LANDSCAPE PHYSIOLOGY: PLASMA METABOLITES, FATTENING RATES AND HABITAT QUALITY IN MIGRATORY WESTERN SANDPIPERS

by

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Landscape physiology: Plasma metabolites, fattening rates and habitat quality in migratory Western Sandpipers

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ABSTRACT

Migratory shorebirds depend on coastal mudflats to feed at stopover sites between flights on migration. It is important to have a reliable technique to measure and compare the habitat quality of migratory stopover sites. One of the primary determining factors underlying an individual's migration success is its ability to refuel at stopover sites. Therefore the best measure of habitat quality in this system is the capacity of stopover sites to support high rates of fattening. Testing for correlations between metabolite levels and site characteristics may provide information regarding which factors determine habitat quality in this system. There were differences in several site characteristics between nine sites within the Georgia Basin/Puget Sound migratory stopover region during northward and southward migrations. Furthermore, there was significant inter-site variation in plasma triglyceride concentrations in the migratory Western Sandpipers (*Calidris mauri*) using these sites. Inter-site variation in triglyceride levels was higher during northward than southward migration. There was within-site consistency between years in site characteristics and triglyceride levels. During northward migration, there was a correlation between triglyceride levels and total macrofaunal prey abundance. In captive birds, metabolite levels reflect mass cycle phase. Glycerol and uric acid levels are significantly related to the rate of mass change. This study demonstrated effects of diet composition on metabolite levels. Future studies on metabolites in free-living systems should consider prey quality. Other areas of research needed to further our understanding of habitat quality of migratory stopover sites for Western Sandpipers are identified.

DEDICATION

To those who believed I could do it and guided me along the way. To the cider, salsa, and yemisir wot that kept me going while I did it. To the sandpipers who made it possible and may they benefit from the work within.

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IN THE BEGINNING...

There has been suggestion in the literature that shorebird populations may be in decline on a global scale (Hitchcock and Gratto-Trevor 1997, Gratto-Trevor et al. 1998, Howe et al. 1989), although data are incomplete for many species, including the Western Sandpiper. Many shorebirds rely on migratory stopover sites, consisting primarily of coastal mudflats, during migration (Bildstein et al. 1991). However, migratory shorebirds must contend with declines in habitat quantity and quality (Senner and Howe 1984, Meyers et al. 1987). Coastal habitats are under constant development pressure and have been under global decline in the past few decades (Dahl 1990, Bildstein et al. 1991). Given the species' dependence on coastal mudflats during migration, there is an *a priori* reason to expect population declines if stopover habitat losses are left unchecked. Accordingly, it is important to measure habitat quality of these migratory stopover sites for shorebirds and to understand the factors that determine habitat quality.

With recent population estimates of 3.5 million (Bishop et al. 2000, Morrison et al. 2001), the Western Sandpiper is one of the most abundant shorebirds migrating along the Pacific coast (Page et al. 1999) and the Georgia Basin/Puget Sound (GB/PS) region (Evenson and Buchanan 1997). The Western Sandpiper is an ideal focal species for local and international conservation strategies and has been identified as a key indicator species for monitoring the health of these marine habitats. As such, determining which habitat types and locations are appropriate for conservation and evaluation of the effectiveness of protecting estuaries in maintaining population targets of Western Sandpipers (and thereby other shorebirds) are identified as key requirements in the most recent Shorebird Conservation Plan of the Canadian Wildlife Service (unpublished report 2003).

Habitat quality is the value or utility of a particular habitat or set of habitats to a particular species or group of species. Land management and conservation recommendations are often based on the assessment and understanding of habitat quality for the species of interest (e.g. Sherry & Holmes 1996, Root 1998, Smith et al.

1998, Roloff et al. 1999, Prenda et al. 2001); this requires an ability to measure habitat quality for the particular system of interest (e.g. Pausas et al. 1995, Doan et al. 1997, Luck 2002). Habitat quality may be determined by multiple ecological, physical, and behavioral factors such as site size, substrate, predation risk, and prey related factors, and identifying and assessing the factors determining habitat quality in a particular system is important (Sutherland 1996). However, the ultimate expression of that habitat quality is found in the condition of the individuals using the site; various parameters of individual condition and population dynamics have proven useful in the measurement of habitat quality (e.g. Morin et al. 1992, Burke et al. 1993, Mason et al. 1995, Belanger and Rodriguez 2002). Within the context of this study, habitat quality of migratory stopover sites for the migrating Western Sandpiper is defined as the ability of the stopover site to contribute to the individual and population's successful migration. One of the primary factors determining migration success in shorebirds is the ability to replenish reserves at stopover sites (Alerstam and Lindstrom 1990). Therefore, habitat quality in this system is defined as the ability of migratory stopover sites to support hyperphagia and high rates of mass gain or fattening, and the most direct way to actually measure the habitat quality in this system is to measure the fattening rates achieved by Western Sandpipers at migratory stopover sites. Plasma metabolites have been shown to be useful indicators of fattening rates in both passerine and shorebird migratory systems (Jenni-Eiermann and Jenni 1994, Williams, et al. 1999, Schaub and Jenni 2001, Guglielmo et al. 2002).

The primary goal of this study was to examine the efficacy of using plasma metabolite concentrations to detect landscape-scale site differences in habitat quality of migratory stopover sites used by the Western Sandpiper. Inter-site variation in physical and biological factors as well as plasma metabolites was tested at nine migratory stopover sites during northward and southward migration within the GB/PS region. The robustness of this technique, particularly the effect of diet composition on metabolite levels, was examined in captive birds.

Study species: Western Sandpiper

The Western Sandpiper (*Calidris mauri*) is a small calidrid shorebird (~25g) that breeds in the arctic and migrates to its nonbreeding grounds, which range from southern California to Peru (Wilson 1994, Figure 1.1). This species is sexually dimorphic, with females larger than males in overall body size (5%) and females having disproportionately longer bills (12%) (Cartar 1984), with culmen length less than 24.2 and greater than 24.8 mm for males and females, respectively (Page and Fearis 1974). The majority of the population migrates along the Pacific Flyway, stopping along the way at stopover sites, mostly coastal mudflats, where they feed in order to replenish their reserves before continuing their long flight (Warnock and Bishop 1998). Mean length of stay for northbound radiomarked individuals using the GB/PS is 2.2 ± 1.2 days (Warnock and Bishop 1998, based on birds relocated at the Fraser River Delta). Residency times of southbound individuals were estimated to be 1-5 days, based on maximum likelihood analysis of resightings of color-marked individuals at Sidney Island, a small site within GB/PS (Butler et al. 1987). There are four major stopover regions between the northern extreme of the overwintering range and the breeding grounds (Warnock and Bishop 1998, Figure 1.1). Each of these stopover regions is comprised of many microsites (stopover sites). This thesis will focus on variation between migratory stopover sites within the GB/PS (Figure 1.1 inset), a migratory stopover area spanning Washington State and British Columbia.

Western Sandpipers spend the majority of the year on the nonbreeding grounds (Wilson 1994). This species exhibits latitudinal segregation between the sexes in their nonbreeding distribution, such that higher proportions of males and females are found at the northern and southern extremes of the nonbreeding range, respectively (Nebel et al. 2002). Adults migrate northward from the overwintering grounds to the breeding grounds and pass through GB/PS between April 20 and 10 May, with males arriving disproportionately earlier than females (Butler et al. 1987). Both second-year and after second year birds occur during this migration stage and can be distinguished by plumage characteristics (Prater et al.1977). Individuals breed within a small geographical area in the arctic (Wilson 1994). Adults depart the breeding grounds to migrate south and pass through GB/PS throughout July, and juveniles depart a month later and pass through GB/PS throughout August (Butler et al. 1987). Because adults

and juvenile migrations are temporally segregated, it is difficult to distinguish potential age and time effects. To avoid this problem as well as to provide an additional independent test of site differences, southward adult and southward juvenile migrations were treated separately. All data were analyzed separately for each of the three migration stages (northward, southward adult and southward juvenile), thus confining comparisons to those sites sampled concurrently.

During migration, Western Sandpipers feed on several taxa of marine invertebrates (see review in Wolf 2001) occurring on exposed intertidal mudflats. The birds are pushed by the rising tide into cove-like areas that retain the last exposed mud. It is in these areas on a rising or falling tide that migrating Western Sandpipers were observed and captured. Because the objective of this thesis is the comparison between stopover sites, the definition of what constitutes a stopover site is critical. A stopover site, in general, refers to a coastal mudflat used by migratory shorebirds, the borders of which are defined by either bird use or a physical barrier that must be crossed to reach the next site. The area within a stopover site can be further subdivided into macrosite and microsites. The macrosite is the entire stopover site, physically defined as the area encompassing from the shoreline out to the zero tide line. Microsites are areas within the macrosite where the birds concentrate to forage during a rising or falling tide (generally within 500m of the shore). Sampling (including blood sampling, behavioral observations, mud core samples and penetrability readings) occurred within a particular microsite. Because of the tide-driven movements of birds between low-tide and high-tide microhabitats within a stopover site, as evidenced by temporal observations as well as contents of fecal samples (see Results Chapter 2), it is clear that shorebird use is not restricted to any particular microsite, but rather the birds use the larger macrosite throughout the course of the tidal cycle. It should be noted, however, that the invertebrate prey base and the behavioral observations exclusively reflect the sampled microsites. While the plasma metabolite concentrations of foraging individuals will reflect food consumption throughout the tide cycle, experimental design was careful to sample individuals during a lengthy feeding bout in the sampled microsite; therefore, plasma metabolite concentrations primarily reflect those occurring at the sampled microsite. Implications and importance of microhabitats within the sampling microsite and the stopover site as a whole will be discussed further in the thesis.

Metabolite assays

The assay used in this study to measure plasma triglyceride and glycerol concentrations is a color endpoint assay (Sigma-Aldrich, Ontario, Canada) consisting of two consecutive reactions on the same plasma sample. During the first reaction, the plasma sample is exposed to Reagent A which reacts with glycerol in a series of stepwise enzymatic reactions to produce a bluish-purple dye. The increase in absorbance due to this color reaction is proportional to the concentration of circulating free glycerol in the plasma sample. During the second reaction, the same plasma sample is exposed to Reagent B which consists of lipoprotein lipase, which cleaves triglycerides, in addition to the ingredients of Reagent A, which generate the same stepwise enzymatic reaction producing a bluish-purple pigment proportional to the concentration of total glycerol in the sample. The concentration of glycerol in the sample at this point, therefore, includes both the originally free (unbound) glycerol as well as the glycerol previously bound in triglyceride. The absorbance of the sample after the first and second reactions is proportional to the free glycerol concentration and the plasma total glycerol concentration of the sample, respectively (Figure 1.3). The concentration of plasma triglycerides, as measured by previously bound glycerol molecules, is the difference between the concentrations of total (Figure 1.3b) and free (Figure 1.3a) glycerol. An understanding of the glycerol-based nature of this assay and the method for calculation of triglyceride concentration based on glycerol concentrations is important for accurate interpretation of triglyceride data and is discussed further in this thesis (See Discussion, Chapter 3).

The assay used in this study to measure plasma uric acid concentration is a color endpoint assay (Wako USA, Richmond, Virginia). The plasma sample is exposed to a single reagent the ingredients of which oxidize the uric acid and cause a color reaction that produces a bluish-purple pigment. The magnitude of the color reaction depends on the amount of hydrogen peroxide produced by the oxidation of uric acid. Therefore, the increase in absorbance due to the color reaction is proportional to the concentration of uric acid in the plasma sample.

Physiological significance of plasma metabolites

Plasma is the fluid responsible for transportation of compounds necessary for ordinary maintenance in the body. The nutrient reserves of an organism are in a constant equilibrium of utilization (catabolism) and generation (anabolism), and this flux is exaggerated in migratory shorebirds by alternating bouts of feasting and fasting (Lindstrom et al. 1999). Anabolism requires compounds to be transported to the site of the generation, and catabolism produces by-products that must either be disposed of or distributed to the appropriate destination; these by-products and precursor molecules are transported through the plasma. Therefore, the plasma concentration of particular metabolites can provide information about the occurrence and magnitude of particular metabolic reactions.

Lipid metabolism occurs in the body with the single objective of converting fat into energy (Guyton and Hall 1996), making the interpretation of lipid metabolite concentrations straightforward (Jenni-Eiermann & Jenni 1998, Guglielmo 1999). Lipids are either absorbed from the diet and incorporated into lipoproteins in the small intestine or are synthesized from fatty acids and incorporated into lipoproteins in the liver (Ramenofsky 1990). During fat deposition, triglyceride molecules, in the form of these lipoproteins, are transported via the plasma to the adipose tissue where they are deposited. Therefore, plasma levels of triglyceride will be elevated during increased fat deposition and should, therefore, correlate with mass gain. During lipolysis (fatty acid mobilization), one glycerol molecule and three fatty acids are released from adipose tissue for each triglyceride molecule that is cleaved. The glycerol is carried through the plasma to either be utilized or discarded. Therefore, plasma levels of glycerol will be elevated during fat utilization and should correlate to mass loss. The biochemical pathways of fat deposition and fat utilization are shown in Figure 1.2.

Fat deposition rates have long been recognized as having an important influence of migration ecology and the resulting departure mass from stopover sites (Alerstam and Henderstrom 1998, Alerstam and Lindstrom 1990). More recently, it has been recognized that shorebirds also burn and restore muscle tissue during migration and stopover, respectively (Jenni and Jenni-Eiermann 1998, Karasov and Pinshow 1998, Piersma et al. 1999). Therefore, a measure of protein metabolism during

stopover is of potential interest. Uric acid is a nitrogenous waste and can, therefore, be used as a measure of protein metabolism. However, unlike lipid metabolism, protein metabolism occurs in the body for several reasons, and uric acid is, therefore, less straightforward to interpret. Proteins, like lipids, have energetic uses in the body but, unlike lipids, also have functional and structural uses. High levels of protein metabolism, as indicated by high levels of uric acid, are not necessarily indicative of protein being catabolized for energy. Jenni-Eiermann and Jenni (1994) have demonstrated that plasma levels of uric acid can be either positively or negatively correlated to physiological condition, depending on whether the origin of the amino acids is diet or tissue, respectively. The interpretation of plasma uric acid levels is further complicated by the effect of dehydration and/or exercise, both of which can reasonably occur during migration, on kidney function (Guglielmo 1999). While incorporating protein metabolism into the assessment of an individual's migration condition is attractive, these factors make the interpretation of uric acid in free-living individuals difficult because an individual with high uric acid could either be ingesting large quantities of amino acids from the diet (and would also be expected to be fattening) or burning muscle for energy (and would also be expected to be burning fat). Furthermore, it is not possible to know the degree of dehydration or exercise an individual is experiencing. These factors are less problematic with captive birds that are given *ad libitum* water and have restricted flight. Furthermore, during the mass loss-gain cycles (see Chapter 4), the gross physiological condition of the individual (feasting or fasting) is known which facilitates the interpretation of the cause of elevated uric acid levels. Therefore, plasma uric acid levels may prove useful to assess protein metabolism in captive individuals. For these reasons, uric acid results will be presented for captive (Chapter 4) but not free-living (Chapter 3) individuals.

Metabolites as an index of habitat quality

Many studies have employed size-corrected body mass in an effort to isolate the portion of body mass due to energetic reserves (instead of structural size) and, as such, serve as an index of body condition in order to answer a wide variety of ecological questions (see reviews in Blem 1990, Brown 1996, Green 2001). Green

(2001) argues that current techniques violate several statistical assumptions and, thus, may generate errors. Merila et al. (2001) further calls in to question the assumption that size-corrected body mass reflects an individual's nongenetic nutritional state and suggests that it may be more genetically influenced than previously thought. Regardless of these cautions, researchers continue to employ size-corrected body mass as an index of condition (e.g. Dubiec & Cichon 2001, Ardia 2002, Lens et al. 2002, Tyeraa & Christensen 2002, Soler et al. 2003). Within the Western Sandpiper system, size-corrected body mass does positively correlate to amount of body fat (Guglielmo et al. 2002), suggesting that size-corrected body mass is indeed a reasonable index for body reserves. Nevertheless, it is a static measure of body reserves and, as such, provides only a single snapshot of physiological condition. For example, if two individuals that arrived to a mudflat weighing 20g and 30g both weighed 25g four days later at the time of capture, the two birds would be in drastically different physiological states, mass loss versus mass gain, respectively; however, mass data at the time of capture would not reflect this difference. Thus, a technique, such as plasma metabolite analysis, which serves as an index for the amount of and direction of mass change (gain or loss) may provide more useful and accurate information that reflects the physiological state of individuals at migratory stopover sites. For a migratory bird, such as the Western Sandpiper, successful completion of migration is a key component to its overall survival. Current migration theory suggests that the ability to accumulate fat reserves during migratory stopover is a primary factor determining migration success (Alerstam and Lindstrom 1990). Therefore, an indication of the degree of fattening achieved by individuals at stopover sites could allow for comparison between stopover sites and could provide imperative information on the utility of these sites to the migratory birds using them.

Study areas – migratory stopover sites

Within the GB/PS migratory stopover region, a total of nine migratory stopover sites (Figure 1.1 inset) were sampled during various combinations of migration stage during 2001 and 2002 (Table 1.1). These sites span 300km (straight line distance between the two farthest sites) and approximately two degrees of latitude. These sites are

within a single migratory stopover region and, together, represent a small portion (~3%) of the species' entire range (60° spread in latitude). These nine sites differ markedly in physical characteristics, bird use, and ecological factors. The site characteristics of each microsite, summarized in Table 1.1, are detailed below. With the exception of Boundary Bay and Robert's Bank, which served as reference sites, the sites are grouped according to the migration stages in which they were sampled, and within each stage are listed in order from north to south as they are presented in Figure 1.1.

Reference sites

Both reference sites are located within the expansive Fraser River Delta which, supports daily peak numbers between 100,000 and 500,000 Western Sandpipers (Butler 1994). The two reference sites are 10km apart and are separated by several beaches (Butler et al. 2002). Therefore, these two sites might be considered part of a single, larger, Fraser River Delta macrosite. These two sites, however, are comprised of markedly different substrates, physical topography, and invertebrate communities (Chapter 2 this volume). Furthermore, Butler et al. (2002) demonstrated that once Western Sandpipers settle within the Fraser River Delta, they tend to stay on that beach or the adjacent beach throughout their stay in the delta; while that study is limited in inference to northward migration, there is no evidence of large between-site movements. Due to the marked differences in site characteristics and high site fidelity, Boundary Bay and Robert's Bank are considered two separate macrosites throughout this study.

Boundary Bay (49°10'N, 123°05') is an expansive (60 km^2) intertidal sandy mudflat within the Fraser River Delta that is located 6km southeast of Ladner, BC. The sampled microsite encompasses the near-shore areas between 88th and 96th Streets along the dike which follows the edge of Boundary Bay. This microsite encompasses the mouth of the dike outflow , which causes at least a portion of the surrounding mud to be consistently covered with up to 5cm of standing water. Cover, defined as objects that obstruct the view of foraging sandpipers that can be potentially used by predators for surprise attacks, exists in the form of a marsh, consisting of several dispersed hummocks, which stretches about 100m from the dike. These hummocks flood under a

3m tide, which occurs at least once and usually twice daily. Physical properties of this site as well as the spatial distribution of invertebrates at this site are detailed by Sewell and Elner (2001). The proximate land is residential and a golf course, and the surrounding land is agricultural. Boundary Bay is used by Western Sandpipers during all three migration stages, although the density of bird use on any given day is higher during northward migration (Butler 1994). During 2001 and 2002, flocks along the tideline within visual proximity of the sampling microsite at Boundary Bay approached 100,000 during peak northward migration. Use of this site during southward migration is continuous throughout July (adults) and August (juveniles), with fewer birds at the site each day. Standardized count data is not available for the Fraser River Delta during southward migration, but daily numbers for this site in 2001 and 2002 peaked between 5000 and 10,000 during southward migration. This is consistent with the finding that, in the Fraser River Delta, there are 10- to 15-fold less individuals during any given day during southward than northward migration (Butler 1994). Boundary Bay was sampled during all three migration stages in both years 2001 and 2002 and served as the reference site to which all other sites were compared.

Robert's Bank (49° 03'N; 123°09'W) is a large (27 km^2) mudflat within the southern Strait of Georgia along the edge of the Fraser River Delta and is located 6km southwest of Ladner, BC. This site, in contrast to Boundary Bay, is largely estuarine in nature and the substrate is predominantly silty clay (Sutherland et al. 2000). The microsite that was sampled is in the high intertidal zone between Brunswick Point, a salt marsh, and Vancouver Port Corporation's causeway. There is minimal vegetation on the mudflat, and the most proximal cover is the dike which borders the site along the entire length of the site. Bird use for this site during sampling was approximately the same as that reported for Boundary Bay. Robert's Bank was sampled during all three migration stages in 2002 and served as a reference site, in addition to Boundary Bay, in that year.

Northward migration

Jensen Access (48°20' 122°27'W) is a WDFW-owned wildlife use area consisting of marsh and mudflat on the northern side of Skagit Bay, 10km west of

Conway, WA. Skagit Bay is a large bay that becomes an expansive (59 km^2) intertidal mudflat at low tide. The sampled microsite runs between two private property lines from the dike out to the bay and consists of 0.2 km^2 of marsh and mudflat. This site has been diked and sprayed for *Spartina anglica* (an invasive exotic grass species) in the past, and the surrounding land is residential and agricultural. This site is fed by freshwater from nearby Brown Slough. There is a gradient of substrates from silty clay near the dike to sandy flats 250m from the dike. There is a marsh of vegetation-topped hummocks which starts 50m from the dike and extends for approximately 200m; this marsh is the first and last mud to be exposed on a falling and rising tide, respectively. The predominant vegetation includes American threesquare. The top of each hummock is vegetated but the sides of the hummocks are exposed. Western Sandpipers were observed feeding in the mud between the hummocks as well as feeding on the side of the hummocks themselves. Skagit Bay, as a macrosite, is reported to be used by 10,000 birds during northward migration (Evanson and Buchanan 1997). During northward migration in 2001 and 2002, Western Sandpipers used this site in mixed flocks with Dunlin, and the numbers of Western Sandpipers foraging at the microsite (the marsh and the border of the sandflats) peaked at 300. This site was sampled during northward migration in both 2001 and 2002.

Jensen Access is a particularly interesting example of the dynamic nature of migratory stopover sites along the Pacific Flyway and the importance of microhabitat classification. The intention was to sample several stopover microsites during each of the three migration stages. This design proved to be impossible due to use of the sites by Western Sandpipers, and the resulting incomplete design was adopted out of necessity. Jensen Access is the most extreme example of differential use and is due to changing microhabitats within the microsite between stages. During southward migration, the hummock marsh, interspersed by exposed mud during northward migration, had grown in completely and was a thick 200m long marsh. Small sections of mud bordered by American Threesquare remain at the edge of the marsh, but exposed mud was restricted primarily to the firmer sandflats at the end of the marsh. The hummocks were densely covered with several species of plants, and there was no exposed mud between the hummocks. Throughout Skagit Bay, there are many sites that experience decreased surface area of mud exposure due to increased vegetation growth during the summer months. During southward adult migration (July) of 2001,

the expansive mudflat to the east of this site, at the mouth of Brown Slough, was being used by approximately 5000 individuals; however, the only birds observed foraging at the microsite that had been used during northward migration were Least Sandpipers, and they were beyond the marsh on the sandflats. Due to the essential nonexistence of this microsite during southward migration, a different site within Skagit Bay (English Boom) was located and sampled during July of both 2001 and 2002.

Jensen Access also serves as an example of the potential for microhabitat dynamics to influence bird use and site characteristics on an annual basis. In addition to the seasonal differences (northward versus southward) in the vegetation density of Jensen Access, the microsite was more densely vegetated in 2002 than 2001. Microhabitat use by Western Sandpipers responded to this vegetation shift in a similar fashion, although to a lesser extreme, in that individuals that visited the microsite foraged primarily on the sandflats along the edge of the marsh and only entered the hummock marsh to feed on one occasion, and it was still relatively close to the edge of the marsh where the hummocks are less dense. Birds only foraged on the sandflats once the tide came up fairly close to the marsh edge, making the window of catching opportunity very narrow. These changes in bird use of the microsite, as dictated by habitat shifts, highlight the importance of microhabitat definition within microsites.

Totten Inlet (47° 06'N; 123°04'W) is an inland mudflat that makes up part of the Kennedy Creek Delta, in the south Puget Sound, 10 km south of Shelton, WA, near Olympia. It consists of a small (ca. 3ha; Buchanan 1988) salt marsh peninsula that jets out into Totten Inlet and is fed by both Kennedy and Schneider Creeks. The sampled microsite consists of the unvegetated mudflats (1 km^2) flanking the sides of the salt marsh and the flyway on the northern tip of the peninsula that birds crossed to move between mudflats on either side. The macrosite includes the mud that stretches out the inlet to the zero tide line, but is still a small (3 km^2) mudflat. The salt marsh, which has a one-meter sharp drop off to the mud flats, as well as the three islands in the inlet, are densely covered in salt marsh vegetation such as *Salicornia*, Cinquefoil, etc., and flood at least once and often twice daily. This site has never been diked and is in pristine condition. The substrate is predominantly fine silt. Obstructive cover is present in the form of the salt marsh and islands as well as forested strips on the western edge of the inlet. The small mudflat between these barriers is about 100 meters wide. Peak

daily counts of Western Sandpipers using Totten Inlet during northward migration as high as 6000 have been reported (Buchanan 1988), though recent counts suggest that numbers during northward peak between 1000 and 2000 (Evenson and Buchanan 1997; J. Buchanan, pers. comm.) During southward migration, this site is used primarily by juveniles; peak numbers are less than 100 for adults (July) and approximately 1000 for juveniles (August) (Buchanan 1988, Evenson and Buchanan 1997, J. Buchanan, pers. comm.). This site was sampled during northward migration in both years.

Southward adult migration

Alice Bay (48°43'N; 122°29'W), owned by Samish Bay Sports Club, is a sheltered inlet off of Samish Bay. Alice Bay is separated from Padilla Bay by Samish Island and is located 3km west of Edison, WA. The tide cuts a deep channel through the inlet dividing the mudflat into two portions, and the sampled microsite is the eastern portion of mudflat between the channel and the road, extending for several hundred meters from the gate out towards Samish Bay, and consisting of 0.1 km^2. This protected inlet feeds out into a more open mudflat (14 km^2) that extends to the zero tide line in Samish Bay. The microsite consists of dispersed marsh throughout the mudflat and is bordered by a short forest edge. The marsh vegetation is primarily *Spartina anglica* and this site has been sprayed to control this invasive species. The spray is washed off by the next incoming tide and did not seem to affect shorebird use of the site except for the human disturbance caused during the spraying. The substrate is silty mud with black oxidative layers. This site is used consistently during southward migration, and numbers during southward adult migration in 2002, when it was sampled, peaked at 500. Peak counts of 500 and 100 individuals have been reported in the past for this site during northward and southward juvenile migrations, respectively (G. Bletsch, unpubl. data). This site is also used by Least Sandpipers, although in fewer numbers (G. Bletsch, unpubl. data).

