 5.1  |
| Goby sp.                               | 8.7        | 32.5 | 3.6  | 22.4 | 10.2 | 10.7 | 3.3  | 1.1  | 2.3  | 0.0  | 5.4  |
| Shrimp                                 | 7.7        | 36.5 | 5.2  | 12.8 | 24.4 | 2.4  | 1.6  | 3.1  | 3.1  | 1.4  | 1.7  |
| <i>Uca</i> sp.                         | 8.6        | 62.6 | 8.8  | 3.9  | 3.5  | 6.1  | 2.6  | 0.2  | 0.0  | 0.6  | 3.1  |
| Crabs                                  | 11.8       | 43.3 | 8.6  | 17.7 | 10.5 | 1.3  | 3.9  | 0.6  | 1.0  | 0.3  | 1.1  |
| Polychaetes                            | 4.0        | 42.8 | 6.4  | 29.1 | 9.8  | 1.3  | 1.0  | 1.0  | 3.2  | 0.4  | 1.1  |
| Average                                | 7.5        | 44.3 | 6.4  | 15.3 | 10.6 | 6.7  | 2.9  | 1.0  | 1.7  | 0.6  | 2.9  |
| <b>Migration stopover (fall)</b>       |            |      |      |      |      |      |      |      |      |      |      |
| <i>Corophium</i>                       | 7.3        | 42.3 | 4.0  | 6.4  | 31.1 | 1.7  | 1.9  | 0.5  | 2.2  | 2.5  | 0.2  |
| Oligochaetes                           | 5.9        | 35.3 | 14.9 | 9.2  | 20.3 | 3.4  | 1.2  | 0.4  | 2.2  | 5.0  | 2.3  |
| Polychaetes                            | 9.9        | 42.1 | 18.8 | 4.4  | 15.0 | 1.8  | 0.6  | 0.1  | 5.8  | 1.6  | 0.1  |
| Average                                | 8.3        | 40.4 | 14.1 | 6.1  | 20.3 | 2.2  | 1.1  | 0.3  | 4.0  | 2.6  | 0.7  |

The lack of a relationship between body mass and fatty-acid composition observed in our study was somewhat surprising. During rapid fattening and mass gain on a low-fat diet (as in the case for Western Sandpipers; Table 4), an increase of endogenously synthesized fatty acids accompanied by the dilution of essential fatty acids would be expected (Napolitano and Ackman 1990). However, in Western Sandpipers the increase in the proportion of monounsaturated fatty acids is independent of body mass, suggesting that seasonal variation of fatty-acid composition is indeed related to time of year or migratory state per se, and is not simply an artifact of rapid fattening.

Western Sandpipers showed significant seasonal variation in the degree of unsaturation of depot fat. Total unsaturation in adults increased by 34% from winter to spring migration, and was 22% higher during the fall migration than in winter. This was mainly due to changes in the proportions of 16:1 and 18:1 fatty acids, which were higher during migration, and their saturated counterparts (16:0 and 18:0). Rate of utilization of fatty acids depends on (1) rate of mobilization from adipose tissue, (2) transport in the blood, (3) rate of uptake by flight muscles, and (4) rate of oxidation within cells. The degree of unsaturation of fatty acids has been shown to influence several of these steps, at least in mammals. Exercise induces a larger increase in the monounsaturated fatty acid oleate (18:1) than other saturated fatty acids in the plasma of dogs (McClelland et al.

1995) and humans (Mougios et al. 1995). In addition, Raclot and Groscolas (1995) demonstrated that the relative mobilization rate of fatty acids *in vitro* increased with the degree of unsaturation and decreased with increasing chain length; mobilization of fatty acids is in the order of 16:1 > 16:0 > 18:1 > 18:0 (Raclot and Groscolas 1993).

The degree of unsaturation has also been shown to affect the rate of oxidation of fatty acids. Oleic acid (18:1) is oxidized more rapidly than other fatty acids in the rat (Leyton et al. 1997), and muscle cells of an antarctic fish (*Gobionotothen gibberifrons*) show a clear preference for the catabolism of monounsaturated fatty acids (Sidell et al. 1995). Whether unsaturated fatty acids are also more easily transported from the circulation into muscle cells, compared with saturated fatty acids, is not known. However, unsaturated fatty acids have a higher solubility in water (Vorum et al. 1992), which would be predicted to allow for a higher concentration of unsaturated free fatty acids in the blood and result in a higher concentration gradient of unsaturated fatty acids into muscle cells. Thus, because monounsaturated fatty acids have been shown to be oxidized preferentially, and possibly are more readily mobilized, it would clearly be beneficial to increase the proportion of monounsaturated fatty acids during periods of high-level aerobic performance. This is consistent with the high levels of monounsaturated fatty acids, primarily oleate (18:1), found in other migratory bird species (Blem

1976) and suggests that a specific fatty-acid composition provides a functional advantage to enhance lipid utilization during migratory flight.

*Intraspecific variation in fatty-acid composition of adipose tissue.*—In the Western Sandpiper, the proportion of monounsaturated fatty acids increased by 58% from winter to spring migration, but premigratory levels of monounsaturated fatty acids were only 31% higher and were intermediate between levels during winter and spring. This indicates that as Western Sandpipers prepare for migration, the level of monounsaturated fatty acids in adipose tissue is gradually increased, but peak levels are not attained until the migratory phase. Thus, our study suggests that biochemical modification of adipose tissue begins during the premigratory period in preparation for long-distance flight and continues as migration progresses. This perhaps reflects a "training" component of physiological adaptation for migration related to, and perhaps caused by, the increased level of flight activity. It is also of interest that juvenile Western Sandpipers showed a similar increase in the proportion of monounsaturated fatty acids during the premigratory period, because juveniles do not undergo premigratory fattening at this site, and most are not thought to migrate north to the breeding grounds in their first year (P. O'Hara pers. comm.).