English Boom (48°16'N, 122°26'W) is an Island County park on the southern shore of Skagit Bay on Camano Island, 3 km northeast of Utsalady, WA. This microsite shares the same macrosite (Skagit Bay) as Jensen Access (see above). This microsite

was located when it became apparent that Jensen Access, as a microsite, does not exist during southward migration due to vegetation filling in the site (see above). The sampled microsite (2 km^2) stretches from the parking lot along the beach over to the second cove, and out from the shore to the mussel bed. The shore is comprised of a few meters of sandy beach along with layers of pebbles and *Fucus*, which dries out during low tide, interspersed with mud along the shore. One meter out from the shore, the mud becomes silty and has moderately dense *Spartina anglica* cover within 5m of shore and is covered with *Ulva* and *Enteromorpha,* the primary vegetation on the mudflat. This algae cover extends approximately one kilometer where it hits a mussel bed and transitions to sandflat. The site is comprised of 500m of beach and three coves, which are the first and last sites of mud during falling and rising tides, respectively. The sampled microsite consists of the western most cove and the mudflat extending out from the cove and beach before reaching the mussel bed. The microsite supports salt marsh vegetation, such as *Triglochin meridima*, *Spirgularia*, and *Salicornia* along the shore. Upland grassy vegetation extends for 100m to the forest edge. Western Sandpipers have been observed feeding along the shore among the rocks and *Fucus*, in the muddy sections close to shore, on the algae-covered mud, and in the mussel beds. They have even been observed feeding in the small creek that feeds into the cove, a freshwater source for this site. There is no local knowledge of Western Sandpipers using this microsite during northward migration. In both 2001 and 2002, during southward adult migration, when it was sampled, the peak count at the sampled microsite was 500. It is not known if this microsite is used by juveniles (August).

Southward juvenile migration

Doug Banks' Flats (49° 07'N; 125°53'W), is a medium-sized intertidal mudflat located 15 km southeast of Tofino, BC, and is part of the larger Tofino mudflats (16 km^2 exposed at low tide) extending through Browning Passage (Butler et al. 1992). The sampled microsite consisted of a small stretch (0.1 km^2) of mudflat extending from the salt marsh, densely vegetated with *Salicornia*, to large forested island. The substrate is silty mud from the marsh shore out to the rock islands where it transitions into sandflats covered with *Ulva*. The microsite is flanked on all sides by a tall forest edge and the

salt marsh is 55m at its widest point. The Tofino mudflats are consistently used by shorebirds in both northward and southward migration and daily peak numbers near 20,000 are reported for both migration stages (Butler et al. 1992). This site was sampled during southward juvenile migration (August) in 2002. Shorebirds used the mudflats visible from this microsite in flocks that peaked at 5000, and Western Sandpipers foraged at the capture site in mixed flocks (with Least Sandpipers and Sanderlings) that peaked at 500.

Sidney Island (48°30'N, 123°20'W) is located in the southern Strait of Georgia, approximately 35 km southwest of Boundary Bay. The microsite includes the small (1 km^2) mudflat surrounding the island out to the zero tide line. The sampled microsite is Calidris Bay, a very small (0.04 km^2) protected mudflat enclosed by a lagoon with a very narrow opening to the Strait. This protected site is proximally bordered by 100m of salt marsh, which grades into 150m of upland shrubs which grades into forest. The substrate is sandy and is densely covered with *Enteromorpha* and *Ulva*. The surrounding salt marsh is predominantly vegetated with *Salicornia*. This site is not known to be used by Western Sandpipers during northward migration. It was historically used during southward migration by both adults (July) and juveniles (August), peaking at 1000 birds (M. Lemon and R. Butler pers. comm.). Use of this site by this species, however, has declined over recent years and is presently thought to be used rarely by adults. This site was sampled during the southward juvenile migration of 2001, during which, the peak count was 350.

False Bay (48°29'N; 123°04'W), owned by the University of Washington, is a sheltered bay that opens into the Straight of San Juan de Fuci on the southwest side of San Juan Island, and is located 6km southwest of Friday Harbor, Washington. It consists of a small (3.5 km^2) mudflat bordered by steep banks the tops of which are forested. The bay consists of four habitats according to surface vegetation and invertebrate taxa: eelgrass/chaetopterid sandbar, *Ulva*/turebillid sandflat, arenicolid sandbar, and *Ulva*/mud shrimp mudflat, starting at the mouth of the bay and working clockwise. Bird use was observed in all four zones, however it was concentrated in and, therefore, catching efforts were focused in the two latter zones. The substrate ranges from fine sand to silty mud but is generally compact and shallow. Large portions of the bay are covered by *Ulva*. Historically, False Bay has been used by

Western Sandpipers during southward migration (July and August) on the order of 200 individuals on peak days (K. O'Reilly unpubl. data). The peak usage observed by Western Sandpipers during July, 2001, was 25 individuals. This site was sampled during southward juvenile migration in 2002, when shorebird numbers peaked at 200. In both adult and juvenile migrations, the Westerns occurred in mixed flocks with Least Sandpipers.

Summary of thesis chapters

This thesis consists of three research chapters that address the primary objectives of this thesis project: 1) test for inter-site variation in ecological and physical factors of multiple migratory stopover sites and foraging behavior of Western Sandpipers using these sites, 2) determine if site differences in fattening rates, as indicated by plasma metabolite concentrations, in the Western Sandpiper at different migratory stopover sites can be detected on a landscape scale, 3) test for correlations between fattening rate and ecological and physical site characteristics on the site level, and 4) determine the effects of captivity, mass trajectory and diet on metabolite values. Each chapter is formatted as a separate manuscript, with an abstract and literature cite section.

Chapter two explores the ecological and physical factors of various migratory stopover sites and the foraging behavior of the Western Sandpipers using the sites, and quantifies the variation between sites. Physical characteristics such as site size and mud penetrability, ecological factors such as prey availability (abundance and taxa representation), prey consumption (as evidence by fecal samples), and behavioral factors such as foraging mode (surface versus deep foraging) and rate are compared between sites.

Chapter three explores the potential utility of plasma metabolite concentration as an index for fattening rates in free-living Western Sandpiper, which can be used to measure and compare habitat quality of different migratory stopover sites. The strong relationship between triglyceride and glycerol concentrations and mass change has been shown in several captive systems. Previous work by this group has also detected a difference in metabolites between two sites (Boundary Bay and Sidney Island). This

chapter tests the robustness of this technique in its ability to detect differences in habitat quality between multiple sites. Correlations will be used to evaluate what proportion of inter-site variation in fattening rates is explained by variation in an ecological factor (prey abundance) and a physical factor (opportunity to forage). Tony D. Williams, Christopher G. Guglielmo, and Robert W. Elner will be co-authors on the submitted version of this manuscript.

Chapter four explores the effects of captivity on metabolite concentrations in Western Sandpipers by comparing levels in birds at the time of capture to levels obtained in the same birds in captivity. We evaluate the effect of mass cycle phase (mass loss, mass gain, and stable mass) on plasma metabolite concentrations. We also examine the effect of diet (low-fat versus high-fat) on metabolite levels during this mass cycle phase. Previous work by our group demonstrated a relationship between rate of mass change and metabolite levels; we further test this relationship on the two different diets in a random-order design controlling for time effects. Tony D. Williams and Christopher G. Guglielmo will be co-authors on the submitted version of this paper.

Chapter five is the concluding chapter that includes a general synthesis of the preceding chapters and suggests directions of future research. This chapter highlights and summarizes the key points illuminated in the thesis. It uses information from all of the data chapters to suggest and discuss seasonal differences in metabolite levels, the factors determining in fattening rates, the feasibility of predicting the rate of mass change from metabolite levels, and the conservation and management implications of this work.

FIGURE LEGENDS

Figure 1.1 Distribution map for the Western Sandpiper, illustrating the breeding (light gray) and nonbreeding (dark gray) ranges for this species. The four major migratory stopover regions along the Pacific Flyway migratory route are indicated by arrows. Inset shows detail of the GB/PS migratory stopover region indicating the nine stopover sites within this region at which Western Sandpipers and underlying ecological factors were sampled. Sites are, from north to south: 1) Doug Banks, 2) Robert's Bank, 3) Boundary Bay, 4) Sidney Island, 5) Alice Bay, 6) False Bay, 7) Jensen Access, 8) English Boom, and 9) Totten Inlet.

Figure 1.2. The avian biochemical pathways of lipid anabolism and catabolism resulting in transport of triglyceride and glycerol, respectively, in the plasma. During fat deposition, triglycerides are transported in the plasma (a), and glycerol is transported through the plasma during fat utilization (b). The plasma concentrations of these molecules are measured and provide information on the physiological condition of the individual.

Figure 1.3 The biochemical reactions involved in the color endpoint assay used to quantify the plasma concentration of free glycerol (a) and total glycerol (b). The plasma concentration of triglycerides is total glycerol (b) minus free glycerol (a).

Table 1.1. Location, tidal source, site type, freshwater input, and migration stage-year sampled for nine migratory stopover sites used by Western Sandpipers within the Georgia Basin/Puget Sound area. Sites have been classified as either protected inlet (PI), protected bay (PB), or open mudflat (OM). Catching seasons are defined by migration stage (northward (N), southward adult (SA), southward juvenile (SJ)) and year (2001 (01), 2002 (02)).

Site	Location	Lat (N)	Long (W)	Tidal Source	Type	Inflow	N01	SA01	SJ01	N02	SA02	SJ02
Boundary Bay	Ladner, BC	49°04'	122°58'	Str. of Georgia	OM	Dyke	X	X	X	X	X	X
Robert's Bank	Ladner, BC	49°03'	123°08'	Str. of Georgia	OM	River				X	X	X
Jensen Access	Conway, WA	48°20'	122°26'	Skagit Bay	OM	Slough	X			X		
Totten Inlet	Shelton, WA	47°06'	123°05'	Puget Sound	PI	Creek	X			X		
Alice Bay	Edison, WA	48°33'	122°29'	Samish Bay	PI	None		X			X	
English Boom	Utsalady, WA	48°16'	122°26'	Skagit Bay	OM	Creek		X			X	
Doug Banks	Tofino, BC	49°07'	125°53'	Browning Passage	OM	None						X
Sidney Island	Sidney Island, BC	48°37'	123°26'	Str. of Georgia	PB	None			X			
False Bay	San Juan Isl, WA	48°29'	123°04'	Str. of Juan de Fuca	PB	Creek						X

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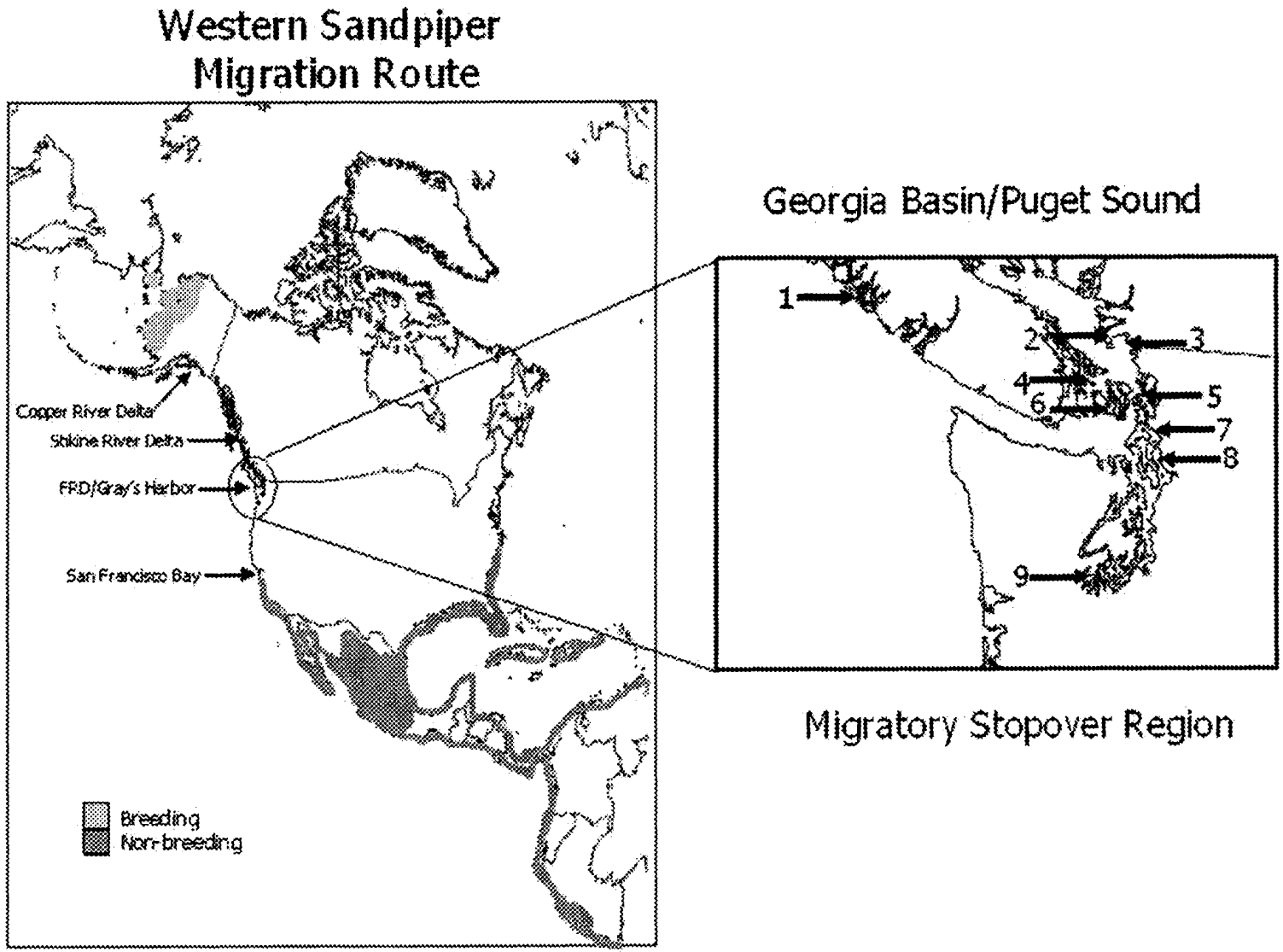
Western Sandpiper
Migration Route
Copper River Delta
Skagit River Delta
FRD/Gray's Harbor
San Francisco Bay
Breeding
Non-breeding
Georgia Basin/Puget Sound
1
2
3
4
5
6
7
8
9
Migratory Stopover Region

Figure 1.1

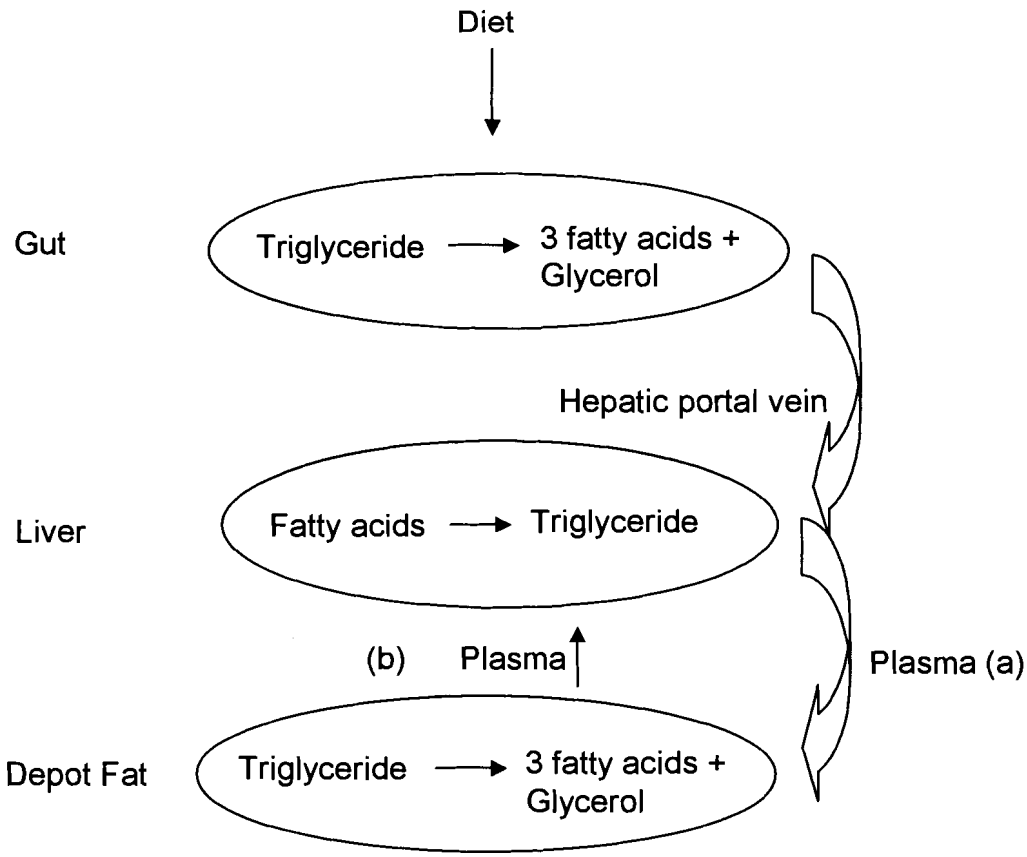
Diet
Gut
Triglyceride
3 fatty acids +
Glycerol
Hepatic portal vein
Liver
Fatty acids
Triglyceride
(b)
Plasma
Plasma (a)
Depot Fat
Triglyceride
3 fatty acids +
Glycerol

Figure 1.2

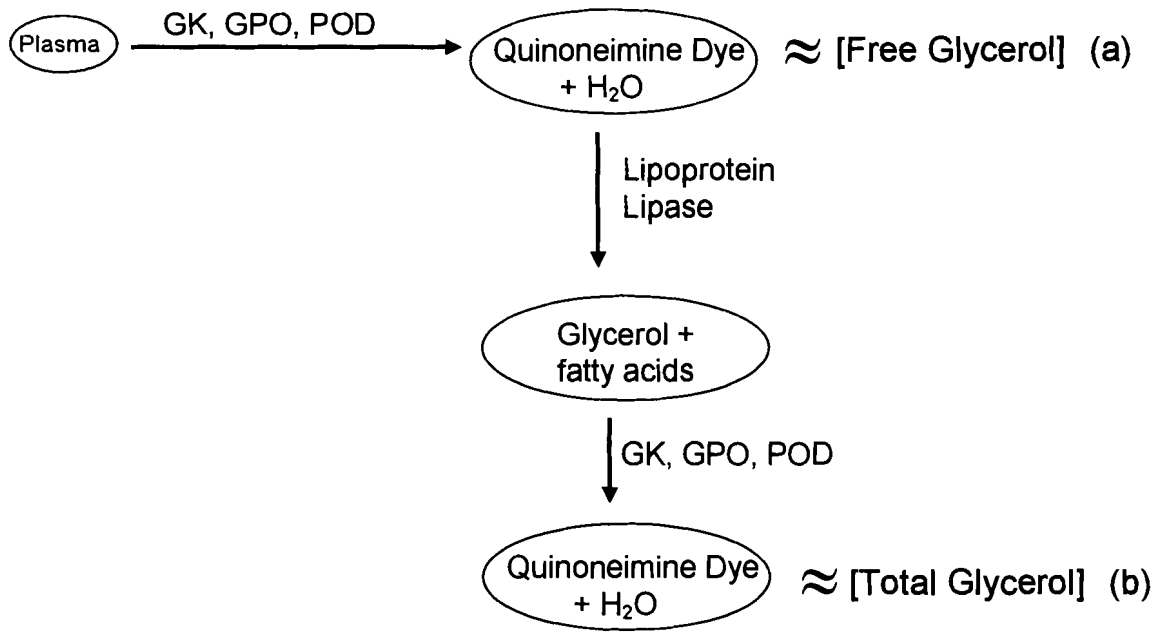
Plasma
GK, GPO, POD
Quinoneimine Dye
+ H_2O
≈ [Free Glycerol] (a)
Lipoprotein
Lipase
Glycerol +
fatty acids
GK, GPO, POD
Quinoneimine Dye
+ H_2O
≈ [Total Glycerol] (b)

Figure 1.3

CHAPTER TWO

INTER-SITE VARIATION IN BIOLOGICAL AND PHYSICAL SITE CHARACTERISTICS AND FORAGING BEHAVIOR OF WESTERN SANDPIPERS USING MIGRATORY STOPOVER SITES

ABSTRACT

During their migratory journey to and from their breeding grounds, Western Sandpipers stop at coastal mudflats along the Pacific Flyway where they feed to replenish their reserves. The capacity of these migratory stopover sites to support hyperphagia in shorebirds is influenced by biological and physical factors such as prey abundance, substrate, and mudflat exposure, and may be reflected in the foraging behavior of the birds using them. The aim of this study is to explore inter-site variability in biological and physical parameters of nine stopover sites within the Georgia Basin/Puget Sound region and in the foraging behavior of Western Sandpipers using these sites during migration. Site differences were detected in multiple factors during all three migration stages. Prey abundance was consistent within sites between years, but foraging mode, foraging rate and sediment penetrability varied among years. A stopover site sampled in all three stages had more prey and softer sediment during northward than southward migrations. In addition, during northward migration, the birds using that site made more foraging attempts per minute and a larger proportion of these attempts consisted of surface feeding modes than during southward migration. We have demonstrated that there is large variation in multiple parameters between migratory stopover sites. Given the high variability of factors that influence habitat quality, we predict that fattening rates achieved by birds using these sites will also be variable (tested in Chapter 3).

INTRODUCTION

There is evidence that plasma metabolite concentrations serve as indicators of fattening rate in migratory birds (Jenni-Eiermann and Jenni 1994, Williams et al. 1999). Furthermore, it has been suggested that fattening rate, as indicated by metabolite levels, may serve as an index of habitat quality of migratory stopover sites (Jenni-Eiermann and Jenni 1998, Williams et al. 1999). Site differences in fattening rates, as indicated by metabolite levels, have been detected in Western Sandpipers on a small scale (Guglielmo et al. 2002) and in passerines on a landscape scale (Schaub and Jenni 2001). During migration, Western Sandpipers use several different migratory stopover sites within the Georgia Basin/Puget Sound (GB/PS) migratory stopover region. Prior to comparing fattening rates between these sites, it is useful to understand if and how these migratory stopover sites differ in prey-related factors and physical factors, and how the foraging behavior of the birds using these sites varies. In this paper, we identify, quantify and compare biological and physical characteristics of stopover sites and the foraging behavior of migratory Western Sandpipers using these sites within GB/PS.

Habitat quality, defined in this system as the capacity of sites to support fattening by Western Sandpipers during stopover (see Chapter 1), may be determined by multiple biological and physical factors (Sutherland 1996). The fattening potential for an individual is likely to be related to the prey available, both in quantity and quality, to that individual at that site. Several studies have demonstrated significant correlations between individual condition, measured by various means, and prey availability (e.g. Shine and Madsen 1997, Thompson et al. 1997, Van Sluvs 1998, Strong and Sherry 2000, Haug et al. 2002, Pinaud and Weimerskirch 2002). During migration, Western Sandpipers forage on a variety of epi- and infaunal invertebrates (Wilson 1994). Zwarts et al. (1990) demonstrated that 40% of the invertebrates eaten by shorebirds pass through a 0.6mm sieve. To address this issue, Sutherland et al. (2000) quantified available prey of two different size classes, meiofauna (63 μm < x < 500 μm) and macrofauna (>500 μm), and demonstrated that Western Sandpipers consume meiofaunal as well as macrofaunal

invertebrates. Prey items representing 17 distinct taxa from 4 phyla have been reported to be consumed by Western Sandpiper during migration (Wolf 2001), demonstrating that this species relies on a large variety of food types to generate and replenish its energy stores during migration. Therefore, the diversity, in addition to abundance, of prey taxa present at a site may impact selection of and/or performance by Western Sandpipers at migratory stopover sites. Here we test for site differences in abundance and diversity of both meiofaunal and macrofaunal prey bases between multiple migratory stopover sites used by Western Sandpipers.

Foraging behavior may influence the transition from prey items in the sediment to fat on the bird causing birds at two different sites with equivalent prey communities to achieve different levels of fat deposition. Foraging selectivity (differential targeting of certain taxa) has been demonstrated in the Western Sandpiper (Sewell 1996, Sutherland et al. 2000, Wolf 2001), and this selectivity has been shown to differ between at least two sites (Wolf 2001). Furthermore, different prey taxa may have different energetic content such that the particular taxa composition of ingested prey may affect the overall fat deposition by the bird. Therefore, differential selectivity between sites may result in differential fattening in spite of similar prey availability. Differences between sites in selectivity may be generated by differential preference of the prey taxa *per se* (i.e. what is eaten) or via differentially preferred foraging modes that target distinct taxa (i.e. how it is eaten). Examining prey consumption via fecal analysis has been successfully used to address the former (Wolf 2001), and foraging mode observations have been used to address the latter (Sutherland et al. 2000). Foraging rate is a similarly important factor as it necessarily affects energetic intake rate. Differences between sites in foraging rates may, therefore, influence the fattening rates achieved at different stopover sites. Here we test for inter-site variation in both prey consumption, as indicated by fecal analysis, and foraging behavior.

The final achievable fat deposition at a particular site may be influenced by several physical factors such as substrate, site size, and mudflat exposure that affect the accessibility of the "available" prey regime to the birds. Sediment penetrability has been demonstrated to correlate to foraging mode (Wolf 2001) and logically influences the accessibility of vertically stratified prey items. Site size, and its association with actual and perceived predation risk, has been offered as an

explanation for differences in individual and group behavior and performance between sites (Ydenberg et al. 2002). While the size of a site influences the amount of mud available for foraging, the tidal cycle at a site determines how many hours of the day the sediment is exposed, allowing sandpipers to forage. The total amount of mud km-hours available during a given day to forage, based on the tide cycle, could influence the ability of individuals to fatten. Here we describe and compare physical characteristics of each migratory stopover site.

In this paper, we assess biological and physical parameters, on a landscape scale, for a total of nine different sites, sampled over two years in the Georgia Basin/Puget Sound area of British Columbia, Canada, and Washington, USA, part of the Pacific Northwest flyway, used by migrating Western Sandpipers. The specific objectives of the study were to a)evaluate the inter-site variation in biological and physical factors between migratory stopover sites and b)determine whether these factors are consistent within sites across years and migration stages.

MATERIALS AND METHODS

Study sites and sample collection

Biological factors were assessed during active foraging bouts of Western Sandpipers at multiple migratory stopover sites in 2001 and 2002 during northward, spring migration (April/May, hereafter referred to as "northward") and southward, fall migration (hereafter referred to as "southward") for both adults (July) and juveniles (August). All of the sampling dates and locations correspond to capture efforts at the same sites (see Chapter 3). Treating southward adult and juvenile migrations as two separate migration stages avoids confounding age and time of year effects and, in addition, allows for a third independent test for site differences. We maintained a constant sampling effort throughout all three migration stages in both years at Boundary Bay (Fraser River Delta), which served as our reference site. In each migration stage, at least one and up to three other sites were sampled concurrently and compared to the reference site. Logistics made it infeasible to collect data for some factors during the sampling period at some sites; mean sampling date and sample sizes of ecological and behavioral factors are listed in Table 2.1 (see Chapter 1 for detailed site descriptions). Not all sites were sampled during all migration

stages and years in part due to differential bird use (see Discussion Chapter 3); only those sites sampled concurrently were compared in subsequent analyses.

Prey base – sediment cores

Sediment cores were taken periodically within the sampling microsites in random locations being actively foraged over by Western Sandpipers in order to evaluate the invertebrate prey base potentially available to Western Sandpipers at each stopover site. Cores were taken to a depth of 40mm using a 60cc syringe modified according to Sutherland et al. (2000). Taking cores to this depth allows comparability with other studies (Sutherland et al. 2000, Wolf 2001) and is appropriate for this system because this depth is longer than the longest Western Sandpiper bill and should include most prey even if they retract into the sediment (Wolf 2001). Cores were either frozen at -20°C within 2 hours of taking the sample or were frozen at -80°C in a cryoshipper for up to 2 weeks and then stored at -20°C until analyzed. In the lab, each core was thawed and sieved in distilled water for meiofaunal (63 μm) and macrofaunal (500 μm) fractions and stored at room temperature in 85% ethanol (Sutherland 2000). Organisms were counted and identified to at least phylum level (e.g. Bacillariophyta [diatoms], Nematoda), but were identified to class level (e.g. bivalvea, foraminifera, gastropoda, insecta, polychaeta), or order (e.g. acari, amphipoda, copepoda, cumacea, ostracoda, tanaidea), whenever possible. For macrofauna fractions, the entire sample was counted. Meiofauna fractions were analyzed according to Wolf (2002), by which 20 randomly selected portions, each representing 0.5% of the plate, were scanned, and the total for all of the sampled portions together was multiplied by 10 to get the total for the entire sample.

Prey diversity was evaluated for both macro- and meiofaunal fractions in terms of the total number of taxa (based on the levels of identification detailed above) and equitability of distribution of individuals among taxa (described in Krebs 1978). Equitability (E, range 0 to 1) is based on the Shannon-Wiener diversity index [$H = -\sum (p_i * \log p_i)$ where p_i is the proportion of total individuals represented by taxa i]. The value of E is calculated as the ratio of H to H_{max}, the species diversity under a maximum equitable distribution ($H_{max} = \log_2 S$, where S is the total number of taxa in a community). The value of E is an indication of how evenly the different taxa are numerically distributed within the community. For example, a site supporting 50

individuals each of 2 different taxa is completely equitable since each taxa is represented by exactly half of the total number of organisms, and this is reflected in its E of 1 (H= -[0.5*log(0.5) + 0.5(log(0.5)]=1; $H_{max}=\log_2(2)=1$; $E=H/H_{max}=1/1=1$). Because sites differ in total number of taxa present (see Results), the H_{max} is different for each site, generating a different potential range of H for each site, making the interpretation of the species diversity index alone difficult to compare between sites. Therefore, we only report values of E (scale 0 to 1 at all sites) for each site.

Neither the total number of taxa present nor the equitability (distribution) reflects which taxa are present at each site. Given the demonstrated selectivity of prey taxa by Western Sandpipers (Sewell 1996, Sutherland et al. 2000, Wolf 2001) and the potential importance of diet composition of prey (see Chapter 4), the representation of macrofaunal taxa (mean percentage of core represented by each taxon) was considered. The identity of the four most represented taxa, those comprising the highest mean percentage of sediment cores, was compared between sites. Furthermore, the measure of prey abundance (total number of organisms per core) utilized in this study does not take into consideration biomass which may be expected to differ significantly between taxa given the differences in size. Therefore, for each migration stage, the abundance (number of organisms per core) of each taxon that was either the most or second most represented taxa (mean percentage of core) for at least one of the sites during that stage was compared between sites.

Prey Consumption – fecal samples

Fecal samples were collected from mist-netted individuals (See Chapter 3) held in modified Tupperware containers with aluminum foil liners while waiting to be processed. Samples were removed from the foil using a spatula and were stored in Eppendorf tubes and were either frozen at -20°C within 2 hours of collection or frozen in a cryoshipper at -80°C for up to 2 weeks and then stored at -20°C until analysis. In the lab, samples were thawed and suspended in 85% ethanol, and items were identified to taxa under a 5-50x dissecting microscope.

Foraging behavior

Observations were made on actively foraging individuals from the general capture location at each site through a spotting scope for 60 seconds or until the focal individual flew away. Observations at Robert's Bank and Boundary Bay were taken by various researchers and assistants, and methods were standardized through practicing observations of video-recordings of foraging sandpipers. For each individual, every foraging attempt was classified as either surface (including single and multiple pecks) or deep (including single or multiple probes). The foraging mode for each individual was calculated as the percentage of total foraging attempts represented by surface feeding. Foraging rate, defined as the total number of attempts per second, was calculated for each individual. It is not possible to see ingestion of prey by the Western Sandpiper during foraging observations, and, as such, foraging rate data are based on foraging attempts and should not be interpreted as intake rates.

Physical site characteristics

Substrate penetrability was measured at all sites at the same time and location of sediment core sampling and foraging observations. No penetrability readings were taken during northward migration in 2001. A 16.3 g steel rod tapered to a conical point was dropped from a height of 9.4 cm down the barrel of a modified 10mL pipette, and the depth of the rod was measured on the pipette. This technique follows the specifications given by Myers et al. (1980) and was further described and used by Wolf (2002).

Site size was calculated by scanning nautical charts into Adobe Photoshop and converting the number of pixels of the site polygon into area (square kilometers) using the scale from the map. The stopover site is physically defined as the area encompassing the mudflat from the shoreline out to the zero tide line; hereafter, the term site size refers to the absolute area of mudflat at each site. The horizontal edge of the site is defined by either physical barriers or the natural borders of the bay. Sampling (including blood sampling, behavioral observations, sediment core samples and penetrability readings) occurred exclusively within a smaller area, which contains the first- and last-exposed sediment, where the birds concentrate to feed

during a falling and rising tide, respectively (see Chapter 1 for microsite descriptions).

The absolute size of a site does not take into consideration the potential differential mudflat exposure due to the tide. Western Sandpipers (whose bills are ~3cm) do not typically forage on intertidal mudflats when they are flooded with water, a condition which can occur for a significant portion of each day. Data on the tidal cycle (tide height at a particular time) was obtained from Wxtide 32 for the tide station closest to each stopover site. The flood height, the height at which zero mud is exposed, for each site was calculated from the tidal cycle data based on personal observation of at what time the site flooded each day. The maximum distance from the shoreline to the zero tide line at each site was measured from nautical charts. The mudflat exposure index (MEI), the mean number of kilometer-hours of mudflat exposed in a 24h period, was calculated based on the distance to shore and the tidal cycle for each site (Ydenberg and Klein, unpubl. data) and represents the temporal and spatial opportunity to forage provided by the stopover site. This calculation is not based on absolute area of sediment exposed but rather on the one-dimensional distance from shore and serves as index for mudflat exposure. Because the tidal cycle varies between migration stages and years, the MEI index was calculated for each migration stage in each year separately.

Statistical analysis

All ecological factors were analyzed separately for each migration stage so that only concurrently sampled sites were compared. Total prey abundance for both meio- and macrofaunal fractions of the core was calculated as the total number of organisms, summed across taxa, per core. There was no overall relationship between total macrofaunal abundance and total meiofaunal abundance ($P > 0.3$, $R^2 = 0.01$); this was also true within each migration stage. Furthermore, the magnitude of meiofaunal abundance is up to 100 fold greater than macrofaunal abundance causing total prey abundance to reflect almost exclusively meiofaunal abundance ($P < 0.0001$, $R^2 = 1.0$) and masking macrofaunal abundance ($P > 0.1$, $R^2 = 0.01$). Therefore, we analyzed each fraction separately and assessed inter-site variation in total macrofaunal and meiofaunal abundance rather than total prey abundance for each site. For each size class, the percentage of total individuals represented by

each taxon in each core was calculated. Site differences in macro- and meiofaunal abundance, foraging mode and rate, and sediment penetrability were tested for year and site using type IV ANOVA, which accounts for missing cells (i.e. a site that was not sample din both years). The year*site interaction was tested based on the two or three sites sampled in both years, and those factors with a significant interaction were analyzed for each year separately; otherwise, the model for that factor was reanalyzed without the interaction term. For each migration stage, inter-site variation in the abundance of each taxon that was either the most or second most abundant taxa for at least one of the sites was tested in the above manner. Seasonal differences (between migration stages) in these biological factors were tested within Boundary Bay, the continuously-sampled reference site, via type IV ANOVA for year and stage. The year*stage interaction was tested, and those factors with a significant interaction were analyzed for each year separately; otherwise, the model for that factor was reanalyzed without the interaction term. For each ANOVA that detected a significant effect, multiple comparisons were tested controlling for experiment-wise error α=0.05 using Bonferroni adjustment, and only significantly different pairs are reported. The percent presence of prey items in fecal samples was calculated as the percentage of samples analyzed containing each taxon. All statistical analyses were performed using SAS 6.0.

RESULTS

Site differences in ecological factors

Prey abundance

During the northward migration (April/May), the year*site interactions for both total macrofaunal and meiofaunal prey abundance, defined as number of organisms per core, were nonsignificant (macrofauna: $F_{2,161} = 1.45$, $P > 0.2$; meiofauna: $F_{2,46} = 3.10$, $P > 0.05$), so it was dropped from the model. Total macrofaunal prey abundance was independent of year ($F_{1,163} = 0.19$, $P > 0.5$) but varied among sites ($F_{3,163} = 31.87$, $P < 0.0001$; Figure 2.1). Boundary Bay and Robert's Bank supported higher macrofaunal prey abundance than both Jensen Access and Totten Inlet ($P < 0.0001$, all cases). Total meiofaunal prey abundance was independent of both year and site ($P > 0.07$, both factors, Figure 2.1).

During southward adult migration (July), the year*site interaction for macrofaunal and meiofaunal prey factors was not significant ($F_{1,179} = 3.43$, $P > 0.05$), so it was dropped from the model. Total macrofaunal prey abundance was independent of year ($F_{1,176} = 0.41$, $P > 0.5$), but varied between site ($F_{3,176} = 37.11$, $P < 0.0001$, Figure 2.1). Alice Bay and Boundary bay had more macrofaunal prey organisms per core than both English Boom and Robert's Bank ($P < 0.0001$, all cases). Meiofaunal prey abundance was independent of year ($P > 0.2$). However, there was a significant site effect on total meiofaunal prey abundance ($F_{3,43} = 44.99$, $P < 0.0001$, Figure 2.1); Alice Bay had more meiofaunal organisms per core than the three other sites ($P < 0.0001$, all sites).

Sampling was unbalanced during southward juvenile migration (August); therefore, each year was analyzed separately. There was significant site variation in total macrofaunal prey abundance in both years (2001: $F_{1,33} = 36.76$, $P < 0.0001$; 2002: $F_{3,128} = 27.70$, $P < 0.0001$); in both years, Boundary Bay had significantly more macrofaunal prey than the other sites ($P < 0.0001$, all cases; Figure 2.1). There was significant site variation in the total meiofaunal prey abundance in 2001 ($F_{1,14} = 7.15$, $P < 0.02$); Sidney Island had more meiofaunal prey than Boundary Bay. In 2002, total meiofaunal abundance was independent of site ($F_{3,27} = 1.15$, $P > 0.3$; Figure 2.1).

Prey diversity

During northward migration, sites in order of increasing macrofaunal diversity (number of taxa per site) were Totten Inlet, Boundary Bay, and Jensen Access in both years (Figure 2.2). In 2002, the difference between sites was less pronounced and Robert's Bank had the same number of taxa as Boundary Bay. Diversity of meiofaunal prey taxa (number of taxa per site) varied between sites; however the differences were small and there was no trend between years except that Totten Inlet consistently had the fewest number of taxa represented (Figure 2.2). There was variation between sites in prey equitability. In both years, the site with the most equitable macrofaunal prey distribution was Jensen Access (Figure 2.3). The trend between the remaining sites varied between years with the most noticeable difference being the decreased equitability of Boundary Bay during 2002. Meiofaunal equitability also varied between sites in both years; however, the trend

between sites was not the same between years and the variation in equitability was less than for macrofauna (Figure 2.3).

During southward adult migration in 2001, Boundary Bay and English Boom had similar numbers of macrofaunal and meiofaunal taxa present. In 2002, Boundary Bay and English Boom both had more macrofaunal taxa present than both Alice Bay and Robert's Bank, while Boundary Bay had more meiofaunal taxa present than the other three sites (Figure 2.2). There was variation between sites in prey equitability. In 2001, the distribution of macrofaunal taxa at English Boom was more equitable than at Boundary Bay. The trend between these two sites was the same in 2002; Robert's Bank had comparable macrofaunal equitability to Boundary Bay, and Alice Bay was considerably less equitable than the other three sites (Figure 2.3). In 2001, Boundary Bay and English Boom had similar meiofaunal equitability. In 2002, the equitability varied between sites and English Boom was more equitable than the other sites (Figure 2.3).

During southward juvenile migration, sites had similar numbers of macrofaunal taxa present during both years (Figure 2.2). In 2001, Boundary Bay and Sidney Island had equal numbers of meiofaunal prey taxa. In 2002, Boundary Bay had more taxa than it did in 2001 and, along with False Bay, had the most meiofaunal taxa (Figure 2.2). Prey equitability varied between sites. The distributions of macrofaunal prey at Doug Banks and False Bay were more equitable than at Boundary Bay and Robert's Bank in 2002 (Figure 2.3). Meiofaunal distribution was most equitable at Boundary Bay in 2001 and at Doug Banks in 2002 (Figure 2.3).

Prey taxa representation

The representation of all prey taxa, as indicated by the average percentage of core represented by each taxon, is reported for macrofaunal prey and meiofaunal prey in Appendix 2.1 and Appendix 2.2, respectively. Statistical analysis of site differences in numerical abundance (number of organisms per core) of each macrofaunal taxa that was either the most or second most represented (mean percentage of core, Appendix 2.1) for at least one site during each migration stage is presented below.

During northward migration, there was noticeable variation between sites in the identity of the four most represented taxa and the relative abundance (mean percentage of core) of each of those taxa (Figure 2.4). Polychaetes and nematodes were universally the most represented (mean percentage of core) taxa at these sites. The year*site interactions for both polychaete and nematode abundance were nonsignificant (polychaete: $F_{2,160} = 1.80$, $P > 0.15$; nematode: $F_{2,160} = 1.53$, $P > 0.2$), so they were dropped from the model. Both of these taxa varied significantly in numerical abundance (number of organisms per core) between sites (polychaete: $F_{3,162} = 30.16$, $P < 0.0001$, nematode: $F_{3,162} = 21.06$, $P < 0.0001$). Both Boundary Bay and Robert's Bank had more polychaetes per core than both Jensen Access and Totten Inlet ($P < 0.0001$, all cases). Robert's Bank had more nematodes per core than the other three sites ($P < 0.0001$, all cases). Polychaete abundance was independent of year ($F_{1,162} = 0.02$, $P > 0.08$), but nematode abundance was greater in 2002 ($F_{1,162} = 7.04$, $P < 0.01$). There was a significant year*site interaction for the remaining four taxa analyzed (ostracod: $F_{2,160} = 12.35$, $P < 0.0001$; foraminifera: $F_{2,160} = 3.37$, $P = 0.04$; cumacean: $F_{2,160} = 10.38$, $P < 0.0001$; insect: $F_{2,160} = 63.37$, $P < 0.0001$), so these taxa were analyzed by year. There was significant inter-site variation in the numerical abundance of ostracods, foraminfera, cumaceans, and insects in both years (2001: $F_{2,43} > 5.30$, $P < 0.01$; 2002: $F_{3,117} = 7.40$, $P < 0.0001$). Jensen Access had more insects and foraminifera per core than the other sites in both years ($P < 0.01$, all cases). Boundary Bay had more ostracods per core than the other three sites in both years ($P < 0.04$, all cases). In 2001, Totten Inlet had more cumaceans per core than Jensen Access and Boundary Bay ($P < 0.0001$), but in 2002, Boundary Bay had more cumaceans per core than both Jensen Access and Robert's Bank which both had more than Totten Inlet ($P < 0.0001$). At each of the sites sampled during both years, the identity of the four most represented taxa was different between years. While the mean number of most taxa varied within sites between years, causing the significant interactions terms, the trend between sites was the same in every case except cumaceans (see above).

During southward adult migration, there was noticeable variation between sites in the four most represented taxa at each site and the relative abundance of each of those taxa (Figure 2.5). Polychaetes and nematodes were universally the most represented taxa at these sites. The year*site interactions for both polychaete and ostracod abundance were nonsignificant (polychaete: $F_{1,152} = 1.87$, $P > 0.15$;

ostracod: $F_{1,152} = 0.93$, $P > 0.3$), so they were dropped from the model. Numerical abundance of both of these taxa varied significantly between sites (polychaete: $F_{3,153} = 29.88$, $P < 0.0001$; ostracod: $F_{3,153} = 5.12$, $P < 0.005$), ($P < 0.003$, both taxa). Both Boundary Bay and Alice Bay had more polychaetes per core than both English Boom and Robert's Bank ($P < 0.001$). Boundary Bay had more ostracods per core than English Boom ($P < 0.005$). Both polychaete and ostracod abundance was independent of year ($P > 0.4$, both taxa). There was a significant year*site interaction for the other two most represented taxa (nematode: $F_{1,152} = 6.47$, $P < 0.02$; copepod: $F_{1,152} = 5.30$, $P < 0.03$), so these taxa were analyzed by year. There was significant inter-site variation in both nematode and copepod abundance in both years (nematode 2001: $F_{1,53} = 23.81$, $P < 0.0001$, copepod 2001: $F_{1,53} = 5.97$, $P < 0.02$; nematode 2002: $F_{3,99} = 9.49$, $P < 0.0001$; copepod 2002: $F_{3,99} = 4.21$, $P < 0.01$); however the trend between sites varied between years. In 2001, Boundary Bay had more nematodes and copepods per core than English Boom ($P < 0.02$, both taxa). However, in 2002, Boundary Bay and English Boom did not differ in nematode abundance in 2002 ($P > 0.05$). In 2002, Alice Bay had more nematodes per core than the other three sites ($P < 0.01$) and English Boom had more copepods per core than Alice Bay and Robert's Bank ($P < 0.02$). At both sites sampled during both years, the identity of the four most represented taxa was different between years.

During southward juvenile migration, there was noticeable variation between sites in the four most represented taxa at each site and the relative abundance of each of those taxa (Figure 2.6). Because sampling was unbalanced during southward juvenile migration, each year was analyzed separately. Polychaetes, ostracods, nematodes, copepods, and amphipods were the most represented taxa during this migration stage; there was no universally most represented taxon. There was no significant difference between sites in numerical amphipod density in either year (2001: $F_{1,35} = 2.70$, $P > 0.1$; 2002: $F_{3,99} = 1.29$, $P > 0.2$). There was significant inter-site variation in numerical abundance of each of the other four taxa in both years (2001: $F_{1,35} = 5.21$, $P < 0.03$; 2002: $F_{3,99} > 8.08$, $P < 0.0001$), with the exception of copepods in 2001 ($F_{1,35} = 0.59$, $P > 0.4$). Boundary Bay had more polychaetes and ostracods per core than Sidney Island in 2001 and all other sites in 2002 ($P < 0.03$, all cases). Sidney Island had more nematodes per core than Boundary Bay in 2001 ($P < 0.03$). False Bay and Robert's Bank both had more

nematodes per core than both Boundary Bay and Doug Banks in 2002 ($P < 0.03$). False Bay had more copepods per core than all other sites in 2002 ($P < 0.002$).

Prey consumption

In 2001, the only year that fecal samples were analyzed, there were differences within each migration stage in the taxa consumed as well as the relative proportion of birds consuming each taxon (Figure 2.7). More taxa were represented in fecal samples during northward migration than southward migrations. A larger proportion of birds ate amphipods at all sites during southward migration than at the sites during northward migration.

Foraging behavior

During northward migration, there was a significant year*site interaction term for both foraging mode and rate (mode: $F_{2, 433} = 60.87$, $P < 0.0001$; rate: $F_{2,433} = 21.61$, $P < 0.0001$); therefore we analyzed each year separately. There was significant variation between sites in both foraging mode and rate in both years ($P < 0.0001$, all cases; Figure 2.8). In 2001, the sites in order of increasing proportion of surface foraging attempts were Robert's Bank, Totten Inlet, and Boundary Bay ($P < 0.0001$, all sites). Similarly, in 2002, the birds at Robert's Bank employed less surface feeding than both Boundary Bay and Totten Inlet ($P < 0.01$, both cases). Although the trend between sites was similar in both years, there was significant intra-site variation between years in foraging mode, with birds employing a greater percentage of surface feeding in 2002 than 2001 at all three sites ($P < 0.0001$, all sites). In both years, the birds at both Boundary Bay and Totten Inlet had higher foraging rates than those at Robert's Bank ($P < 0.05$, both years). At Boundary Bay, the birds had higher foraging rates in 2001 ($F_{1, 178} = 29.49$, $P < 0.0001$), while at Robert's Bank, the birds had higher foraging rates in 2002 ($F_{1, 201} = 255.40$, $P < 0.0001$); there was no difference between years in foraging rate at Totten Inlet ($F_{1, 49} = 1.86$, $P > 0.1$).

During southward adult migration, there was a significant year*site interaction term for both foraging mode and rate (mode: $F_{1, 298} = 4.43$, $P < 0.04$; rate: $F_{1, 298} = 5.54$, $P < 0.02$); therefore, we analyzed each year separately. Foraging mode was independent of site in both years ($P > 0.08$, both years; Figure 2.8). In 2001, there

was significant site variation in foraging rate ($F_{1, 123} = 50.79$, $P < 0.0001$); the birds at Boundary Bay made more foraging attempts per minute than those at English Boom. However, in 2002, foraging rate was independent of site ($F_{2\ 175} = 1.74$, $P < 0.1$). At Boundary Bay, foraging mode was independent of year ($F_{1, 170} = 0.17$, $P < 0.5$), but foraging rates were significantly higher in 2001 than 2002 ($F_{1, 170} = 5.43$, $P < 0.02$). At English Bay, there was significant intra-site variation within year in foraging mode ($F_{1, 109} = 5.93$, $P < 0.02$), with the birds in 2002 employing a larger percentage of surface feeding that in 2001; foraging rate was independent of site ($F_{1, 109} = 3.65$, $P > 0.05$).

During southward juvenile migration (August), foraging observations were only taken at one site during 2001, preventing analysis for site variation in that year. In 2002, there was significant inter-site variation in foraging mode ($F_{2, 127} = 5.24$, $P < 0.01$; Figure 2.8); birds at Boundary Bay foraged more surficially than those at Doug Banks ($P < 0.01$). In contrast, foraging rate was independent of site ($F_{2, 127} = 0.41$, $P > 0.5$).

Sediment penetrability

During northward migration in 2002, there was significant site effect in sediment penetrability ($F_{3,189} = 503.31$, $P > 0.0001$, see Table 2.2) with sites in order of increasing substrate softness being Boundary Bay, Jensen Access, Robert's Bank, and Totten Inlet ($P < 0.0001$, all cases); sediment penetrability was not measured in 2001.

During southward adult migration, there was a significant year*site interaction term for sediment penetrability ($F_{1, 370} = 46.39$, $P < 0.0001$); therefore, we analyzed each year separately. In 2001, sediment penetrability was independent of site ($F_{1,156} = 2.76$, $P > 0.05$, Table 2.2). In 2002, there was significant inter-site variation ($F_{3,214} = 57.95$, $P < 0.0001$; Table 2.2), with sites in order of increasing softness being Boundary Bay, English Boom and Roberts Bank being similar, and Alice Bay ($P < 0.0002$, all cases). There was intra-site variation between years at both sites that were sampled in both years (Boundary Bay: $F_{1,96} = 473.18$, $P < 0.0001$; English Boom: $F_{1, 205} = 24.04$, $P < 0.0001$), with the sediment being softer in 2001 at both sites.

During southward juvenile, there was a significant site effect on sediment penetrability in both years (2001: $F_{1,63} = 16.03$, $P < 0.0002$; 2002: $F_{3,184} = 25.59$, $P < 0.0001$; Table 2.2). In 2001, Boundary Bay was softer than Sidney Island; in 2002, the sites in order of increasing softness were Boundary Bay, False Bay, and both Robert's Bank and Doug Banks which did not differ ($P < 0.01$, all significant pairwise comparisons). There was significant within-site variation in sediment penetrability between years at Boundary Bay, the only site sampled in both years ($F_{1,23} = 138.49$, $P < 0.0001$), with the sediment being softer in 2001 than 2002.

Size and mudflat exposure

The stopover sites sampled during each migration stage vary in macrosite size (Table 2.3). There is a four-fold difference in size between the smallest and largest site sampled in southward adult; the difference is even more dramatic during northward (30-fold) and southward juvenile (60-fold). Totten Inlet, Alice Bay, and Sidney Island were the smallest sites sampled during northward, southward adult, and southward juvenile, respectively; Boundary Bay was the largest site sampled. The sampling microsites are similar for each site and are all relatively small ($< 1km^2$). There are also inter-site differences in maximum distance to shoreline and flood height (Table 2.3). There were large differences in the km-hours of mudflat exposed at the different sites (Table 2.2). These values, in general, varied little between years although the values for English Boom varied more between years than the other sites. There was a significant positive relationship between the absolute site size (km^2) and the maximum distance to shoreline ($P < 0.02$, $R^2 = 0.59$), suggesting that, for these nine sites, an MEI based on the uni-dimensional distance variable is an adequate proximate for mudflat exposure. There was no relationship between site size and flood height ($P > 0.5$, $R^2 = 0.05$).

Seasonal differences in biological and physical factors

To avoid the complication of different sites sampled during different migration stages, we tested for inter-seasonal variation (between migration stages) in biological and physical factors within Boundary Bay, which was sampled for most factors during all migration stages of both years (see Table 2.1 for sample sizes).

Prey abundance

Within Boundary Bay, the year*stage interaction for numerical macrofaunal and meiofaunal abundance was nonsignificant (macrofauna: $F_{2,178} = 0.84$, $P > 0.4$; meiofauna: $F_{2,46} = 3.10$, $P > 0.05$), so it was dropped from the model. Total macrofaunal and meiofaunal abundance (number of organisms per core) were independent of year ($P > 0.09$, both size fractions). Both factors varied significantly between migration stages (macrofauna: $F_{2, 174} = 3.35$, $P < 0.04$; meiofauna: $F_{2,44} = 7.05$, $P < 0.003$, Figure 2.9). Boundary Bay had lower macrofaunal prey abundance in northward migration than in southward adult migration ($P < 0.04$); the site had more meiofaunal organisms per core during northward than southward adult and juvenile migrations ($P < 0.01$, both stages).