Adult Western Sandpipers had a significantly lower proportion of monounsaturated fatty acids during fall migration than during spring migration, but juveniles exhibited a level of unsaturation similar to that of spring migratory adults. Age differences in fatty-acid composition of birds have not been identified before, and the increased unsaturation in juvenile Western Sandpipers in fall, compared with adults, was unexpected. Whether this difference is due to altered lipid metabolism in young birds is unknown. Both age classes use terrestrial habitats (tundra) during the breeding season and consume significant amounts of larval dipterans (Holmes 1972). However, adults and pre fledging juveniles quickly move to tidal mudflats adjacent to breeding areas, and they also stage on intertidal habitats prior to fall migration at least one month before reaching the Fraser River delta (B. Sandercock pers. comm.). Thus, even though adults migrate approximately one month earlier than ju-

veniles (Wilson 1994), diet differences seem unlikely to explain the age difference in fatty-acid profiles because both adults and juveniles use intertidal habitats extensively prior to fall migration. One obvious difference between adults and juveniles is the rapid development phase that juveniles complete just prior to migration. Western Sandpiper chicks hatch during the first week in June and are approximately two months old when embarking on their first fall migration (Wilson 1994). This requires extremely rapid growth and development of all physiological systems, which perhaps gives rise to ontogenetic effects on lipid biosynthesis and/or utilization (Ricklefs et al. 1998).

We also detected a significant sex difference in the proportion of monounsaturated fatty acids in Western Sandpipers during spring migration. Females had more unsaturated adipose tissue, and the proportion of monounsaturated fatty acids was 11% higher in females than in males. Similar sex differences in fatty-acid composition in relation to reproduction also have been noted in Canada Geese (*Branta canadensis*), where breeding females had significantly higher levels of monounsaturated fatty acids than did males (Thomas and George 1975). The fact that this difference is observed only during the spring migration (immediately prior to reproduction) in Western Sandpipers suggests that this phenomenon is linked to egg production. Western Sandpipers begin egg laying approximately 10 days after arrival on the breeding grounds (Holmes 1972, Wilson 1994), suggesting that females must arrive in adequate physiological condition to commence egg development immediately. Increased quality of depot fat may be necessary for the mobilization of endogenous lipids and deposition into the developing eggs. Preliminary evidence in poultry suggests a link between monounsaturation of lipid stores and reproductive success. Dietary supplementation of turkey hens with oil containing large quantities of oleic acid resulted in increased proportions of this fatty acid in yolk lipids, increased embryo viability, and increased hatchability of fertile eggs (Vilchez et al. 1990). Thus, the increased monounsaturation of depot fat may impart a higher degree of unsaturation to egg lipids and increase chances of survival of the developing embryo. Because male Western Sandpipers arrive on the breeding grounds earlier than females (Senner et al.



1981) and do not have the physiological demands of egg production, they may not require enhanced unsaturation of endogenous reserves observed in females.

*Fatty-acid composition and diet.*—Western Sandpipers exhibit seasonal changes in fatty-acid composition that are similar to those observed in other migratory birds. Studies on Wood Thrushes, Dark-eyed Juncos (*Junco hyemalis*), and Mallards, for example, report an increase in the degree of unsaturation of adipose lipids (primarily due to an increase in oleate) in relation to migration (Bower and Helms 1969, Heitmeyer and Fredrickson 1990, Conway et al. 1994). This variation in fatty-acid composition usually has been attributed to changes in diet, a notion that has been confirmed with free-living (Conway et al. 1994) and captive passerines (Morton and Liebman 1974).

In our study, prey species collected from the Western Sandpiper's wintering site (Panama) had a higher proportion of saturated fatty acids than did prey from a migratory stopover site (Boundary Bay). In addition, these prey species contained less 18:1 and more 18:0 fatty acid at the southerly wintering site. These differences in degree of saturation and fatty-acid composition of prey therefore paralleled, and might partly explain, the seasonal changes in depot fatty-acid composition observed in Western Sandpipers. Nevertheless, even on the wintering grounds, Western Sandpipers showed an increase in level of unsaturation and in the amounts of 16:1 and 18:1 fatty acids in their fat stores.

Bairlein (1996) suggested that migratory birds select different food types to promote accumulation of fat of increased unsaturation. Diet selection based on fatty-acid composition has been demonstrated in mammalian hibernators, which tend to prefer foods rich in polyunsaturated fatty acids (Frank 1994, Bozinovic and Mendez 1997). Nothing is known about diet selection in Western Sandpipers, but given the large differences in fatty-acid composition of potential prey items, particularly in Panama, this clearly warrants further investigation. Even if dietary fatty-acid composition partly determines the bird's fatty-acid composition, because total lipid content of the prey species of sandpipers is so low (1 to 4%), endogenous physiological factors are likely to play an important role in determining fatty-acid compo-

sition of depot lipids. Napolitano and Ackman (1990) compared the fatty-acid composition of Semipalmated Sandpipers with that of their main prey item (*Corophium volutator*) and reached a similar conclusion. Although some fatty acids of *Corophium* were detected in the birds' lipids, most depot lipids seemed to be derived from endogenous synthesis of fatty acids, resulting in the birds having a fatty-acid profile that was significantly different from that of their diet. Similarly, in Western Sandpipers provided with synthetic diets with variable fatty-acid composition, not all fatty acids were deposited equally into their adipose tissue (O. Egeler unpubl. data). Therefore, at least in these migratory shorebirds, seasonal variation in fatty-acid composition of depot fat associated with migration does not appear to be caused solely by changes in diet.

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