Prey diversity

The number of macrofaunal taxa present at Boundary Bay varied between migration stages; there were more taxa present in southward adult migration than northward and southward juvenile migrations in both years (Figure 2.9). In general, the equitability of macrofaunal prey was similar across stages and years, with the exception of northward migration in 2002, which was less equitable, and southward adult migration in 2001, which was more equitable than the other sampling periods (Figure 2.9).

The number of meiofaunal taxa present at Boundary Bay varied between migration stages; however there was no consistent trend between years. There was the same number of meiofaunal taxa at this site in both years during northward migration, but there were more types of taxa in 2002 than 2001 during both southward adult and juvenile migrations (Figure 2.9). There was also variation within Boundary Bay in the equitability of meiofaunal prey distribution. In both years, the equitability changed between stages, but there was no consistent trend between years (Figure 2.9). During all three stages, the site had higher equitability in 2002 than 2001.

Prey taxa representation

Within Boundary Bay, the year*stage interactions for both polychaete and ostracod abundance (number of organisms per core) were nonsignificant (polychaete: $F_{2,120} = 1.65$, $P > 0.15$; ostracod: $F_{2,120} = 3.17$, $P = 0.05$),so they were dropped from the model. Ostracod abundance varied between stages ($F_{2,122} = 19.90$, $P < 0.0002$), but polychaete abundance was independent of stage ($F_{2,122} = 1.22$, $P > 0.2$). There was a year effect in the abundance of both of these taxa (polychaete: $F_{1,122} = 8.29$, $P < 0.005$; ostracod: $F_{1,122} = 22.49$, $P < 0.0001$), with polychaetes being more abundant per core in 2002 and ostracods more abundant per core in 2001. There was a significant year*stage interaction for the other two most represented taxa (nematode: $F_{2,120} = 6.78$, $P < 0.002$; copepod: $F_{2,120} = 5.15$, $P < 0.01$), so these taxa were analyzed by year. There was significant inter-stage variation in nematode abundance in both years ($P < 0.02$, both years); however the trend between sites varied between years. In 2001, there were more nematodes per core at Boundary Bay in southward adult than southward juvenile migrations ($P < 0.02$), but in 2002, there were more nematodes per core during northward migration than both southward adult and juvenile migrations ($P < 0.01$, both cases). Copepod abundance was independent of stage in 2001($P > 0.2$), but varied between stage in 2001 ($P < 0.0001$), with there being more in southward adult than both northward and southward juvenile migrations.

Prey consumption

Within Boundary Bay in 2001, the only year fecal samples were analyzed, a larger proportion of birds consumed amphipods in both southward migrations than northward migration (Figure 2.7). In addition, birds consumed more prey types in northward than both southward migrations.

Foraging behavior

Within Boundary Bay, the year*stage interactions for each foraging factor was nonsignificant (mode: $F_{1,421} = 1.88$, $P > 0.15$; rate: $F_{1,421} = 0.30$, $P > 0.5$), so they was dropped from the model. There was significant variation between migration stage in both foraging mode and rate (mode: $F_{2,422} = 37.53$, $P < 0.0001$; rate: $F_{2,422} = 9.19$, P

< 0.0001; Figure 2.10). Birds at Boundary Bay foraged more surficially and had higher foraging rates during northward than both southward adult and juvenile migrations ($P < 0.0005$, all cases). Foraging mode was independent of year ($F_{1, 422} = 0.38$, $P > 0.5$). Foraging rate varied significantly between years ($F_{1, 422} = 23.89$, $P < 0.0001$); birds using the site in 2001 made more foraging attempts than those using it in 2002. Foraging rate also varied significantly between migration stages ($F_{2, 422} = 9.19$), $P < 0.0001$); birds using this site made more foraging attempts during northward migration than both southward adult and juvenile migrations ($P < 0.0001$, all cases). Foraging observations were only made at Robert's Bank during northward migration, preventing inter-stage comparisons for that site.

Sediment penetrability

Within Boundary Bay, there was a significant year*stage interaction term for sediment penetrability ($F_{1, 148} = 5.44$, $P < 0.03$); therefore we analyzed each year separately. In 2001, sediment penetrability was independent of migration stage ($F_{1, 24} = 0.09$, $P > 0.5$), though it should be noted that this factor was not measured during northward migration in 2001. In 2002, there was a significant stage effect on sediment penetrability ($F_{2, 124} = 88.11$, $P < 0.0001$), with stages in order of increasing softness being northward, southward juvenile, and southward adult ($P < 0.02$, all cases).

DISCUSSION

Our most important findings were that 1) site differences were detected in numerical abundance and diversity of two size fractions of prey (meio- and macrofauna), prey consumption, foraging behavior, and physical site characteristics during all three migration stages, 2) prey abundance was consistent within sites between years, but foraging behavior mode and rate and sediment penetrability varied among years, 3) fecal analysis provides information on prey consumption for entire stopover site in contrast to the prey information from sediment cores which is restricted to the sampling microsite, 4) stopover sites had less numerically abundant macrofaunal prey and softer sediment (higher penetrability), and the birds had higher foraging

rates and foraged more surficially during northward migration than southward migrations.

Detecting site differences: possible confounding factors

While the prey-related and behavioral factors assessed in this study logically influence realized fattening rates, there are some implicit assumptions and lacking information. While there is a theoretical relationship between intake rate and fat deposition, the measurement of intake rate of Western Sandpipers has proven logistically difficult in the field (e.g. Wolf 2001). It is occasionally possible to see polychaetes during bouts of Western Sandpiper foraging and to determine if a foraging attempt was successful (D. Seaman, pers. obs.); however, the diet of the Western Sandpiper includes prey items not visible during observations. For example, *Corophium* amphipods, an important prey taxon consumed by at least 30% at all sites and up to 100% of all individuals at some sites, consumed by Western Sandpipers at two of the sites sampled in this study measure less than 5mm (Wolf 2001). Similarly, there is documented evidence for consumption by Western Sandpipers of meiofaunal taxa (Sutherland 2000, Wolf 2001) which by definition are less than 0.5 mm. Therefore, it is not visually possible to determine total intake rate but rather only the rate of foraging attempts. Differential rates of foraging success between sites would influence the fattening rates achieved at those sites, and would weaken the correlation between fattening rates and foraging rate; this represents an unknown confounding variable in this study.

Underlying the analyses of inter-site variation in prey-related factors and foraging behavior is the implicit assumption that assimilation of ingested prey by the digestive system is the same for birds at all sites. Stein (2002) demonstrated that gut morphology is plastic during migration and influenced by diet quantity and quality. It has been demonstrated from gut analysis that, during foraging on mudflats, Western Sandpipers ingest substrate particles (Stein 2002), which affect gut morphology by diluting the energy density of the diet resulting in an increase in digestive capacity (Karasov 1996). The sites sampled in this study vary widely in substrate (e.g. sandy versus silty clay), and Quammen (1982) demonstrated that some sand particles are large enough to not be ingestible or to block ingestion of food items; therefore, differential proportions of sand particles in sediments may

result in different amounts of sediment being ingested at different sites. This could, in turn, cause differences in digestive capacity between sites, introducing a confounding factor. If prey base and substrate at different sites create dissimilar intestinal conditions in the birds, the digestive efficiency may vary between sites, yielding differential fattening between sites even if the prey selected at the sites is the same in both quality and quantity.

Another confounding variable is the fact that observed behavioral differences (e.g. foraging mode, foraging rate, prey selection) may be due to the natural preferences of individuals that chose the site. Site-independent preferences may be the result of a) preferential selection of certain sites by different age and sex classes, b)site selection driven by individual preferences based on individual bill morphometrics (See Elner and Seaman 2003), and/or c) differential site selection driven by distinct sub-populations arising from segregation on the breeding grounds instead of site selection by individuals from within one large population. It is clear that on the species level, Western Sandpipers are able to fatten sufficiently during migration using a variety of foraging techniques at sites that vary in prey base. This may be indicative of the Western Sandpiper either being an adaptable opportunist able to function adequately under a variety of conditions or having sub-populations comprised of specialist individuals each able to function optimally (only) at a particular microhabitat type. Elner and Seaman (2003) propose research directions to tease apart these two possibilities. In either scenario, the diversity of stopover sites within a single migratory stopover region would be valuable to the migratory Western Sandpiper.

Many of the ecological, behavioral, and physical parameters assessed in this study are inter-related and it is difficult to tease apart the influences of each factor on the realized fattening rates at stopover sites. A mechanism by which some site characteristics influence the ability to fatten, or autocorrelations between factors, may not be intuitive. Sediment penetrability has been demonstrated to explain inter-site variation in the foraging mode utilized by individuals at different stopover sites (Wolf 2001). Different foraging modes have been shown to target different taxa (Sutherland et al. 2000), and if these taxa differ in nutritional content, the fat deposition realized at a site may be influenced by the softness of the sediment.

Similarly, physical site characteristics may not seem to be intuitively correlated to foraging rate. However, it has been demonstrated that individuals alter their behavior to moderate perceived risk (Lima and Dill 1990) and, in particular, may shift their activity budgets to include more vigilance behavior, resulting in lower foraging rates (Altendorf et al. 2001); therefore differential perceived risk between sites may result in differential foraging rates and, therefore, different realized fattening rates. The primary factor determining perceived predation risk for mudflat-foraging sandpipers has been suggested to be the distance to obstructive cover that may provide protection to approaching predators (Wolf 2001, Ydenberg et al. 2002). Our study demonstrated that, in general, larger sites have a greater maximum distance to cover; therefore, site size may indirectly influence realized fattening rates. Ideally, all parameters should be evaluated together in order to reveal potential trade-offs and correlations between factors that may influence the realized differences in fattening rates. In the above example, analysis of risk alone according to a predation-minimizing model would predict that birds foraging at the more dangerous site would achieve lower fattening rates (Alerstam and Lindstrom 1990). However, overall higher fattening rates may be realized at the more dangerous site if softer substrates at the more dangerous site allow for less energetically demanding foraging modes (Wolf 2001) or if prey availability or quality is higher at the more dangerous site, either of which may compensate for lower foraging rates at the risky site. Analysis of site differences in prey availability in terms of energy content (both at the site and taxa levels) would lend great insight to this issue.

Site differences in stopover site characteristics

Prey base

All factors related to the available prey base that were measured in this study differed between stopover sites in all three migration stages, except meiofaunal prey abundance which only differed between sites during southward adult migration in 2002 when Alice Bay had substantially more diatoms per core, by the thousands, than other sites. Due to the time-intensive nature of quantification of meiofauna, sample sizes were low (n=5-8 per site), providing low power of detection for inter-site variation; therefore, it is difficult to assess inter-site variation in this size fraction of prey. The rest of the discussion on inter-site variation in prey base will be restricted

to the macrofauna fraction. During all three migration stages, Boundary Bay had higher numerical prey abundance (number of macrofaunal organisms per core) than the other sites sampled. However, Boundary Bay was less equitable than at least one site during each migration stage; this is consistent with a previous study documenting the high level of spatial variability in invertebrates at Boundary Bay (Sewell and Elner 2001). The most represented taxon (mean percentage of core) during northward and southward adult migrations was polychaetes; there was no universally most represented taxon during southward juvenile migration. Boundary Bay had more polychaetes and ostracods per core than the other sites sampled in all three migration stages. Jensen Access had more insects per core than the other three sites during northward migration and was the only site that had insects as one of the four most represented taxa; this highlights the extreme difference in prey base available in the hummock marsh at this site. Quantified prey base-related factors were independent of year except for equitability which had a year effect during northward migration. While neither the total numerical prey abundance per core nor the abundance per core of particular taxa differed within sites between years, the ranking order of most represented taxa at each site did vary annually.

Prey consumption

There was inter-site variation in the identity and relative proportion of taxa consumed, as indicated by fecal samples. This variation was in part reflective of differences between sites in available prey; however, the prey consumed did not exclusively reflect the prey available at the microsites. For example, whereas Jensen Access in 2001 supported twelve macrofaunal prey taxa, only five taxa were detected in fecal samples, indicating prey selectivity by birds. Fecal analysis also provided information of where, specifically, the birds are foraging. For example, at Boundary Bay, only six prey taxa were present in the sediment cores whereas at least seven taxa were consumed according to prey types detected in fecal samples; this suggests that individuals were feeding in other microhabitats before arriving to the sampling microsite. Although amphipods were absent from sediment cores at Boundary Bay and Totten Inlet, and not very abundant at Jensen Access, between thirty and sixty percent of individuals at all three sites had consumed amphipods. This, along with the fact that there are more taxa represented in the fecal samples

than in sediment cores at some sites suggests that individuals are feeding on different taxa out at lower tide prior to coming in to feed into the last-exposed sections of mudflat where we captured them. Consistent with this conclusion, sediment cores analyzed from Boundary Bay ~2.5km from shore (Wolf 2001) had more amphipods per core than those form the near-shore microsite sampled in our study.

Fecal analysis has been used to discern what portion of the available prey base is consumed by the species of interest and use this difference as an index for prey selectivity by shorebirds (Wolf 2001); however, it is only an adequate measure of consumption for those taxa represented in the feces in proportion to their consumption. The interpretation of fecal samples should take into consideration the differential proportion of hard parts of prey. Soft-bodied organisms (e.g. nematodes) are rarely represented in fecal samples and their absence should not be interpreted as lack of consumption. While Sutherland et al. (2000) did not detect removal of nematodes by Western Sandpipers, given that previous studies have documented (via gut content analysis) evidence of nematode consumption by Western Sandpipers during migration (see Wolf 2001 for review), it is not possible to determine from fecal samples alone whether birds at the sites in our study consumed soft-bodied prey such as nematodes. The disparity in geographical representation between the fecal samples and the sediment cores (i.e. fecal samples reflect foraging over a larger area) that was demonstrated in this study represents a further limitation to utility of using fecal analysis to measure prey selectivity. However, this disparity may increase the scope of utility of the technique to assess differences in prey consumption at the site level. The expertise required and logistical difficulty of quantifying fecal samples was a limitation in this study; the ability to test for correlations between fattening rates, as indicated by plasma metabolites, and quantitative prey consumption data is an important area of future investigation.

Foraging behavior and substrate

There were inter-site differences in both foraging mode and rate; however, there were no universal trends between sites and both factors varied between years in at

least one migration stage. Furthermore, foraging mode and rate did not vary between sites together (i.e. within a migration stage there was inter-site variation in mode but not rate, or vice versa).

There was inter-site variation in sediment penetrability during all migration stages and years, except for the two sites sampled during southward adult migration in 2001. There was inter-annual variation in substrate softness within sites. During southward juvenile migration in 2002, the substrate at Boundary Bay was softer than that at Sidney Island; this is in contrast to the finding that Sidney Island had substantially softer substrate than Boundary Bay during the same migration stage in 1999 (Wolf 2001; see below for further discussion about this study). This may be due to differences in sampling microsite between these two studies; the sampling area was identical at Sidney Island but Wolf (2001) sampled Boundary Bay ~2.5km out from shore compared to the near-shore microsite in our study. With the exception of this case, Boundary had the hardest substrate of all the sites sampled during all migration stages and years.

Comparison to other studies

Several studies have demonstrated inter-site variation in some aspect of intertidal or subtidal macrofaunal prey base (e.g. abundance, diversity, and/or taxa representation) between two or three sites on a small scale (<25 km apart) (e.g. Davidson 1989, Corbisier 1991, Wong et al. 2000). In addition to differences in prey availability, Wong et al. (2000) also detected inter-site differences in prey consumption by and foraging behavior of Little Egrets using two different sites (16 km apart) in Hong Kong. Studies have also detected inter-site variation in macrofaunal prey on a landscape scale similar to that represented by the sites in our study (300km, 2° latitude). Warwick et al. (1991) detected inter-site variation in macrofaunal prey abundance and biomass between 6 estuaries spanning 200km along the southwestern coast of Britain; the study area covers 2° in longitude in contrast to the latitudinal spread of the sites in our study. In contrast to our study, in which the prey base varied between all pairs of sites sampled concurrently, the only significant differences in prey base in the study by Warwick et al. (1991) were between the Severn estuary and the other 5 estuaries, which are on a completely different waterway from the Severn. Swennen et al. (1982) did not statistically

analyze inter-site variation but presented evidence of differences in macrofaunal prey abundance and biomass between 5 intertidal mudflats spanning 150km and 2° in longitude along the northern coast of Surinam. Donn and Cockcroft (1989) presented evidence of differences in prey abundance and biomass between two intertidal sites 100km and 1° latitude apart on the Namibian coast, although inter-site variation was not statistically tested. The study that is most comparable to ours in geographic scale detected similar inter-site variation in macrofaunal prey abundance and number of taxa between 35 intertidal sites spanning 150km and 1.5° latitude along the coast of Kuwait (Al Bakri et al. 1997). None of these studies tested for inter-annual variation in prey base within sites. Furthermore, to our knowledge, our study is the first to test for inter-site variation in the available prey base between multiple intertidal mudflats on this geographic scale in North America.

The only other study to test for site differences in ecological and physical site characteristics between stopover sites within the GB/PS and the foraging behavior of the birds using the sites was conducted in 1999 by Wolf (2001). There are several key differences in experimental design and results between these two studies. Our study tested for inter-site variation between nine different stopover sites within all three migration stages for two consecutive years; in contrast Wolf (2001) compared two sites during only southward migration, focusing on juvenile migration. There were differences in the species list generated for Boundary Bay by these two studies. Noticeably, in our study we did not find bivalves or sand dollars which were present in sediment cores sampled by Wolf (2001); however, we did find cumaceans and tanaids which were not detected by Wolf. These differences may be explained by the previously described difference in geographical position of the microsite sampled; Wolf (2001) sampled far off-shore near the low-tide line while we sampled near shore during rising and falling tides.

Furthermore, we detected differences in total macrofaunal numerical prey abundance per core between these two particular sites (as well as all other sites), while Wolf (2001) found that the total number of organisms present in sediment cores was similar between sites; this difference may also be related to differences in method. In the study by Wolf (2001), each taxon was classified into either the meiofaunal or macrofaunal size fraction (e.g. copepods and ostracods were always meiofauna and polychaetes and amphipods were always macrofauna. Due to this

difference, total macrofaunal prey abundance values are not compatible between the two studies because they include different lists of taxa. In the study by Wolf (2001), for each sediment core taken, macrofauna was analyzed for the entire core, but meiofaunal was only analyzed for the 1mm surface slice taken off the top of the core. The lack of polychaetes and amphipods in meiofaunal samples analyzed by Wolf (2001) suggests that these taxa are never found in the top 1mm of the sediment. It is possible that, similarly, the lack of detection of copepods, ostracods, and nematodes in the macrofaunal fractions in the study by Wolf is because those taxa are never found below the surface. However, because only the epifaunal slices were analyzed for meiofauna in that study, it is not possible to determine. Furthermore, in our study it is not possible to determine whether any of the meiofaunal organisms were in deeper parts of the sediment. In our study, there were organisms of taxa considered by Wolf to be exclusively meiofaunal (e.g. ostracods) which were abundant in sizes larger than 0.5mm. Because the reported differential targeting of taxa by different foraging modes (Sutherland et al. 2000) has potential ramifications for the determination of achieved fattening rates as well as potential individual morphometric-based specialization (Elner and Seaman 2003), it is important in future studies to determine whether particular taxa are obligate surface- or burrow-dwellers. Determination of the vertical distribution of prey availability will also prove valuable to the questions raised by Elner and Seaman (2003).

Conclusion

We found significant variation in several site characteristics such as prey abundance and diversity, foraging mode and rate, substrate softness, and mudflat exposure index (opportunity to forage) between different migratory stopover sites. Some of these factors also varied between years and migration stages. All of these factors are expected to influence the habitat quality of these migratory stopover sites. It, therefore, stands to reason that these nine migratory stopover sites within the GB/PS stopover region differ in habitat quality (i.e. the capacity to support fattening) for Western Sandpipers during stopover. There is also evidence that habitat quality may vary seasonally (migration stages) and inter-annually. Based on these findings, we predict that fattening rates, as indicated by plasma metabolite concentrations,

would, too, vary between stopover sites. To address this prediction, we measured plasma metabolite concentrations of birds foraging at these sites during the three different migration stages to evaluate inter-site, inter-seasonal, and inter-annual variation in fattening rates (Chapter 3).

ACKNOWLEDGEMENTS

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FIGURE LEGEND

Figure 2.1. Inter-site variation in macrofaunal (>500μm) prey abundance [top] and meiofaunal (63μm<x<500μm) prey abundance [bottom] at several migratory stopover sites used by Western Sandpipers during northward (left), southward adult (middle) and southward juvenile (right) migrations in 2001 (squares) and 2002 (circles).

Figure 2.2. Inter-site variation in total number of macrofaunal (>500μm) prey [top] and meiofaunal (63μm<x<500μm) prey taxa at several migratory stopover sites used by Western Sandpipers during northward (left), southward adult (middle) and southward juvenile (right) migrations in 2001 (black) and 2002 (white).

Figure 2.3. Inter-site variation in equitability of macrofaunal (>500μm) [top] and meiofaunal (63μm<x<500μm) [bottom] prey taxa at several migratory stopover sites used by Western Sandpipers during northward (left), southward adult (middle) and southward juvenile (right) migrations in 2001 (black) and 2002 (white).

Figure 2.4 Macrofaunal prey taxa representation at several migratory stopover sites used by Western Sandpipers during northward migration in 2001 (top) and 2002 (bottom).

Figure 2.5 Macrofaunal prey taxa representation at several migratory stopover sites used by Western Sandpipers during southward adult migration in 2001 (top) and 2002 (bottom).

Figure 2.6 Macrofaunal prey taxa representation at several migratory stopover sites used by Western Sandpipers during southward juvenile migration in 2001 (top) and 2002 (bottom).

Figure 2.7 Prey consumption expressed as the percentage of samples (individuals) containing each taxa at several migratory stopover sites used by Western Sandpipers during northward (left), southward adult (middle) and southward juvenile (right) migrations.

Figure 2.8 Inter-site variation in mean foraging mode (top) and foraging rate (bottom) by Western Sandpipers at several migratory stopover sites during northward (left), southward adult (middle) and southward juvenile (right) migrations in 2001 (squares) and 2002 (circles).

Figure 2.9 Variation between migration stages (N=northward, SA=southward adult, SJ=southward juvenile) in prey abundance (top), number of prey taxa (middle) and taxa equitability (bottom) of macrofaunal (>500μm) [left] and meiofaunal (63μm<x<500μm) [right] prey fractions sampled within Boundary Bay, a migratory stopover site used by Western Sandpipers in 2001 (squares) and 2002 (circles).

Figure 2.10 Variation in foraging mode [top] and foraging [bottom] rate between migration stages (N=northward, SA=southward adult, SJ=southward juvenile) within Boundary Bay, a migratory stopover site used by Western Sandpipers in 2001 (squares) and 2002 (circles).

Table 2.1 Mean sampling julian date and sample sizes of macrofaunal and meiofaunal prey abundance, foraging observations, and sediment penetrability measured at several migratory stopover sites during northward (N), southward adult (SA), and southward juvenile (SJ) migrations in 2001 and 2002.

Site	Stage	Year	Date	Macro	Meio	For. Obs.	Pen.
Boundary Bay	N	2001	118.7	30	8	43	0
Jensen Access	N	2001	122.2	12	8	0	0
Robert's Bank	N	2001	124.0	0	0	43	0
Totten Inlet	N	2001	123.4	5	5	25	0
Boundary Bay	N	2002	120.2	30	8	137	30
Jensen Access	N	2002	127.2	30	8	6	82
Robert's Bank	N	2002	120.8	31	8	160	30
Totten Inlet	N	2002	120.6	30	8	26	51
Boundary Bay	SA	2001	189.6	22	8	60	21
English Boom	SA	2001	190.6	31	8	65	246
Alice Bay	SA	2002	200.3	30	8	20	21
Boundary Bay	SA	2002	191.1	44	8	72	77
English Boom	SA	2002	191.6	30	8	46	70
Robert's Bank	SA	2002	199.8	30	8	0	40
Boundary Bay	SJ	2001	230.0	16	8	73	5
Sidney Island	SJ	2001	223.0	21	8	22	60
Boundary Bay	SJ	2002	223.9	44	8	38	20
Doug Banks	SJ	2002	229.9	25	7	38	49
False Bay	SJ	2002	218.9	28	8	18	40
Robert's Bank (R)	SJ	2002	223.5	39	8	0	79

Table 2.2. Mean sediment penetrability (cm ± 95% CI) and mudflat exposure index (MEI, km*hr) at several migratory stopover sites used by Western Sandpipers during northward (N), southward adult (SA), and southward juvenile (SJ) migrations in 2001 and 2002.

Site	Stage	Year	Penetrability	MEI
Boundary Bay	N	2001	N/A	26.42
Jensen Access	N	2001	N/A	25.73
Totten Inlet	N	2001	N/A	3.61
Boundary Bay	N	2002	0.32±0.03	26.13
Jensen Access	N	2002	0.91±0.06	23
Robert's Bank	N	2002	1.89±0.09	24.91
Totten Inlet	N	2002	2.75±0.13	3.43
Boundary Bay	SA	2001	1.97±0.14	25.06
English Boom	SA	2001	1.85±0.08	26.74
Alice Bay	SA	2002	1.89±0.30	11.98
Boundary Bay	SA	2002	0.78±0.04	27.90
English Boom	SA	2002	1.45±0.09	22.18
Robert's Bank	SA	2002	1.47±0.17	27.84
Boundary Bay	SJ	2001	1.92±0.41	26.02
Sidney Island	SJ	2001	1.29±0.08	1.92
Boundary Bay	SJ	2002	0.46±0.08	27.12
Doug Banks	SJ	2002	1.11±0.14	5.32
False Bay	SJ	2002	0.81±0.12	5.63
Robert's Bank	SJ	2002	1.22±0.08	27.16

Table 2.3 Physical site characteristics including size of macrosite (entire site from shore to zero tide line), maximum distance from shoreline at zero tide, tidal height at which zero mud is exposed, length of intertidal cover (vegetation on sediment surface utilized by foraging shorebirds) and length of upland cover (obstructive cover not utilized by shorebirds).

Site	Macrosite Size (km^2)	Maximum Distance (m)	Flood Height (m)	Intertidal Cover (m)	Upland Cover (m)
Alice Bay	14.31	1560	2.58	500	5
Boundary Bay	60.11	4100	4.00	100	∞
Doug Banks	7.88	750	2.74	0	55
English Boom	58.93	2800	2.59	0	125
False Bay	3.57	890	1.82	0	∞
Jensen Access	58.93	2800	2.44	200	∞
Robert's Bank	27.25	4590	3.60	0	∞
Sidney Island	1.13	360	2.40	0	250
Totten Inlet	3.28	400	3.81	0	500

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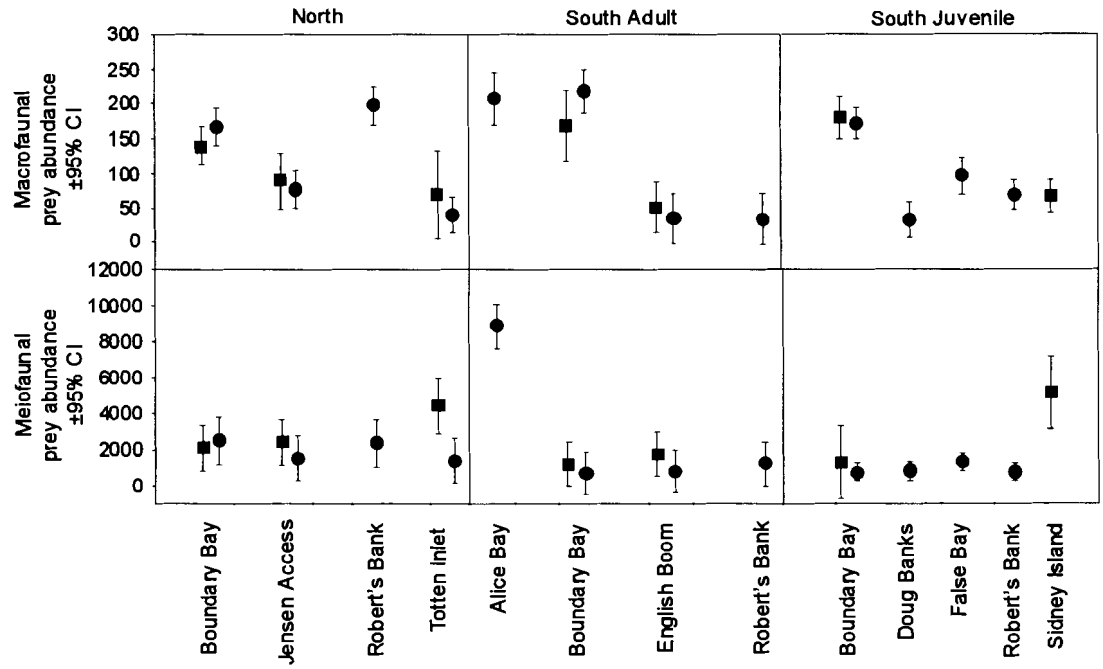

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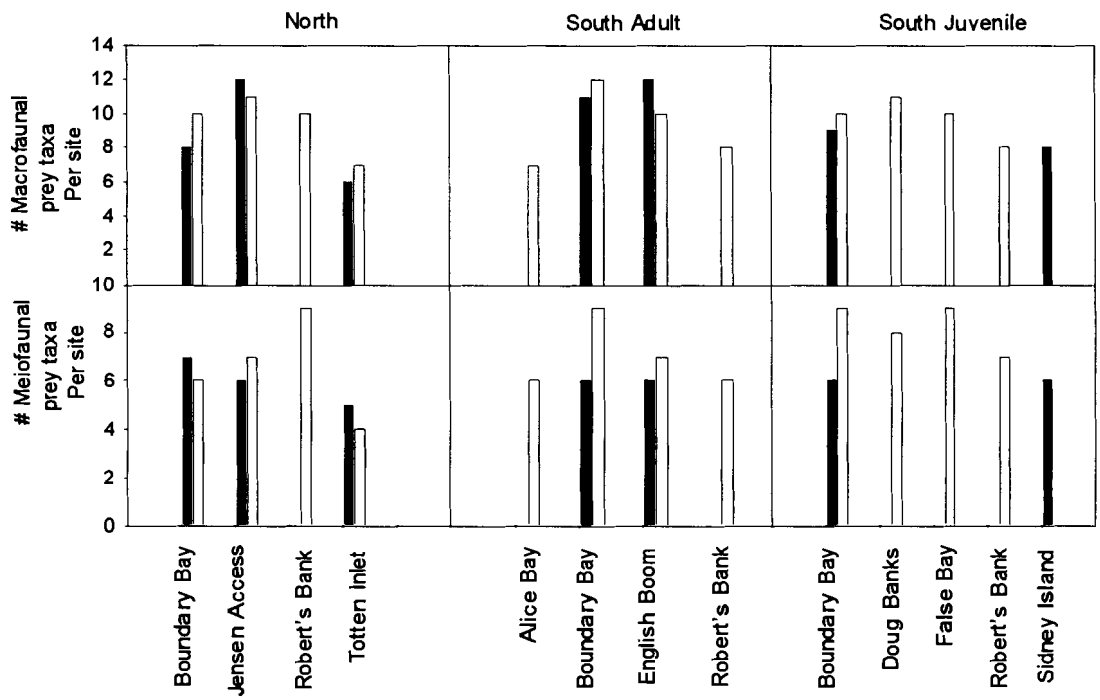

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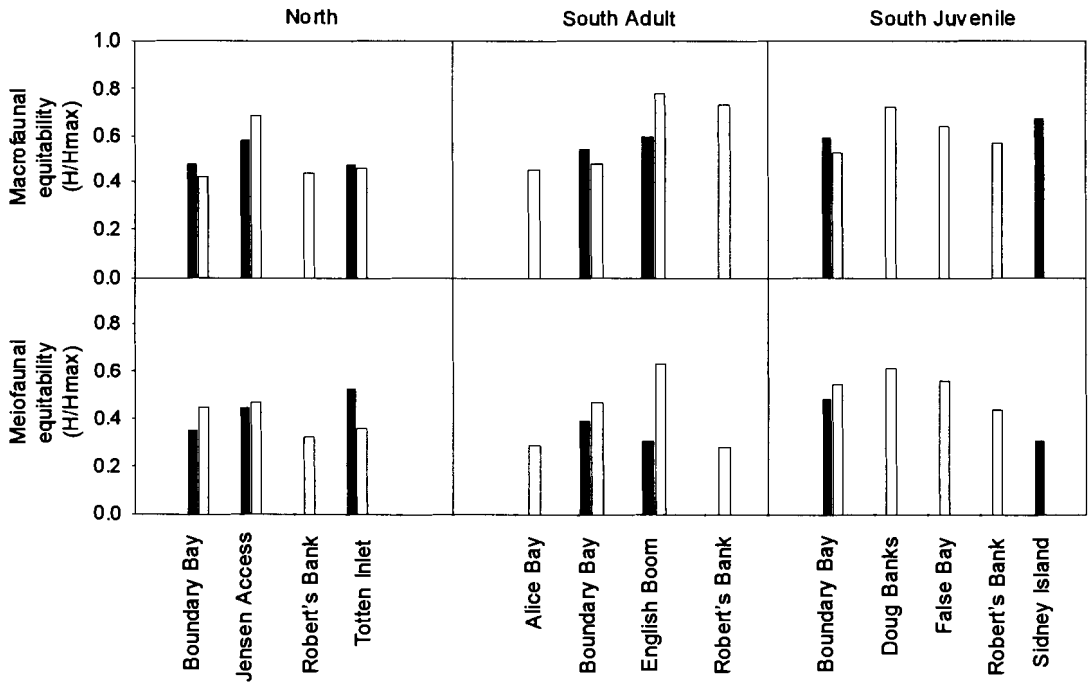

North
South Adult
South Juvenile
Macrofaunal equitability (H/Hmax)
Meiofaunal equitability (H/Hmax)
1.0
0.8
0.6
0.4
0.2
0.0
Boundary Bay
Jensen Access
Robert's Bank
Totten Inlet
Alice Bay
Boundary Bay
English Boom
Robert's Bank
Boundary Bay
Doug Banks
False Bay
Robert's Bank
Sidney Island

Figure 2.3

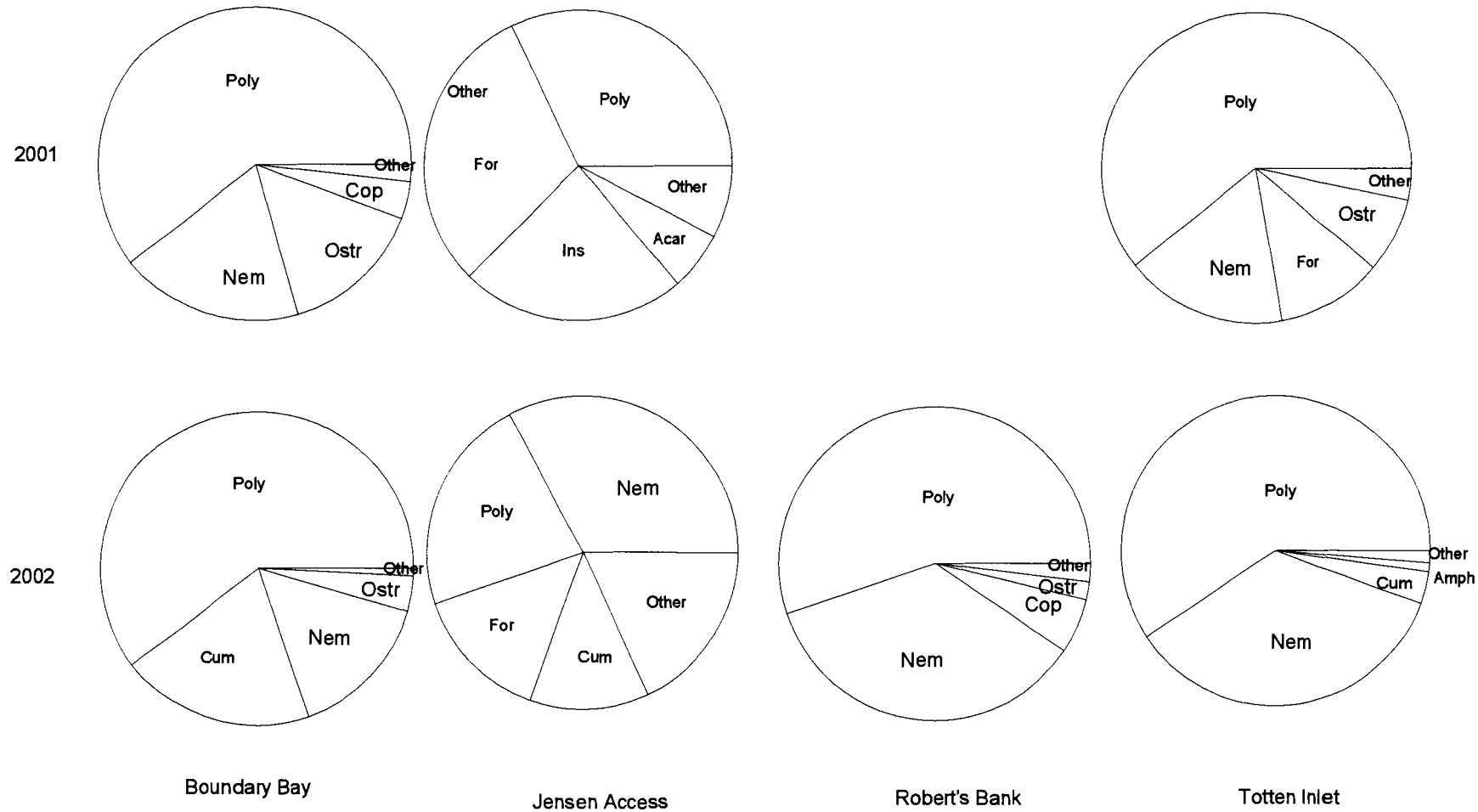
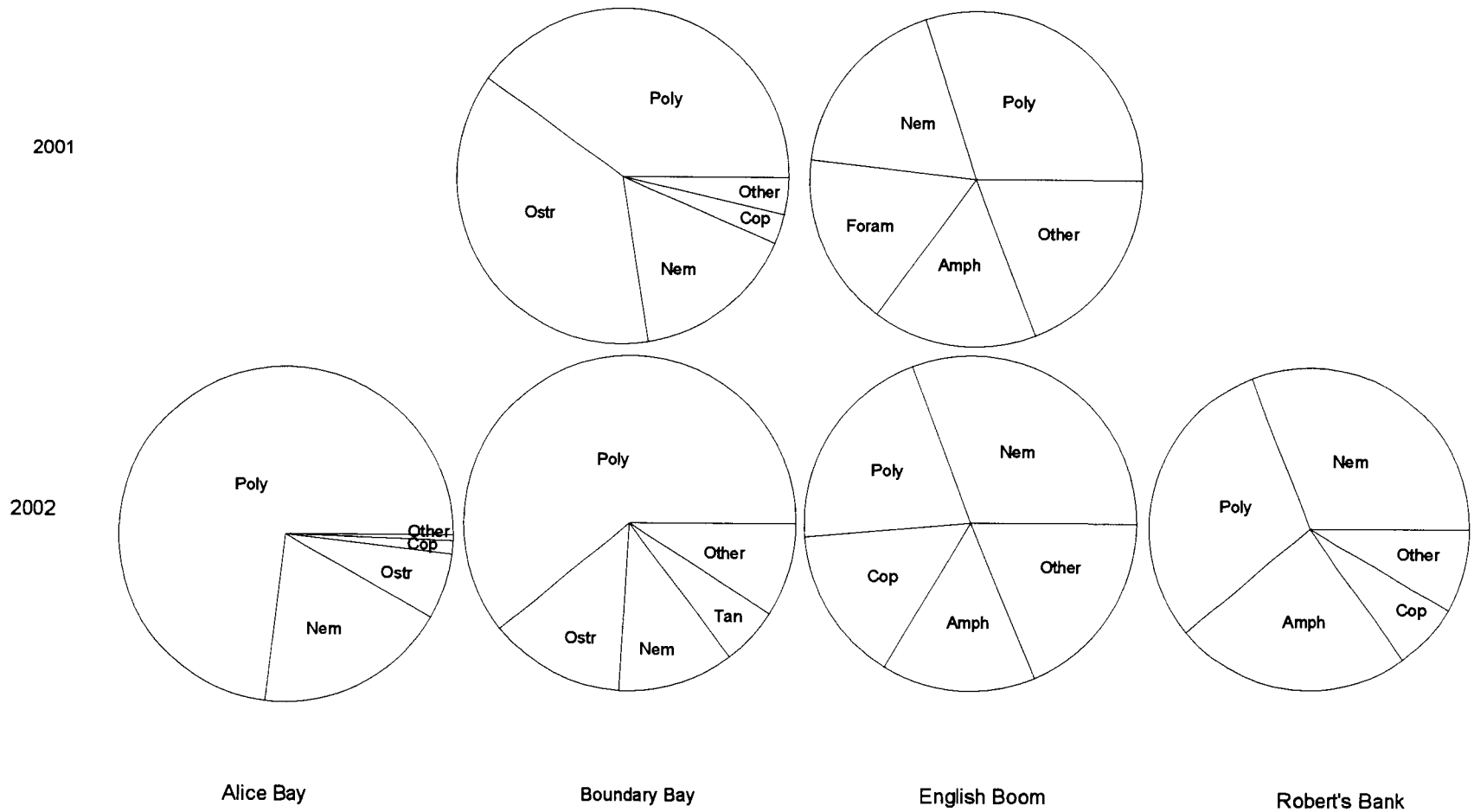

2001
2002
Poly
Other
Cop
Ostr
Nem
Poly
Other
For
Other
Acar
Ins
Poly
Other
Ostr
Nem
For
Poly
Other
Ostr
Cum
Nem
Poly
Nem
Poly
For
Cum
Other
Poly
Other
Ostr
Cop
Nem
Poly
Other
Amph
Cum
Nem
Boundary Bay
Jensen Access
Robert's Bank
Totten Inlet

Figure 2.4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Other
Cop
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Poly
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Foram
Amph
Other
Poly
Other
Cop
Ostr
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Poly
Other
Ostr
Nem
Tan
Poly
Nem
Cop
Amph
Other
Poly
Nem
Other
Amph
Cop
Alice Bay
Boundary Bay
English Boom
Robert's Bank

Figure 2.5


2001
2002
Poly
Other
Nem
Ostr
Tan
Nem
Poly
Other
Amph
Ostr
Poly
Other
Nem
Ostr
Tan
Cop
Amph
Other
Poly
Cum
Nem
Cop
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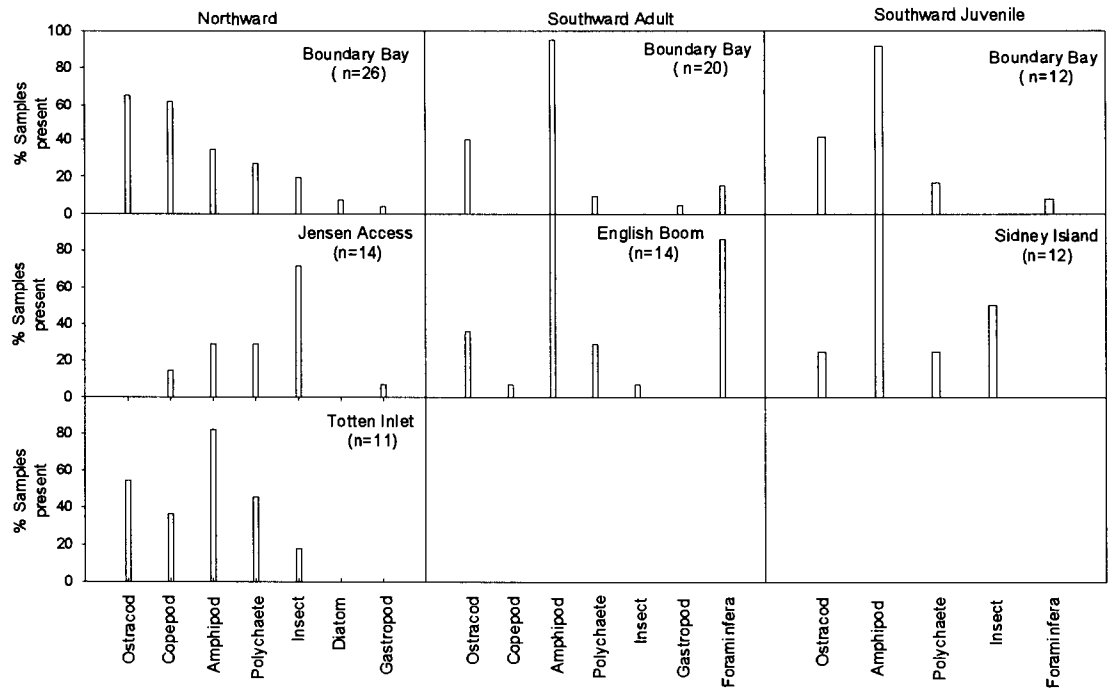
Alice Bay
Boundary Bay
English Boom
Robert's Bank
Sidney Island

Figure 2.6



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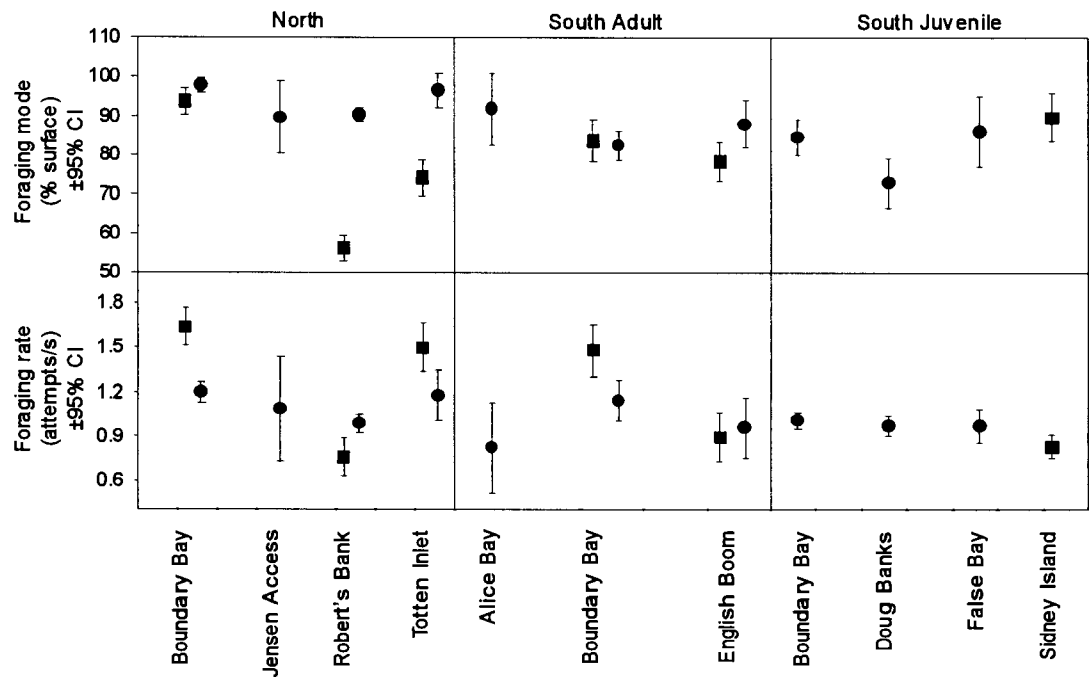

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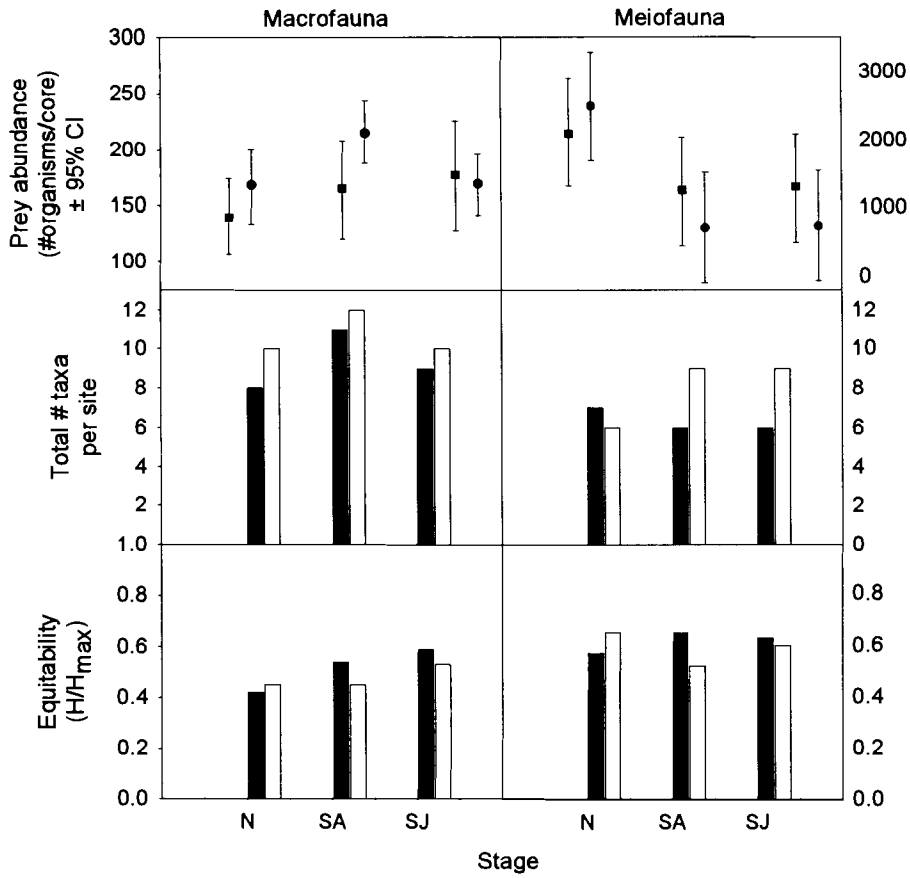
Macrofauna
Meiofauna
Prey abundance (#organisms/core) ± 95% CI
300
250
200
150
100
3000
2000
1000
0
Total # taxa per site
12
10
8
6
4
2
1.0
0
Equitability (H/Hmax)
0.8
0.6
0.4
0.2
0.0
N
SA
SJ
Stage

Figure 2.9

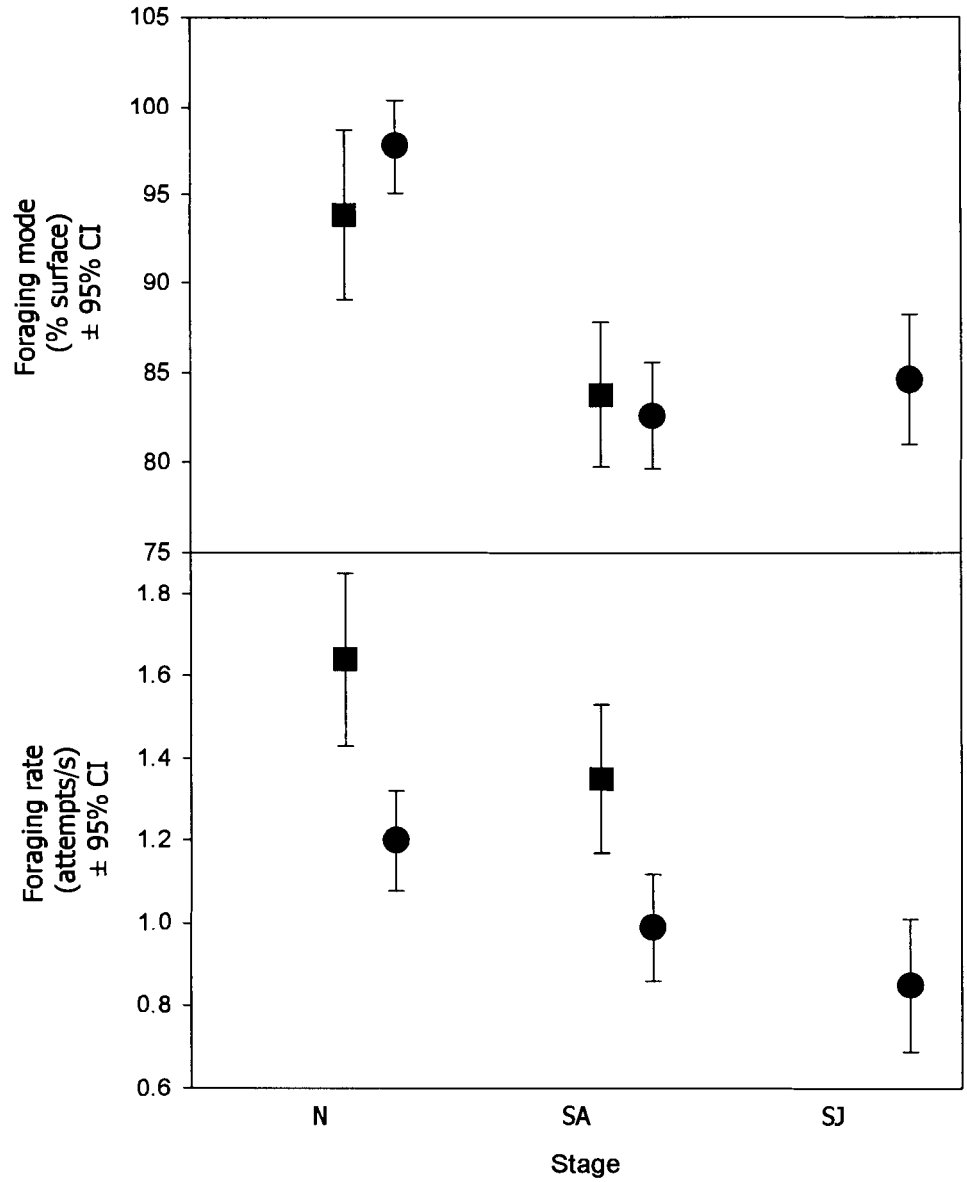
105
100
95
90
85
80
75
Foraging mode
(% surface)
± 95% CI
1.8
1.6
1.4
1.2
1.0
0.8
0.6
Foraging rate
(attempts/s)
± 95% CI
N
SA
SJ
Stage

Figure 2.10

Appendix 2.1

Representation of prey taxa (mean percentage ± 95% CI of core represented by each taxa) for macrofaunal (x > 500µm) fractions of sediment cores taken at several migratory stopover sites used by Western Sandpipers. Sampling stages are defined by migration stage (northward (N), southward adult (SA), southward juvenile (SJ)) and year (2001 (01), 2002 (02)). Only those taxa with over 1% for at least one site are included.

Site	Stage	n	Acari	Amphipod	Copepod	Cumacean	Foram	Insect	Bivalve	Nematode	Ostracod	Polychaete	Tanaid
B	N01	30	0.00 ± 0.00	0.00 ± 0.00	3.84 ± 0.92	0.00 ± 0.00	0.05 ± 0.04	1.55 ± 0.49	0.11 ± 0.06	14.85 ± 1.87	18.70 ± 2.80	60.84 ± 4.07	0.00 ± 0.00
J	N01	12	6.22 ± 1.25	0.73 ± 0.73	3.43 ± 2.53	0.09 ± 0.09	30.41 ± 7.26	23.57 ± 5.91	0.09 ± 0.09	1.24 ± 0.77	1.68 ± 0.66	32.24 ± 6.49	0.18 ± 0.18
T	N01	5	0.63 ± 0.63	0.00 ± 0.00	0.00 ± 0.00	2.66 ± 1.32	11.26 ± 6.97	0.00 ± 0.00	0.00 ± 0.00	16.94 ± 4.92	7.77 ± 2.72	60.74 ± 11.08	0.00 ± 0.00
B	N02	30	0.00 ± 0.00	0.31 ± 0.19	0.07 ± 0.04	19.78 ± 3.25	0.10 ± 0.07	0.03 ± 0.03	0.00 ± 0.00	15.16 ± 1.78	3.69 ± 0.86	60.63 ± 3.81	0.21 ± 0.11
J	N02	30	0.87 ± 0.42	0.11 ± 0.08	0.06 ± 0.06	12.48 ± 1.96	14.17 ± 4.39	7.03 ± 2.95	1.03 ± 0.30	32.84 ± 4.29	0.22 ± 0.15	22.47 ± 2.65	8.72 ± 2.36
R	N02	31	0.00 ± 0.00	1.85 ± 0.53	1.12 ± 0.26	5.65 ± 0.92	0.10 ± 0.04	0.01 ± 0.01	0.03 ± 0.02	35.43 ± 2.50	0.03 ± 0.03	55.20 ± 3.11	0.57 ± 0.16
T	N02	30	0.00 ± 0.00	3.35 ± 1.06	0.08 ± 0.08	0.42 ± 0.26	0.77 ± 0.40	0.00 ± 0.00	0.00 ± 0.00	35.07 ± 3.05	0.92 ± 0.32	59.38 ± 3.13	0.00 ± 0.00
B	SA01	22	0.00 ± 0.00	0.98 ± 0.64	2.88 ± 1.02	0.17 ± 0.13	0.78 ± 0.24	0.29 ± 0.15	0.13 ± 0.06	15.85 ± 2.31	37.57 ± 3.56	40.14 ± 3.20	1.04 ± 0.64
E	SA01	31	1.22 ± 1.08	15.93 ± 4.85	3.29 ± 1.10	0.18 ± 0.18	16.74 ± 5.19	8.00 ± 3.15	3.66 ± 1.48	18.07 ± 4.67	0.07 ± 0.07	30.10 ± 5.35	2.09 ± 0.68
A	SA02	30	0.06 ± 0.04	0.26 ± 0.22	1.29 ± 0.53	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.11	0.00 ± 0.00	18.78 ± 3.42	6.34 ± 4.39	73.05 ± 4.97	0.00 ± 0.00
B	SA02	44	0.00 ± 0.00	3.27 ± 0.73	5.09 ± 0.86	0.09 ± 0.04	0.56 ± 0.27	0.02 ± 0.01	0.01 ± 0.01	11.32 ± 1.08	13.13 ± 2.25	60.89 ± 4.03	5.60 ± 1.10
E	SA02	30	0.00 ± 0.00	14.88 ± 2.77	20.55 ± 3.48	2.28 ± 1.29	6.71 ± 2.61	0.17 ± 0.13	8.17 ± 1.86	30.83 ± 3.92	0.31 ± 0.17	15.02 ± 2.17	1.09 ± 0.56
R	SA02	30	0.00 ± 0.00	23.77 ± 4.33	6.66 ± 1.44	0.15 ± 0.15	2.82 ± 1.07	0.00 ± 0.00	0.00 ± 0.00	30.96 ± 5.36	0.56 ± 0.56	30.23 ± 4.78	4.85 ± 1.83
B	SJ01	16	0.00 ± 0.00	3.35 ± 1.13	1.17 ± 0.39	0.00 ± 0.00	0.32 ± 0.22	0.23 ± 0.11	0.00 ± 0.00	6.45 ± 1.34	40.96 ± 5.12	35.58 ± 5.98	11.90 ± 4.56
S	SJ01	21	0.08 ± 0.08	16.51 ± 4.98	4.81 ± 2.04	0.00 ± 0.00	6.13 ± 2.67	1.91 ± 1.27	0.00 ± 0.00	41.28 ± 5.07	10.45 ± 2.99	18.83 ± 3.60	0.00 ± 0.00
B	SJ02	44	0.00 ± 0.00	7.56 ± 1.90	1.00 ± 0.19	0.01 ± 0.01	0.63 ± 0.30	0.04 ± 0.03	0.00 ± 0.00	8.28 ± 1.16	14.63 ± 2.09	56.40 ± 4.00	11.38 ± 2.08
D	SJ02	25	1.00 ± 1.00	20.56 ± 4.55	42.83 ± 5.32	7.94 ± 2.67	1.49 ± 0.50	1.24 ± 0.44	0.98 ± 0.65	6.24 ± 1.51	4.16 ± 2.14	12.62 ± 2.86	0.94 ± 0.54
F	SJ02	28	0.17 ± 0.17	5.47 ± 1.67	28.15 ± 4.72	0.77 ± 0.26	0.30 ± 0.12	0.00 ± 0.00	0.19 ± 0.17	37.60 ± 5.22	18.75 ± 3.21	8.47 ± 1.96	0.14 ± 0.10
R	SJ02	39	0.00 ± 0.00	5.03 ± 1.69	4.46 ± 0.96	0.46 ± 0.24	0.51 ± 0.23	0.00 ± 0.00	0.00 ± 0.00	50.42 ± 3.80	0.91 ± 0.82	31.90 ± 4.15	6.30 ± 2.28

Appendix 2.2

Representation of prey taxa (mean percentage ± 95% CI of core represented by each taxa) for meiofaunal (63µm > x > 500µm) fractions of sediment cores taken at several migratory stopover sites used by Western Sandpipers. Sampling stages are defined by migration stage (northward (N), southward adult (SA), southward juvenile (SJ)) and year (2001 (01), 2002 (02)). Only those taxa with over 1% for at least one site are included.

Site	Stage	n	Acari	Copepod	Cumacean	Diatom	Foram	Nematode	Ostracod	Polychaete
B	N01	8	0.00 ± 0.00	5.00 ± 1.67	0.00 ± 0.00	22.85 ± 7.90	0.20 ± 0.20	69.69 ± 6.47	1.83 ± 1.17	0.39 ± 0.26
J	N01	8	0.00 ± 0.00	0.92 ± 0.40	0.00 ± 0.00	58.42 ± 13.40	2.83 ± 2.20	32.74 ± 10.23	0.06 ± 0.06	0.00 ± 0.00
T	N01	5	0.00 ± 0.00	2.79 ± 1.16	0.00 ± 0.00	44.93 ± 8.22	0.56 ± 0.35	51.63 ± 7.42	0.09 ± 0.09	0.00 ± 0.00
B	N02	8	0.26 ± 0.26	6.65 ± 1.61	0.00 ± 0.00	25.63 ± 10.21	0.32 ± 0.21	66.88 ± 9.67	0.00 ± 0.00	0.26 ± 0.13
J	N02	8	0.25 ± 0.18	5.04 ± 1.77	0.00 ± 0.00	61.16 ± 11.44	2.02 ± 1.67	31.07 ± 11.19	0.28 ± 0.20	0.19 ± 0.13
R	N02	8	2.58 ± 1.31	11.77 ± 3.25	0.11 ± 0.11	56.81 ± 11.18	0.26 ± 0.18	26.87 ± 6.87	0.20 ± 0.20	1.29 ± 1.16
T	N02	8	0.00 ± 0.00	0.29 ± 0.20	0.00 ± 0.00	85.26 ± 2.28	2.50 ± 1.10	11.95 ± 1.61	0.00 ± 0.00	0.00 ± 0.00
B	SA01	8	0.00 ± 0.00	6.50 ± 3.02	0.00 ± 0.00	11.80 ± 5.37	0.11 ± 0.11	80.10 ± 5.52	1.22 ± 0.43	0.27 ± 0.14
E	SA01	8	0.00 ± 0.00	3.34 ± 1.20	0.00 ± 0.00	65.55 ± 13.04	1.01 ± 0.40	29.87 ± 12.13	0.07 ± 0.07	0.17 ± 0.17
A	SA02	8	0.08 ± 0.04	3.58 ± 1.03	0.00 ± 0.00	88.27 ± 3.48	0.19 ± 0.08	7.67 ± 2.57	0.00 ± 0.00	0.19 ± 0.10
B	SA02	8	0.32 ± 0.32	7.76 ± 3.79	5.70 ± 5.70	5.98 ± 2.29	0.00 ± 0.00	72.67 ± 7.87	3.63 ± 2.13	3.72 ± 2.12
E	SA02	8	1.85 ± 1.03	35.31 ± 8.30	0.00 ± 0.00	35.86 ± 6.27	0.63 ± 0.24	25.45 ± 5.50	0.51 ± 0.35	0.40 ± 0.40
R	SA02	8	0.00 ± 0.00	5.19 ± 2.21	0.00 ± 0.00	49.15 ± 13.67	0.00 ± 0.00	45.04 ± 13.29	0.34 ± 0.34	0.12 ± 0.12
B	SJ01	8	0.00 ± 0.00	8.68 ± 4.57	0.00 ± 0.00	16.00 ± 4.18	0.00 ± 0.00	71.78 ± 7.13	2.71 ± 0.73	0.00 ± 0.00
S	SJ01	8	0.00 ± 0.00	5.52 ± 2.43	0.00 ± 0.00	78.23 ± 5.83	0.97 ± 0.44	14.76 ± 4.70	0.31 ± 0.15	0.00 ± 0.00
B	SJ02	8	0.19 ± 0.19	10.30 ± 2.68	0.00 ± 0.00	23.74 ± 7.97	0.35 ± 0.23	53.19 ± 9.00	5.67 ± 1.07	5.66 ± 3.19
D	SJ02	7	0.00 ± 0.00	18.26 ± 5.16	0.00 ± 0.00	41.17 ± 9.23	0.90 ± 0.42	33.60 ± 9.46	3.30 ± 0.97	1.09 ± 0.41
F	SJ02	8	0.34 ± 0.18	25.64 ± 7.25	2.70 ± 2.70	25.24 ± 7.45	0.11 ± 0.11	44.56 ± 5.96	1.25 ± 0.67	0.12 ± 0.12
R	SJ02	8	0.00 ± 0.00	6.09 ± 2.81	0.00 ± 0.00	48.07 ± 13.91	0.26 ± 0.26	37.64 ± 14.74	7.41 ± 7.11	0.40 ± 0.40

CHAPTER THREE

LANDSCAPE PHYSIOLOGY: SITE DIFFERENCES IN FATTENING RATES, AS INDICATED BY PLASMA METABOLITES IN FREE-LIVING MIGRATORY WESTERN SANDPIPERS (*CALIDRIS MAURI*)

ABSTRACT

Migratory shorebirds stopover on coastal mudflats to feed during migration. One of the primary factors determining an individual's migration success is its ability to refuel (i.e. deposit fat) at these stopover sites. Therefore, habitat quality for migratory shorebirds may be defined as the capacity of stopover sites to support high rates of fattening, and the best measure of habitat quality would be an indication of the degree of fattening achieved during stopover. Previous studies have shown that plasma triglyceride concentrations correlate with mass change in captive birds and can be used to predict rate and direction of mass change in free-living birds. In this study, we demonstrate significant site differences in triglyceride levels in migratory Western Sandpipers (*Calidris mauri*) using stopover sites within the Georgia Basin/Puget Sound region. Stronger site effects were detected during northward than southward migration. Metabolite data provide information not obtainable by mass data alone. Analysis of ecological and physical site characteristics suggests that a) there was a positive relationship between plasma triglyceride levels and total macrofaunal prey abundance, b) there was a positive relationship between plasma triglyceride levels and an index of mudflat exposure (i.e. the site-specific number of kilometer-hours available for foraging), but c) there was no trend in triglyceride levels according to stopover site size. This is the first study to test for correlations between plasma metabolite levels and prey-related factors in free-living birds.

INTRODUCTION

Land management and conservation decisions often rely on the assessment of the value of a particular habitat or set of habitats to a particular species or community, and the characterization of "habitat quality" needs to be based on sound scientific data or else valuable sites may be overlooked or misinterpretations may be made (e.g. Sutherland 1998). Here we test the validity of using a physiological technique, plasma metabolite analysis (Jenni-Eiermann and Jenni 1994, Williams et al. 1999;) to measure and compare habitat quality for different migratory stopover sites used by the long-distance migrant Western Sandpiper (*Calidris mauri)*. Habitat quality is a necessarily complex concept dependent on the species, community or system for which it is being assessed, and is influenced by a multitude of underlying ecological factors such as the size of a site, predation risk, and prey related factors (Sutherland 1996). For migratory birds, such as the Western Sandpiper, one of the primary determining factors underlying an individual's migration success and condition upon arrival on the breeding grounds is its ability to replenish the reserves used as fuel for flight at migratory stopover sites (Alerstam and Lindstrom 1990). Thus, habitat quality in this system is defined as the capacity of migratory stopover sites to support hyperphagia and high rates of mass gain or fattening. In this paper, we use plasma metabolite concentrations to test for inter-site variation in habitat quality and test for correlations between metabolite levels and biological and physical site characteristics .

Fat deposition (mass gain) and fat utilization (mass loss) involve synthesis of metabolic by-products and precursor molecules which are transported via the plasma, and several studies have shown that changes in these various plasma metabolites can be used to estimate rates of mass change (e.g. Jenni-Eiermann and Jenni 1994, Jenni-Eiermann and Jenni 1996, Gannes 2001, Schaub and Jenni 2001). For Western Sandpipers, Williams et al. (1999) showed that the two metabolites that are the strongest predictors of mass change in captive individuals are triglyceride and glycerol. Furthermore, plasma triglyceride was the only metabolite to show significant variation between migration stages in free-living Western Sandpipers (Guglielmo et al. 2002).

Williams et al. (1999) suggested that plasma metabolite analysis might not only provide information on rates of mass change in individual birds, but that it might also be used to compare rates of mass change among populations in relation to habitat quality (see also Schaub and Jenni 2001). Consistent with this idea, Guglielmo et al. (2002) reported a difference in triglyceride levels in Western Sandpipers using two different stopover sites, Boundary Bay and Sidney Island, BC (35 km apart).

In this paper, we used plasma metabolite analysis to investigate variation in fattening rates on a landscape scale over two years within the Georgia Basin/Puget Sound region of British Columbia, Canada, and Washington, USA (part of the Pacific Northwest flyway used by migrating Western Sandpipers). The specific objectives of the study were to determine whether, a) there are significant inter-site differences in plasma metabolite concentrations, b) individual sites are characterized by consistent metabolite concentrations across years and migration stages, and c) site-related variation in metabolite levels correlates with variation in biological and physical site characteristics.

METHODS

Study sites and sample collection

Western Sandpipers were captured at multiple sites in 2001 and 2002, during northward, spring migration (April/May, hereafter referred to as "northward") and southward, fall migration (hereafter referred to as "southward") for both adults (July) and juveniles (August) using mist nets (Avinet, Dryden, NY, USA). We treated southward adult and juvenile migration as two separate migration stages since this allows for a third independent test for site differences and, in addition, avoids confounding age and time of year effects. We maintained a constant mist-netting effort throughout all three migration stages in both years at Boundary Bay (Fraser River Delta), which served as our reference site. In each migration stage, at least one and up to three other sites were sampled concurrently and compared to the reference site. Location, physical characteristics, and sampling periods for each site are detailed in Table 1.1 (see also Chapter 1 for more detailed descriptions). Not all sites were sampled during all migration stages and years in part due to differential bird use (see Discussion); only those sites sampled concurrently were compared in subsequent analyses. Banding and

all animal handling was carried out under USFWS, WDFW, Environment Canada, and Simon Fraser University Animal Care permits.

Mist nets were constantly in view during banding and time to blood sampling was recorded from when birds first hit the net. All birds were weighed and blood sampled (up to 300 μL) via brachial venipuncture. Blood was kept cool, centrifuged at 5000 RPM for three minutes within 2 hours of sampling, and the plasma was either stored at -20°C immediately or frozen and transported in liquid nitrogen for up to two weeks and then stored at-20°C until assayed. The culmen and tarsus were measured, and (in 2002 only) each individual was given a fat score from 0-6 (according to Helms and Drury 1960) in 0.5 increments. Individuals were sexed by culmen length (male < 24.2 mm, female > 24.8 mm, intermediate values = unknown; Page and Fearis 1971) and aged by plumage characteristics (according to Prater et al. 1977). Macrofaunal prey abundance (number of organisms per core) was measured throughout the sampling period during active foraging bouts of Western Sandpipers in the same location as the banding effort (see Chapter 2 for methods). Mudflat exposure index, the mean number of kilometer-hours of mudflat exposed in a 24h period, represents the opportunity to forage provided by each site and was calculated according to methods described in Chapter 2.

Metabolite Assays

Free glycerol and triglyceride were assayed via sequential color endpoint assay (Trinder reagent A and B, respectively, Sigma-Aldrich Canada, Oakville, Ontario), using 5 μL of sample with 240 μL and 60 μL of reagents A and B respectively, with a reading taken at 540nm after 10 minutes 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, Denmark) and read with a microplate spectrophotometer (Biotec 340EL). Each plate was run with a standard curve based on a serial dilution of 2.54 mmol glycerol (Sigma-Aldrich), and a 19-day old hen plasma pool used to calculate inter-assay coefficient of variation. Inter-assay coefficients of variation were 6.6% and 3.7% (n=15), and intra-assay coefficients of variation were 3.2% and 3.9% (n=6) for glycerol and triglyceride, respectively.

Statistical Analysis

Both metabolite levels were non-normally distributed, so we transformed the data using ln(metabolite+0.5) to make them normally distributed (Shapiro-Wilks, $P > 0.4$, all cases). There was no correlation between plasma triglyceride levels and plasma glycerol levels ($P > 0.8$) so these metabolites were analyzed separately. In order to only compare those sites sampled concurrently, all analyses were performed by migration stage (northward, southward adult, or southward juvenile). After correcting for body size, as indicated by tarsus length, body mass was compared between stopover sites using type III analysis of covariance (ANCOVA). We confirmed the significant relationship between both metabolites and both body mass and bleed time (Guglielmo et al. 2002; see Results), so metabolite levels were compared between sites using type III ANCOVA, with body mass and bleed time as covariates. Metabolite values are reported as least squares means, and these corrected triglyceride and glycerol values will hereafter be referred to as triglyceride and glycerol concentrations, respectively. For body mass and metabolites, the year*site interaction was tested based on the two or three sites sampled in both years, and those factors with a significant interaction were analyzed for each year separately; otherwise, the model for that factor was reanalyzed without the interaction term. Seasonal differences (between migration stages) in body mass and metabolites were tested within Boundary Bay in both years and Robert's bank in 2002 via type III ANCOVA for year and stage. The year*stage interaction was tested, and those factors with a significant interaction were analyzed for each year separately; otherwise, the model for that factor was reanalyzed without the interaction term. For each ANCOVA that detected a significant effect, multiple comparisons were tested controlling for experiment-wise error $\alpha=0.05$ using Bonferroni adjustment, and only significantly different pairs are reported. The relationship between triglyceride levels and stopover site characteristics were tested on site means independently for each factor, within northward and southward migration separately, using Pearson's correlation for total macrofaunal prey abundance and linear regression for mudflat exposure index. Jensen Access during northward migration 2001 was an ecological anomaly (see Discussion for more details) and, as such, was excluded from the analyses. Graphical representations of the data include Jensen Access 2001, but that data point was excluded from the calculation of the major axis of correlation (for prey abundance) and

the regression line (for mudflat exposure index). All statistical analyses were performed using SAS 6.0.

RESULTS

Effects of bleed time, body mass, and sex

Bleed time, defined as the number of minutes between capture and blood sampling, averaged 16.8 ± 11.4 min, and 95% of the bleed times were less than 36 minutes. Plasma triglyceride levels were negatively related to bleed time ($F_{1,464} = 8.63$, $P < 0.005$, $m = -0.005$), and plasma glycerol levels were positively related to bleed time ($F_{1,464} = 7.63$, $P < 0.01$, $m = 0.003$). Overall, plasma levels of both metabolites were mass dependent: glycerol levels were negatively related to mass ($F_{1,463} = 15.75$, $P < 0.001$, $m = -0.013$), triglyceride levels were positively related to body mass ($F_{1,463} = 75.81$, $P < 0.0001$, $m = 0.048$), and there was no difference in slope between sexes ($F_{1,449} = 0.40$, $P > 0.5$). There were no significant site differences for either culmen or tarsus for any migration stages in either year except for culmen during southward adult migration in 2002 ($F_{3,58} = 3.77$, $P = 0.02$). Given that birds at Robert's Bank (75% male) have shorter bills than those at Boundary Bay (8% male), this difference can most likely be explained by the highly skewed sex ratio and small sample size in this capture sample. Sex ratios (expressed as percentage male) of blood sampled birds each year at each site during northward, southward adult and southward juvenile migrations are reported in Table 3.1. Overall, both corrected metabolite values were independent of sex ($P > 0.2$, both metabolites). Furthermore, there was no significant relationship between sex and either metabolite ($P > 0.1$, all cases) within site for those sites with the largest sample sizes for each migration stage in 2002, (see Table 3.2), which allow the most robust test of sex differences. These analyses suggest that any biased sex ratio in the capture sample will not affect the outcome of the analysis for site differences in metabolite levels, and this is consistent with findings from a previous study (Guglielmo et al. 2002). We therefore pooled sexes for all subsequent analyses which allowed us to include metabolite data for individuals of unknown sex. Overall, there was no significant difference in either corrected metabolite value between after-second year adults (ASY)

and second year adults (SY, born the summer of the year prior to catching) (P>0.1, both metabolites). We therefore pooled values for ASY and SY adult individuals in northward and southward adult migrations.

Overall, triglyceride levels were positively related to fat score ($F_{1,319}$ = 58.21, P < 0.0001, m = 0.113) and glycerol levels were negatively related to fat score ($F_{1,319}$ = 31.61, P < 0.0001, m = -0.053). Plasma triglyceride level was positively related to fat score during each migration stage (P < 0.01, all stages). Glycerol levels, however, were only significantly correlated to fat score during southward adult and juvenile migrations (P < 0.02, both stages); during northward migration, glycerol levels were independent of fat scores (P > 0.4).

Site differences in body mass and metabolites

Northward migration

During the northward migration (April/May) there was a significant year*site interaction term for triglyceride levels ($F_{2, 199}$ = 7.79, P < 0.001); therefore we analyzed each year separately. Triglyceride levels varied among sites in both years (2001: $F_{2,57}$ = 8.85, P < 0.002; 2002: $F_{3, 140}$ = 10.59, P < 0.0001; Figure 3.1). In 2001, birds at Boundary Bay had higher triglyceride levels than those at Jensen Access (P < 0.002). In 2002, Boundary Bay, Robert's Bank and Jensen Access all had higher triglyceride levels than Totten Inlet (P < 0.005 all cases), but Jensen Access did not differ from Boundary Bay (P > 0.5). There was no intra-site difference in triglyceride levels between years for two of three sites (Boundary Bay and Totten Inlet, P > 0.2 both sites), but there was a significant difference for Jensen Access (P < 0.0001). The year*site interaction term for glycerol levels was not significant ($F_{2,199}$ = 1.73, P > 0.15), so it was dropped from the model. There was a significant year effect ($F_{1, 201}$ = 7.16, P < 0.01), with values being higher in 2002. There was a marginally significant site difference in glycerol levels ($F_{3, 201}$ = 2.79, P < 0.05), with birds at Jensen Access having higher corrected glycerol values than those at Boundary Bay (P = 0.04). The year*site interaction term for size-corrected body mass was not significant ($F_{2,194}$ = 2.76, P > 0.05), so it was dropped from the model.

Size-corrected body mass was independent of both year ($F_{1, 196} = 0.09$, $P > 0.7$) and site ($F_{3, 196} = 2.01$, $P > 0.1$, Figure 3.1).

Southward adult migration

During southward adult migration (July), for both metabolites, the year*site interaction was not significant (triglyceride: $F_{1,107} = 2.89$, $P > 0.05$; glycerol: $F_{1,107} = 1.75$, $P > 0.15$), so it was dropped from the model. Triglyceride levels were independent of year ($F_{1, 108} = 0.23$, $P > 0.5$), but there was a marginally significant site effect ($F_{3, 108} = 2.66$, $P = 0.05$, Figure 3.2). Birds at Alice Bay had marginally higher triglyceride levels than those at Boundary Bay ($P = 0.05$). Glycerol levels were independent of both year and site ($P > 0.1$, both variables). There was a significant year*site interaction term for size-corrected body mass ($F_{1,100} = 19.52$, $P < 0.0001$); therefore we analyzed each year separately. In both years, there was significant inter-site variation in size corrected body mass (2001: $F_{1,43} = 5.94$, $P < 0.02$; 2002: $F_{3,56} = 9.09$, $P < 0.0001$; Figure 3.2). In 2001, birds at English Boom were heavier than those at Boundary Bay; in contrast, in 2002, the birds at Boundary Bay were heavier than those at English Boom ($P < 0.002$), and, in addition, the birds at both Alice Bay and Boundary Bay were heavier than those at Robert's Bank ($P < 0.02$, both cases). There was a significant year effect within the two sites that were sampled in both years (Boundary Bay: $F_{1, 37} = 14.74$, $P < 0.001$; English Boom: $F_{1, 37} = 5.16$, $P < 0.03$), with birds in 2002 being heavier than those in 2001 at Boundary Bay and vice versa for English Boom.

Southward juvenile migration

Sampling was very unbalanced during southward juvenile migration (August); therefore we analyzed each year separately. Triglyceride levels were independent of site in 2001 ($F_{1, 28} = 2.79$, $P > 0.1$), but there was significant inter-site variation in 2002 ($F_{3, 103} = 3.35$, $P < 0.03$; Figure 3.3); juveniles at Robert's Bank had higher values than those at Boundary Bay ($P < 0.03$). Glycerol levels were independent of site in 2001 ($F_{1, 28} = 1.64$, $P > 0.2$), but there was significant inter-site variation in 2002 ($F_{3,103} = 5.47$, $P < 0.002$, Figure 3.3); juveniles at False Bay had higher values than those at both Boundary Bay

and Robert's Bank ($P < 0.004$ both cases). There were no significant differences between years within Boundary Bay in either metabolite ($P > 0.1$, both metabolites). In both years, there was a significant site difference in size-corrected body mass (2001: $F_{1,29} = 10.15$, $P < 0.005$; 2002: $F_{3,104} = 9.20$, $P < 0.0001$; Figure 3.3). In 2001, birds at Boundary Bay were heavier than those at Sidney Island, and in 2002, birds at Robert's Bank were heavier than those at Boundary Bay and False Bay ($P < 0.005$ both cases). There was a significant year effect within Boundary Bay ($F_{1,38} = 4.89$, $P < 0.04$), with birds using the site in 2001 being heavier than those using it in 2002.

Inter-seasonal differences in body mass and metabolites within sites

To avoid the complication of different sites sampled during different migration stages, we looked for inter-seasonal variation (between migration stages) within Boundary Bay, sampled in each stage in both years, and within Robert's Bank, sampled during each stage in 2002. Within Boundary Bay, the year*stage interaction for both triglyceride and glycerol levels was nonsignificant (triglyceride: $F_{2,148} = 3.07$, $P = 0.05$; glycerol: $F_{2,148} = 1.36$, $P > 0.2$), so it was dropped from the model. Triglyceride levels were independent of both year ($P > 0.05$) and migration stage ($P > 0.5$, Figure 3.4). Glycerol levels were independent of year, but there was a significant stage effect ($F_{2,150} = 12.97$, $P < 0.0001$; Figure 3.4). Individuals using the site during northward migration had higher glycerol levels than those using it during southward adult and juvenile migrations ($P < 0.05$, both cases). There was a significant year*stage interaction term for size-corrected body mass ($F_{2,147} = 11.41$, $P < 0.0001$), so we analyzed years separately. In both years, birds using the site during different migration stages differed significantly in size-corrected body mass (2001: $F_{2,67} = 3.13$, $P = 0.05$, 2002: $F_{2,80} = 18.81$, $P < 0.0001$; Figure 3.4), though the difference in 2001 was marginal. In 2002, birds using Boundary Bay during both northward and southward adult migrations were heavier than those using it during southward juvenile migration ($P < 0.0001$, both cases).

Similar trends were detected within Robert's Bank in 2002. There was no significant stage effect on triglyceride levels ($F_{2,109} = 2.28$, $P > 0.1$), but there was a significant difference between migration stages in glycerol levels ($F_{2,109} = 24.27$, $P < 0.0001$). Birds using Robert's Bank during northward migration had higher glycerol

levels than those using the site during both southward adult and juvenile migrations ($P < 0.01$, both cases). There was a significant effect of stage on size-corrected body mass at Robert's Bank ($F_{2,110} = 4.90$, $P < 0.01$); birds using the site during both northward and southward juvenile migrations were heavier than those using it during southward adult migration ($P < 0.02$ both cases).

Relationship between metabolites and stopover site characteristics

The migratory stopover sites sampled in this study were found to vary significantly between sites in prey-related factors, foraging behavior, and physical factors during all three migration stages (Chapter 2, this volume). During northward migration, there was a positive relationship between the mean total macrofaunal prey abundance (per core) and mean triglyceride levels based on site averages ($r = 0.80$, $P = 0.05$; Figure 3.6a). There was a significant positive relationship between mean plasma triglyceride levels and mudflat exposure index ($R^2 = 0.84$, $P < 0.01$, $m = 0.02$; Figure 3.6b). There was no significant relationship between mean triglyceride levels and either factor during southward migration ($P > 0.2$, both factors).

DISCUSSION

Our most important findings were that 1) site differences were detected in triglyceride levels between migratory stopover sites during all three migration stages, 2) site differences were stronger for triglyceride levels than glycerol levels and these two metabolites were not correlated, 3) site differences in triglyceride levels were more pronounced during northward than southward migration, 4) there was inter-annual consistency in metabolite levels within sites, except Jensen Access which reflected changes in microhabitat, and 4) variation in triglyceride levels during northward migration was positively correlated to prey abundance and foraging opportunity (mudflat exposure index).

This study clearly demonstrates inter-site variation in plasma triglyceride levels of migratory Western Sandpipers within the Georgia Basin Puget Sound. Several studies suggest that plasma triglyceride levels are related to the rate of mass change in migratory birds (Jenni-Eiermann and Jenni 1994, Williams et al. 1999, Jenni and Schwilch 2001) and, as such, serve as an indicator of fattening in free-living individuals (Jenni-Eiermann and Jenni 1996, Jenni-Eiermann and Jenni 1998, Gannes 2001, Schaub and Jenni 2001, Guglielmo et al. 2002). Therefore, we contend that in this study plasma triglyceride levels are indicative of the relative degree of fattening achieved by migratory Western Sandpipers during stopover.

Plasma triglyceride levels were consistent within site between years, with the exception of Jensen Access where the microhabitat varied between years. Furthermore, triglyceride levels were independent of migration stage within the two reference sites, Boundary Bay and Robert's Bank. Both sites supported similar triglyceride levels during northward (adult) and southward adult migrations but differed between sites during southward juvenile migration. This may suggest age-dependent (juvenile vs. adult) variation in plasma triglyceride levels. In contrast to triglyceride levels, size-corrected body mass varied within both Boundary Bay and Robert's Bank between migration stages; however the pattern of variation was not consistent between the two sites. This suggests that while birds of variable mass use these sites during different stages, each site supports similar triglyceride levels regardless of migration stage. In addition, in every case, the significance of site differences in size-corrected body mass and triglyceride levels were opposite (i.e. if there was difference in mass, there was no difference in triglyceride levels and vice versa). This highlights the difference in information provided by each technique; size corrected body mass reflects which birds are using the site while triglyceride levels reflect what the birds do once arriving at a particular site.

Biological significance of metabolite levels

Lipids, either absorbed from the diet or synthesized in the liver, have the predominate destination of oxidation for energy (Ramenofsky 1990, Guyton & Hall 1996). This unambiguous function of fat allows for straightforward interpretation, and

the synthesis of multiple studies has developed the clear biological interpretation of plasma triglyceride levels in several high-fattening migratory systems (Jenni-Eiermann & Jenni 1998, Guglielmo 1999). High levels of plasma triglyceride are indicative of an individual in the process of depositing lipids in adipose tissue. The positive relationship between plasma triglyceride levels and mass change has been demonstrated in captive systems of both passerines and shorebirds (Jenni-Eiermann and Jenni 1994, Williams et al. 1999). Plasma triglyceride concentrations have also been shown to vary between migration stages in both systems (Jenni & Jenni-Eiermann 1996, Guglielmo et al. 2002).

Plasma concentrations of endogenous glycerol have been less extensively examined in free-living systems than plasma triglyceride and the relationship between glycerol levels and physiological condition seems less clear. Glycerol levels of migrating passerines did not differ between actively foraging and fasted individuals; however, elevated glycerol levels were detected in recently flown (and fasted) individuals (Jenni-Eiermann and Jenni 1991), suggesting that the activity level of an individual may impact glycerol metabolism. Similarly, Gannes (2001) detected elevated levels of glycerol in recently arrived passerine migrants. Both of these studies suggest that a non-foraging factor (i.e. exercise) may impact realized glycerol levels; this fact may complicate the interpretation of glycerol levels in free-living migratory systems. Furthermore, Gannes (2001) detected differences in several plasma metabolites between four passerine species with varied life history traits; glycerol was the only metabolite (of 6) that did not differ between species. In our study, in contrast to triglyceride levels, only marginal inter-site variation in glycerol levels was detected and glycerol levels were year-dependent during northward migration. Furthermore, within Boundary Bay and Robert's Bank, glycerol levels were higher during northward than southward migration. The between-year and between-stage differences in glycerol levels may be due to differences in activity level (e.g. amount of flying during foraging bouts) or proportion of new arrivals sampled.

While glycerol is a metabolic component of lipid metabolism, and should be straightforward to interpret, the biological significance of plasma glycerol levels has proven more problematic. Although there was a significant negative relationship between plasma glycerol levels and mass change in captive western sandpipers (Williams et al. 1999), glycerol levels did not vary between migrating and overwintering free-living Western Sandpipers (Guglielmo et. al. 2002). Given the positive and

negative correlations obtained in captive systems between mass change and plasma levels of triglyceride and glycerol, respectively, one would expect a negative correlation between triglyceride and glycerol levels. The lack of correlation between glycerol and triglyceride levels demonstrated in our study is evidence of the lack of understanding of the complete physiological picture behind glycerol. In free-living Western Sandpipers, there are individuals with high levels of both triglyceride and glycerol. We are confident in the interpretation that these birds are undergoing fat deposition. The traditional interpretation of glycerol concentrations would suggest that these individuals are also utilizing their fat stores. Alternatively, it is possible that either glycerol serves another physiological function that is not yet understood or that the efficiency of assimilation of dietary lipids affects the glycerol levels, making their interpretation difficult. This suggests that the relationship between plasma triglyceride and glycerol levels may be important in interpreting the physiological condition of an individual. However, combining these two values into a single predictive value would be premature due to our lack of understanding in this relationship.

Detecting site differences: possible confounding factors

Sampling issues

Microhabitat use. Triglyceride levels differed between years during northward migration at Jensen Access. This is possibly explained by changes in features of the microhabitats used by birds between the two years. In 2001, catching occurred within the 200m stretch of marshy hummocks where birds congregated to forage on the first and last exposed bits of mud during a falling and rising tide, respectively. The flock flew in from or departed to sections of the larger Skagit Bay; this is in contrast to the gradual arrival and dispersal from the low tide line that occurs at most sites. While foraging in the marsh, birds pecked and probed directly into the sides of the hummocks which contained a more terrestrial prey base including insects and mites (see Chapter 2). During southward migration, this hummock marsh was completely vegetated and was not available to foraging shorebirds; this is the reason that birds within Skagit Bay were sampled at two different sites, Jensen Access and English Boom, during northward and southward migrations, respectively. During northward migration in 2002, the marsh at

Jensen Access still consisted of exposed mud between the hummocks; however, it was much more densely vegetated than in 2001, and birds concentrated their foraging on the firmer sandy flats along the edge of the marsh, rarely venturing into the hummock marsh. Birds foraging in the hummock marsh during northward migration in 2001 had lower triglyceride levels than birds at other sites during that season. However, in 2002, the birds foraging on the sandflats within the same macrosite had metabolite levels similar to the other sites sampled during that season. This difference in microhabitat most likely explains the inter-annual variation in metabolite levels at this site. The fact that this difference in microhabitat was detected by plasma triglyceride levels is further evidence of the robust ability of this technique to reflect the habitat quality of stopover sites used by free-living migratory individuals at or near the location of capture. While the two different microhabitats sampled within Jensen Access (i.e. 2001 versus 2002) did not differ in total macrofaunal prey abundance, the hummock marsh prey base had more terrestrial taxa (e.g. insects) than the sandflats. The hummock marsh that was foraged in 2001 represents an ecological anomaly and the sandpipers achieved unexpectedly low triglyceride levels given the prey abundance. This site demonstrates the importance of definition of microsites as well as the dynamic nature of migratory stopover sites.

Capture dates. Because not all sites were sampled concurrently, if there is an increasing or decreasing trend in metabolites with date within a migration stage, it is possible that the site effect is in fact an artifact of a within stage temporal effect rather than an actual difference between sites. Mean capture dates, expressed as julian date, did vary at the different sites within each migration stage (Table 3.2). Within Robert's Bank and Boundary Bay, which were sampled continuously, there was a positive relationship between triglyceride levels and capture date (i.e. late caught birds had higher triglyceride levels than early caught birds) during northward and southward adult migration. However, during northward migration, the mean capture date is earlier at Boundary Bay which supported higher triglyceride levels than both Jensen Access and Totten Inlet (which had later capture dates) in 2001. This is the opposite trend than that expected if the site difference were due to capture date. Furthermore, sampling effort at Boundary Bay was continuous to allow for same-time comparison of all sites with this reference site. All significant pairwise site differences in metabolites include Boundary Bay, a comparison that is independent of capture date biases. The only exception is

during southward adult migration in 2002 when, while capture effort at Boundary Bay was continuous, actual catching at Boundary Bay and Alice Bay did not overlap. Because birds at Alice Bay had higher triglyceride levels than those at Boundary Bay, and birds at Alice Bay were caught later in the season, the difference in triglyceride levels between these two sites could potentially be explained by capture date. However, mean capture date at Alice Bay was also later than that at English Boom which did not differ in capture date from Boundary Bay, but triglyceride levels of birds using Alice Bay and English Boom were not significantly different. Therefore, if the difference in triglyceride levels between Alice Bay and Boundary Bay was a spurious result due to difference in capture date, birds at Alice Bay should also have higher triglyceride levels than those at English Boom (also caught earlier) and they were not. While it can not be definitively ruled out, there is no substantial evidence that site differences in triglyceride levels in this study are due to a confounding capture date effect.

Sex biases

Sex ratios of captured individuals did depart from 1:1 at several sites, although in most cases not dramatically. Biased sex ratios may originate from either differential use of the sites by different sex and age classes (observed at some sites) and/or sampling bias. Given time constraints involved in concurrently sampling multiple stopover sites within one migration stage, sample sizes are often relatively low in our study. Pooling metabolite levels for all individuals within a site increases the power to detect differences between sites, the primary objective of this study. There is no evidence from our results of a difference in corrected metabolite levels between sexes, a conclusion that is support by a previous study within substantial sample sizes (Guglielmo et al. 2002).

Mass change not equal to fat change

One potential confounding factor inherent in using plasma lipid metabolites as an index for mass change is the underlying implication that all mass change is in the form of change in fat mass. Several studies have documented that migration and migratory stopover involve utilization and restoration, respectively, of non-fat tissues such as

muscle and organs (Jenni and Jenni-Eiermann 1998, Karasov and Pinshow 1998, Piersma et al. 1999, Battley et al. 2000, Jenni and Jenni-Eiermann 2000). This brings into question the utility of plasma triglyceride levels as an index for mass change if, in fact, a portion of that mass change is in non-lipid tissues. In the Western Sandpiper, it has been determined that up to 40 percent of mass change is due to change in lean or muscle mass (Guglielmo and Williams 2003). However, this change in lean and muscle mass only occurred after the onset of flight exercise (Guglielmo and Williams 2003), suggesting that it may represent a smaller proportion of total mass change at stopover sites. The correlation between triglyceride levels and subcutaneous fat loads (i.e. fat scores) found in our study suggests that the plasma concentration of this metabolite does increase with high levels of deposited fat. There is a positive correlation between plasma triglyceride levels and mass change in captive Western Sandpipers (Williams et al. 1999), indicating that triglyceride levels are a good predictor of total mass change even if a portion of that mass is not due to change in lipid tissue. In our study, triglyceride levels also correlated to muscle load (i.e. muscle score) (Seaman and Williams unpubl. data). There is no biochemical reason that plasma triglyceride levels would fluctuate according to muscle metabolism, and this relationship is probably due to fat and muscle being correlated. If fat deposition coincided with muscle catabolism, then the mass lost due to loss of muscle mass would confound the relationship between total mass change and lipid metabolite levels. Given that fat and muscle scores are correlated in this system, this is not a likely possibility.

Explanation of variation in metabolite levels

A portion of the variation in triglyceride levels between stopover sites during northward migration was explained by the variation in total macrofaunal prey abundance. The positive nature of the correlation coefficient is suggestive of a positive relationship between these two variables on a landscape scale. A statistical shortcoming of this analysis is that the Pearson correlation does not take into consideration the error inherent in both variables (B. Smith, pers. comm.). However, due to small sample sizes and large variation, particularly in prey abundance which has been previously reported in this system (Sewell and Elner 2001), there was not sufficient power to analyze this data using a model that takes standard error into consideration (B. Smith, pers. comm.).

While the correlation is only marginally significant, there is an evident trend that birds achieving the highest degree of fattening did so at the sites containing the most prey. We recognize that this measure of prey abundance is a gross measure of total availability and does not consider prey selectivity which has been demonstrated be important in this system (Sutherland et al. 2000, Wolf 2001). Furthermore, total macrofaunal abundance is only one of many prey-related factors that may determine habitat quality. However, to our knowledge, this is the first study to report a positive correlation between plasma metabolite levels and any measure of food availability in free-living birds.

The statistically significant positive relationship between triglyceride levels and mudflat exposure index during northward migration suggests that the spatial and temporal opportunity to forage partially influence the degree of fattening achieved by birds using migratory stopover sites. The mudflat exposure index is empirically calculated based on distance to shore and tidal height for the sampling period of each analysis, and the recalculation of the index for the same sampling period would not yield different results; therefore, the linear regression analysis for this factor does not violate the assumption of no error for the x variable. To our knowledge, the influence of the opportunity to forage at migratory stopover sites on fattening has not previously been tested. However, it is not unexpected that birds would be able to achieve higher rates of fattening at sites at which they are able to forage over a larger area for more hours over a 24h period.

Guglielmo et al. (2002) demonstrated that Western Sandpipers foraging at Sidney Island, a small protected site, had higher fattening rates than those at Boundary Bay, a large open mudflat. Lissimore et al. (1999) further demonstrated that shorebirds using the smaller Sidney Island are lighter than those using the larger Boundary Bay, and Ydenberg et al. (2002) hypothesized that this represented a trade-off between high fattening potential and risk of predation. Our study, however, does not provide any evidence of a more general trend of small sites supporting high fattening rates for lighter individuals. During northward migration, Western Sandpipers foraging at Totten Inlet, a small protected inlet of similar size to Sidney Island, had lower triglyceride levels than those at Boundary Bay, an open expansive mudflat; body mass did not differ between the two sites. Totten Inlet (~3 km^2) and Sidney Island (~1 km^2) are both very small sites with much smaller maximum distance to cover (~400m, both sites) than the larger

Boundary Bay (~60 km^2, 4100m to cover; Chapter 2, this volume). Distance to obstructive cover that may provide protection to approaching predators has been used as an index for the intrinsic danger level of a site (Wolf 2001, Ydenberg et al. 2002). While the abundance of Peregrine Falcons, the primary predator in this system, in the GB/PS is higher during the southward juvenile (August) than the northward (April/May) migration of Western Sandpipers, there is a peak in falcon numbers during northward migration (Lank et al. 2003). Therefore, both Totten Inlet and Sidney Island would appear to have similar intrinsic danger levels and be relatively more dangerous to foraging Western Sandpipers than Boundary Bay. This suggests that, in contrast to the risk-dependent trade-off suggested for Sidney Island during southward juvenile migration, birds chose to use Totten Inlet during northward migration, even though it is more dangerous and supports lower fattening rates. Totten Inlet was the only small (~3 km^2) site sampled during northward migration (the other three sites ranged from ~30-60 km^2; Chapter 2, this volume). Therefore, it is not possible to determine if this result is representative of a size-related trend or if it is restricted to this particular set of sites.

During southward adult migration, Alice Bay (~15 km^2), the smallest of the sites sampled, supported marginally higher triglyceride levels than the larger Boundary Bay (~60 km^2). However, the triglyceride levels of birds at Alice Bay did not differ from those at English Boom (~60 km^2) or Robert's Bank (~25 km^2), both similarly expansive mudflats. Furthermore, the birds using Alice Bay did not differ in body mass from those at Boundary Bay and were heavier than those at Robert's Bank. During southward juvenile migration, in contrast to data presented by Guglielmo et al. (2002), no difference in triglyceride level was detected between Sidney Island and Boundary Bay. Samples sizes for these two sites were lower in the present study providing lower power to detect a site difference; however, differences were detected in this study during northward migration with similar sample sizes. The only difference in triglyceride levels detected during southward juvenile migration during this study was between Robert's Bank and Boundary Bay, both large sites. There was no difference in body mass or triglyceride levels between birds at False Bay, a small (~3 km^2) protected site, and those at Boundary Bay. While the sample size for False Bay was low (n=7), there was enough power to detect a difference in glycerol levels between these two sites, suggesting sufficient power of detection.

The data from this study demonstrates that birds don't universally achieve a higher degree of fattening at small sites and that the body mass of individuals using sites of different sizes is not consistently size-dependent. We have provided evidence of at least one site at which site selection is not driven by the risk-dependent trade-off suggested by Ydenberg et al. (2002). Furthermore, there may be differences between migration stage (northward, southward adult or southward juvenile) in the relationship between site size and fattening.

Comparison to other studies

In the only other study to test site differences in metabolite levels on a landscape scale (Schaub and Jenni 2001, see further discussion below), the plasma triglyceride levels reported are actually total plasma glycerol which includes both glycerol previously bound as triglyceride (the values reported in our study) and endogenous non-bound glycerol (analyzed separately in our study). Several studies on metabolite levels in free-living systems measure triglyceride including free glycerol (Jenni-Eiermann and Jenni 1991, Jenni and Jenni-Eiermann 1996, Jenni-Eiermann and Jenni 1996, Schaub and Jenni 2001). The rationale for not subtracting out free glycerol includes an increase in sample size due to the larger plasma volumes needed for the glycerol analysis (Schaub and Jenni 2001), a strong correlation between total and free glycerol levels (Schaub and Jenni 2001), and low values of free glycerol compared to total glycerol (Jenni and Jenni-Eiermann 1996, Jenni-Eiermann and Jenni 1996, Schaub and Jenni 2001). The method used in our study (and by Gannes 2001) is a two-reaction assay that gives both free and total glycerol levels for a single (5 μL) plasma sample. In our study, free and total glycerol levels are also strongly correlated ($r = 0.95$, $n=468$); however, the mean percentage of total glycerol represented by free glycerol in this study was 23.2%. None of the studies using total glycerol as an estimate for triglyceride levels report the percentage of total glycerol represented by free glycerol in their system. Furthermore, none of the studies comment on the amount of inter-individual variation in the percentage of free glycerol, which, if high, is far more problematic. In our study, the percentage of free glycerol was quite variable between individuals, ranging from 3.1% to 76.9%, with a standard deviation of 12.3%. Similar ranges and variation were found within migration stage and site. Our findings suggest that, at least in our system, total

plasma glycerol levels include potentially very large amounts of metabolites not targeted for fat deposition. Therefore, for this system, triglyceride levels should be based on true plasma triglyceride levels (i.e. total minus free glycerol). Before applying this technique to other systems, we would advise careful evaluation of the magnitude and variability of the percentage of free glycerol.

The only heretofore published test of differences in plasma metabolite levels between multiple sites was presented by Schaub and Jenni (2001), in which they detected site differences in a fattening index, calculated from principle component analysis of plasma levels of ß-hydroxy-butyrate and triglyceride (including free glycerol), in free-living migratory passerines. Inter-site variation in glycerol levels was not tested. One key difference between these two studies is the geographic scale tested. The sites sampled by Schaub and Jenni (2001) span 9 countries and 3 continents whereas the sites in our study are all within a single migratory stopover region. In addition, the only variables tested for possible explanation of the inter-site variation in fattening index were a) latitude and b) distance to ecological barriers. Neither of these parameters was tested in our study because a) there are no ecological barriers along the migration flyway within the GB/PS stopover region, and b) the sites in our study area span a much smaller range in latitude (2 degrees versus 47 degrees). Schaub and Jenni (2001) did not test correlations with ecological site characteristics; our study is the first attempt to correlate site differences in metabolite levels to the prey bases at those sites. Furthermore, the sites in Schaub and Jenni (2001) were not all collected in the same year, and only two of these sites are sampled in more than one year and, therefore, does not assess or consider potential within-site inter-annual variation in triglyceride levels. While Schaub and Jenni (2001) did test for differences in the inter-site variation in triglyceride levels between migration stages (see below), they did not analyze for stage effect on the fat index values themselves. Given that they sampled five sites during both northward and southward migration, there is a unique opportunity to test for differences in fattening index between migration stages at multiple sites across a much larger geographic range. The test for differences in triglyceride levels between migration stages in our study was restricted to two sites of close geographical proximity.

Seasonal variation in site differences

We found less inter-site variation in triglyceride levels during northward than southward migration. In contrast to our findings, Schaub and Jenni (2001) found no significant difference between migration stages in the variation between sites in fattening index in migrating passerines. In our study, while site differences were detected during all three migration stages, differences during southward migration, both adult and juvenile, were only detected during 2002 when four sites were sampled, and, even so, the differences were statistically less significant. This consistently stronger signal in site differences in northward migration may indicate inherent differences in migration strategy and/or physiology between northward and southward migration. One possible explanation for this disparity in site effect between migration stages may be that the sites sampled during southward migration happen, by chance, to all support similar fattening rates whereas the sites selected for northward happen to vary in their capacity to support hyperphagia. Given the vast difference in several ecological factors at these sites in both northward and southward migration (see Chapter 2), and the significant correlation of triglyceride levels to ecological factors in northward migration, when fattening rates did vary (this chapter), this does not seem a likely possibility.

Another possibility is that the relative lack of inter-site variation during southward migration is due to less optimization of stopover site capacity to support fattening. Metabolite concentrations of individuals using a particular site do not measure the maximum potential fattening rates supported by that site, but rather the fattening rates that are actually achieved by individuals using that site at the time of sampling; these values might be quite different. Observed fattening rates are no doubt a trade-off between the energetic potential of the site (prey availability) and the target departure mass which is optimized according to speed of migration and length of stopover (Alerstam and Lindstrom 1990). Differential expression of fattening potential between migration stages may occur due to the fact that less time is spent on northward than southward migration. Northward migration in the Western Sandpiper is more temporally compressed than southward migration, in that northbound adults pass through the GB/PS over a 3 week period whereas southbound individuals (adults and juveniles) pass through the area over a 2 month window (Butler et al. 1987). The temporally compressed nature of the northward migration is further evidenced by the fact that 10- to 15-fold more individuals are counted on any given day during northward than southward

migration (Butler 1994, based on data for the Fraser River Delta). The minimization of migration time has been attributed to an effort at early arrival on the breeding grounds (Lindstrom and Alerstam 1992). Ruthrauff (2002) demonstrated that, at Kanaryaraq, Alaska, birds nesting earlier than the median nest initiation date had a higher nest success rate. Therefore, birds may be under selective pressure to arrive to the breeding grounds early and the proximal mechanism may be selection for more temporally compressed migration. If northbound birds are operating at maximum metabolic capacity, individuals would be more likely to express their full physiological potential and, likewise, triglyceride levels achieved by individuals at stopover sites may be more likely to express the maximum potential of that site. The higher fattening rates achieved by individuals captured later in the season, when the temporal pressure is more extreme, is consistent with this hypothesis. In contrast, during southward migration, if birds are not under this temporal pressure, individuals may be less likely to express their maximum potential and individual fattening rates may be less reflective of site potential, causing there to be less variation between sites. Plasma triglyceride levels were correlated to foraging opportunity, as measured by mud exposure index, during northward but not southward migration. This is consistent with the explanation that birds fully optimize the potential of a site to support fattening during northward but not southward migration. Stein (2002) showed significantly increased intestinal enzyme activity during northward migration, suggesting that northward migration may, indeed, be more metabolically demanding than southward migration. Seasonal variation in hormone concentrations of migratory Western Sandpipers (O'Reilly and Wingfield 1995) is further evidence for the physiological difference between northward and southward migrations. More information about the difference between these migration stages is needed to elucidate the differences in the ability to detect site differences in corrected metabolite levels between these two stages.

Conservation Implications and Conclusions

This study has highlighted the dynamic nature of migratory stopover sites and the influence that these changes in site structure can have on a site's capacity to support fattening by Western Sandpipers. Future studies should take into consideration the dynamic nature of stopover sites and the potential influence of habitat change and the

resulting changes in utilization of these sites by shorebirds. We have demonstrated the ability to detect differences in habitat quality, as measured by plasma triglyceride levels, between multiple migratory stopover sites within a single stopover region. There is evidence that differential ability of these sites to support fattening is partially determined by inter-site variation in both in macrofaunal prey abundance and the opportunity to forage (mud exposure index). Further investigation is needed to further understand which factors influence the degree of fattening achieved by migratory shorebirds at stopover sites.

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FIGURE LEGEND

Figure 3.1. Inter-site variation in mean a)plasma triglyceride levels, b)plasma glycerol levels and c)size-corrected body mass in free living Western Sandpipers caught at several migratory stopover sites during northward migration in 2001 (squares) and 2002 (circles).

Figure 3.2. Inter-site variation in mean a)plasma triglyceride levels, b)plasma glycerol levels and c)size-corrected body mass in free living Western Sandpipers caught at several migratory stopover sites during southward adult migration in 2001 (squares) and 2002 (circles).

Figure 3.3. Inter-site variation in mean a)plasma triglyceride levels, b)plasma glycerol levels and c)size-corrected body mass in free living Western Sandpipers caught at several migratory stopover sites during southward juvenile migration in 2001 (squares) and 2002 (circles).

Figure 3.4. Intra-site cross-seasonal variation in mean a)plasma triglyceride levels, b)plasma glycerol levels and c)size-corrected body mass in free living Western Sandpipers caught at Boundary Bay, BC, during three migration stages (N=northward, SA=southward adult, SJ=southward juvenile) in 2001(squares) and 2002 (circles).

Figure 3.5. Triglyceride levels in free-living Western Sandpipers correlate to a) total macrofaunal prey abundance and b) foraging opportunity, as indicated by the mudflat exposure index, during northward migration in 2001 (closed symbol) and 2002 (open symbol) at several migratory stopover sites: Boundary Bay (triangle), Totten Inlet (square), Robert's Bank (upside down triangle), and Jensen Access (circle). Plotted values are site averages. The line represents the major axis of correlation for prey abundance (a) and the regression line for mudflat exposure index (b). The data for Jensen Access 2001 was excluded from analyses.

Table 3.1. Mean capture julian date, sample sizes and sampling sex-bias (% male) of Western Sandpipers caught at migratory stopover sites during northward (N), southward adult (SA), and southward juvenile (SJ) migrations in 2001 and 2002.

Site	Stage	Year	Date	Birds	% Male
Boundary Bay	N	2001	118.7	31	53.33
Jensen Access	N	2001	122.2	12	45.45
Robert's Bank	N	2001	124.0	0	N/A
Totten Inlet	N	2001	123.4	21	57.89
Boundary Bay	N	2002	120.2	44	51.16
Jensen Access	N	2002	127.2	28	59.26
Robert's Bank	N	2002	120.8	48	57.45
Totten Inlet	N	2002	120.6	27	53.85
Boundary Bay	SA	2001	189.6	29	38.46
English Boom	SA	2001	190.6	19	44.44
Alice Bay	SA	2002	200.3	13	46.15
Boundary Bay	SA	2002	191.1	13	8.33
English Boom	SA	2002	191.6	31	38.71
Robert's Bank	SA	2002	199.8	13	75
Boundary Bay	SJ	2001	230.0	13	38.46
Sidney Island	SJ	2001	223.0	19	42.11
Boundary Bay	SJ	2002	223.9	28	46.43
Doug Banks	SJ	2002	229.9	20	57.89
False Bay	SJ	2002	218.9	7	42.86
Robert's Bank	SJ	2002	223.5	53	46.15

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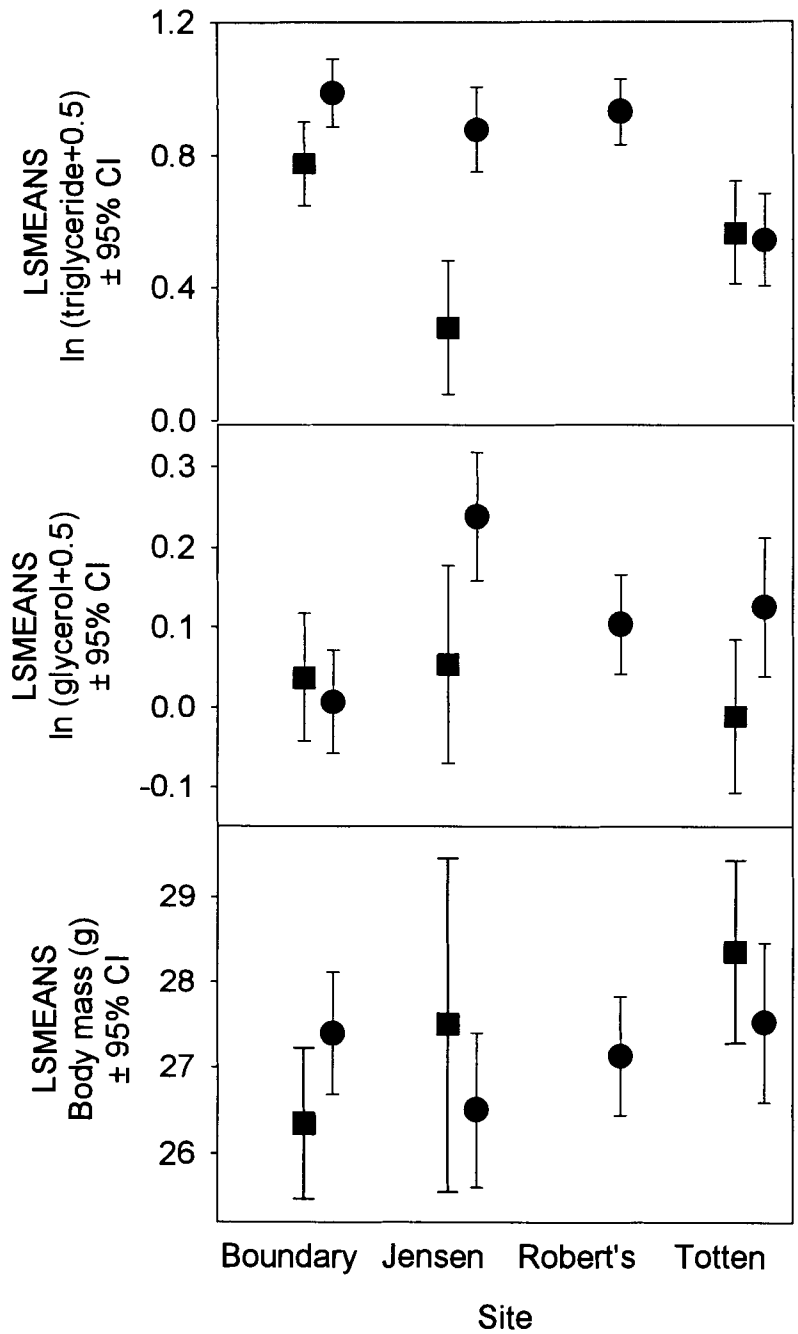
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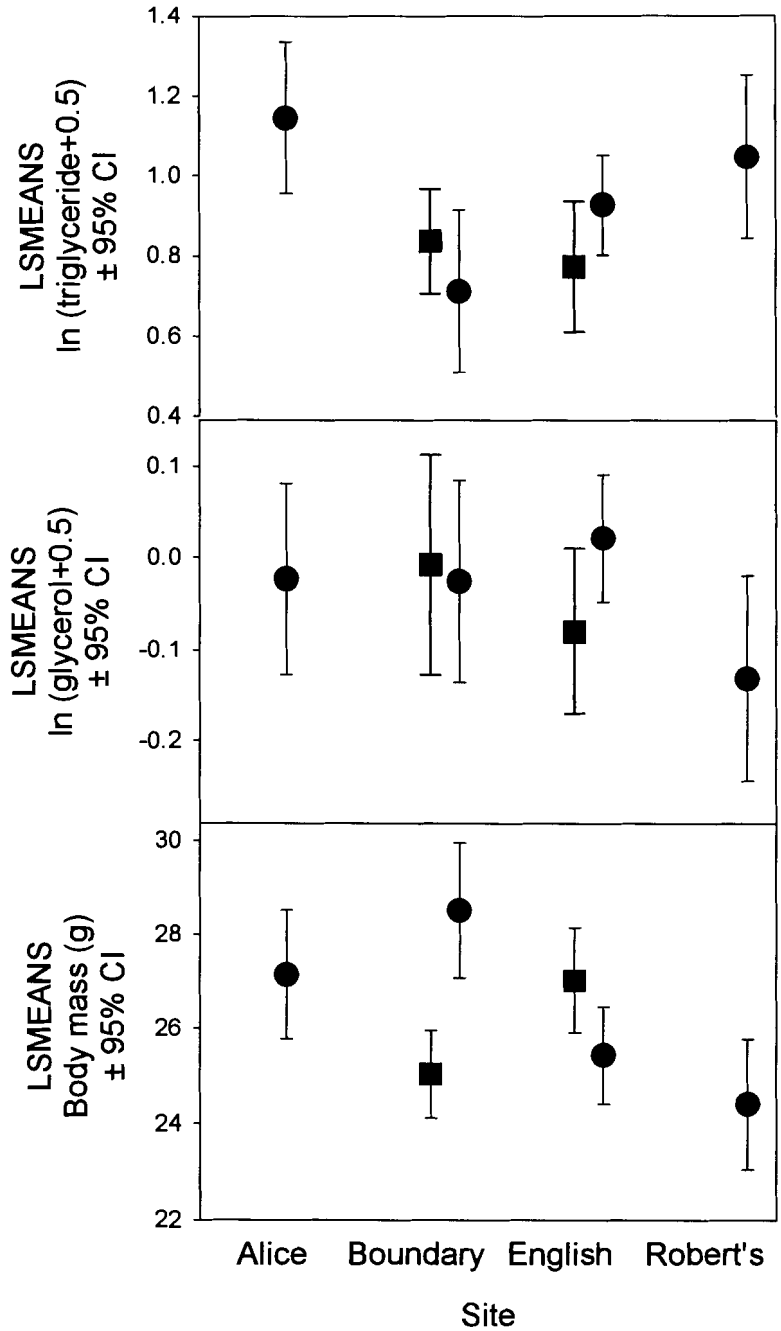
ln (triglyceride+0.5)
± 95% CI
1.2
0.8
0.4
0.0
LSMEANS
ln (glycerol+0.5)
± 95% CI
0.3
0.2
0.1
0.0
-0.1
LSMEANS
Body mass (g)
± 95% CI
29
28
27
26
Boundary
Jensen
Robert's
Totten
Site

Figure 3.1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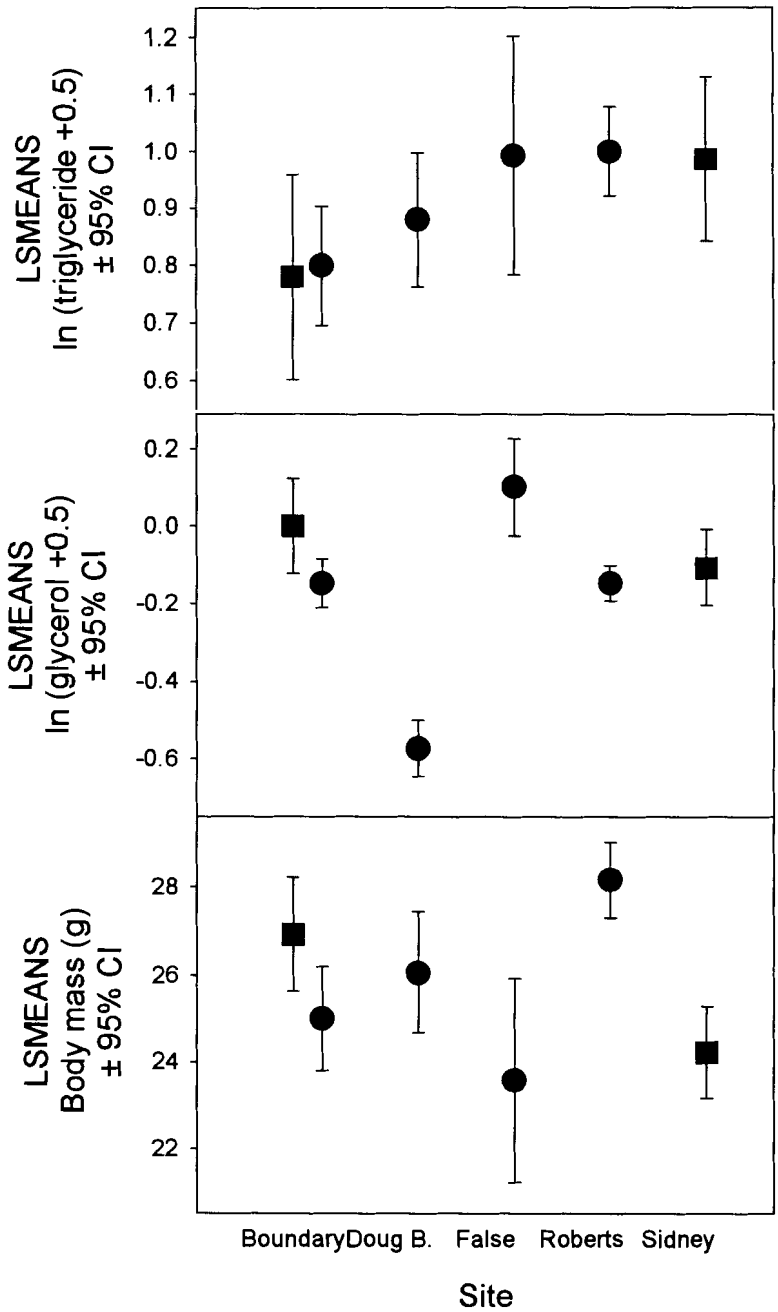
LSMEANS
ln (glycerol+0.5)
± 95% CI
0.1
0.0
-0.1
-0.2
LSMEANS
Body mass (g)
± 95% CI
30
28
26
24
22
Alice
Boundary
English
Robert's
Site

Figure 3.2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± 95% CI
1.2
1.1
1.0
0.9
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0.7
0.6
LSMEANS
ln (glycerol +0.5)
± 95% CI
0.2
0.0
-0.2
-0.4
-0.6
LSMEANS
Body mass (g)
± 95% CI
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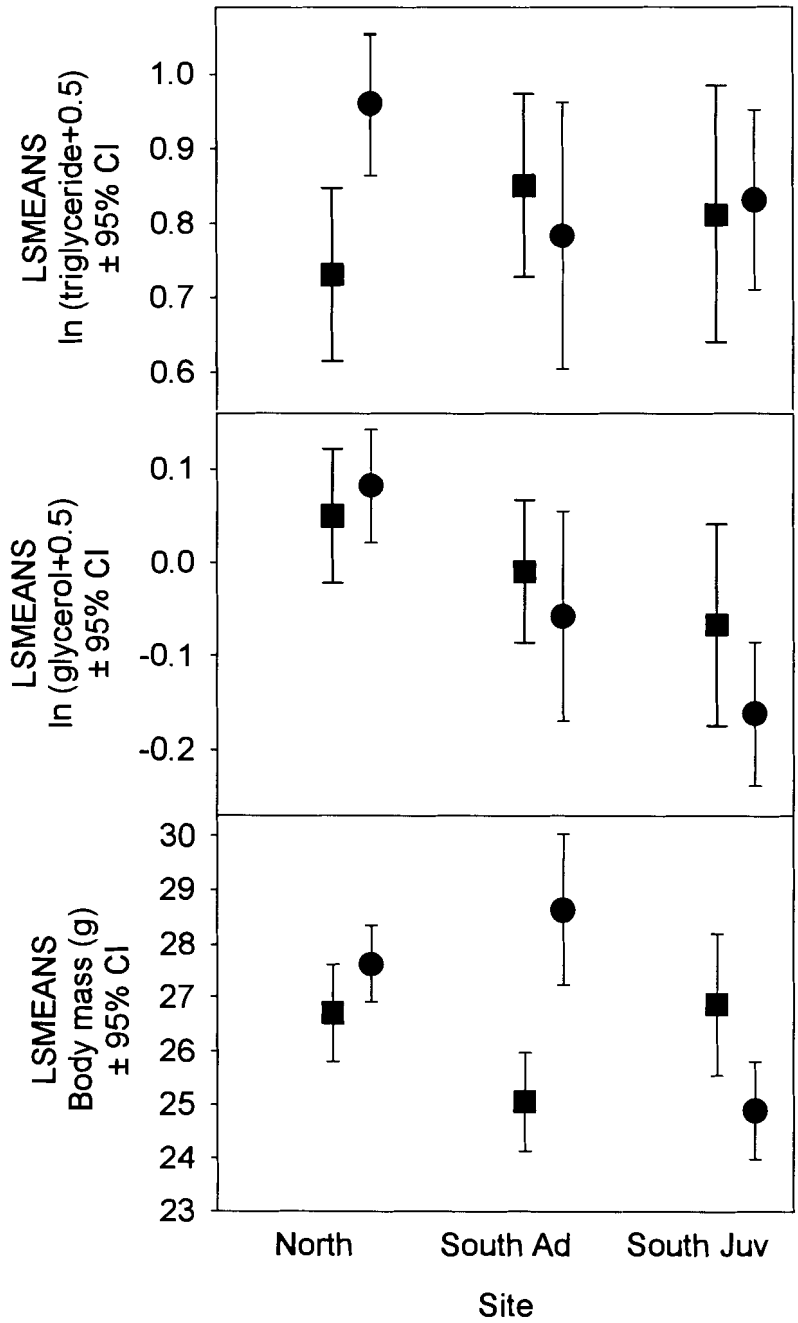
26
24
22
Boundary
Doug B.
False
Roberts
Sidney
Site

Figure 3.3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± 95% CI
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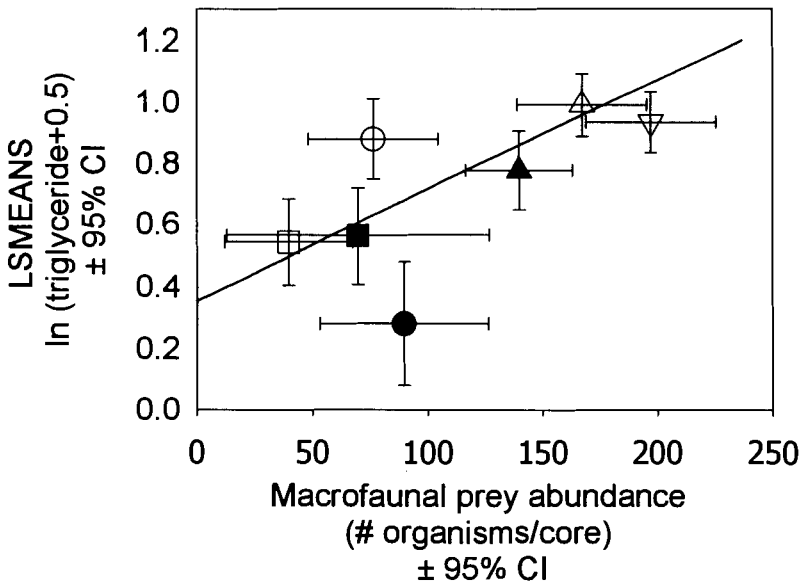
LSMEANS
ln (glycerol+0.5)
± 95% CI
0.1
0.0
-0.1
-0.2
LSMEANS
Body mass (g)
± 95% CI
30
29
28
27
26
25
24
23
North
South Ad
South Juv
Site

Figure 3.4


LSMEANS
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± 95% CI
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
Macrofaunal prey abundance
(# organisms/core)
± 95% CI

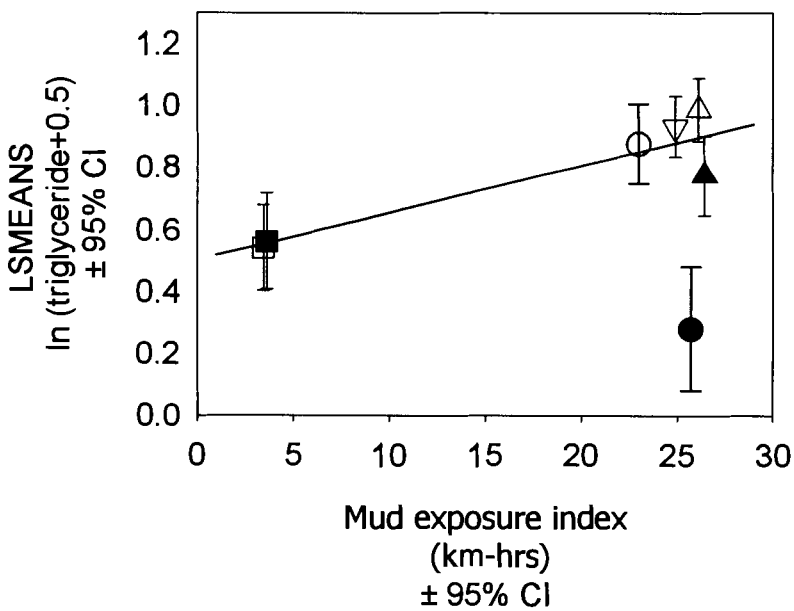
LSMEANS
ln (triglyceride+0.5)
± 95% CI
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
Mud exposure index
(km-hrs)
± 95% CI

Figure 3.5

CHAPTER FOUR
THE EFFECTS OF CAPTIVITY, MASS TRAJECTORY AND DIET QUALITY ON PLASMA METABOLITES IN WESTERN SANDPIPERS

ABSTRACT

Plasma metabolite concentrations have been used to test the effects of several factors on fuel utilization in avian migrants; these studies biologically interpret metabolite concentrations based on validation experiments conducted on captive individuals. The effect of captivity on avian plasma metabolite concentrations has not yet been tested. Similarly, the effect of diet composition on metabolite levels has not to date been tested. In this study, we test for the effects of captivity, mass cycle phase (i.e. mass loss or gain) and diet (i.e. low-fat or high-fat) on plasma metabolite levels in captive Western Sandpipers. Mass trajectories during the mass loss and gain cycle were different between the two diets, and birds achieved higher rates of mass loss on the low-fat diet, and this was reflected in metabolite profiles. Birds gaining mass on the low-fat diet had similar metabolite profiles as at the time of capture (free-living), but metabolite levels of birds gaining mass on the high-fat diet differed from the time of capture. Metabolite profiles reflected the different phases of the mass cycle and differed within phase between the two diets. Glycerol was negatively related and uric acid was positively related to the rate of mass change; triglyceride was independent of the rate of mass change. There was a significant diet effect on the relationship between the rate of mass change and both triglyceride and uric acid levels; the relationship between glycerol and mass change was independent of diet. This study highlights the effect of diet composition on plasma metabolite profiles in sandpipers and we consider the implications of this for application of laboratory-based validation studies to free-living birds.

INTRODUCTION

Plasma metabolite concentrations have been widely used to understand how fuel utilization by avian migrants is affected by several factors including feeding state (e.g. fasting; Jenni-Eiermann and Jenni 1991, Jenni-Eiermann and Jenni 1997, Totzke et al. 1999, Alonso-Alvarez and Ferrer 2001), diurnal feeding patterns (Jenni and Jenni-Eiermann 1996), molt (Jenni-Eiermann and Jenni 1996, Totzke and Bairlein 1998, Jenni-Eiermann et al. 2002), annual cycle (e.g. migration or nonbreeding; Mori and George 1978, DeGraw et al. 1979, Guglielmo et al. 2002, Jenni-Eiermann et al. 2002), flight (Jenni-Eiermann and Jenni 1991, Bairlein and Totzke 1992, Gannes 2001), and migration distance (Jenni-Eiermann and Jenni 1991, Jenni and Jenni-Eiermann 1998, Gannes 2001). Furthermore, inter-site variation in fuel utilization during migratory stopover has been tested via metabolite analysis (Schaub and Jenni 2001, Guglielmo et al. 2002; Chapter 3, this volume). In these studies, the biological interpretation of metabolite concentrations in the free-living individuals was based on relationships between metabolite concentrations and the rate of mass change established in captive individuals (e.g. Jenni-Eiermann and Jenni 1994, Williams et al. 1999). However, Lambrechts et al. (1999) demonstrated that the behavior of non-domesticated captive individuals differs from that of their free-living conspecifics and cautions against the use of captive populations to make inferences about free-living systems. For example, DeGraw et al. (1979) found that captive metabolite concentrations in White-crowned Sparrows differed from those in free-living individuals. To our knowledge, the effect of captivity on plasma metabolite concentrations has not been tested in shorebirds. Here we test for differences within individual Western Sandpipers between the free-living (i.e. time of original capture) and captive states.

Plasma metabolite concentrations have been shown to reflect mass cycle phases (e.g. mass loss or mass gain) in captive migrant passerines (Jenni-Eiermann and Jenni 1994). The difference in metabolite concentrations between mass cycle phases artificially induced via diet manipulation has not yet, to our knowledge, been

tested in shorebirds. Plasma metabolite levels have been shown to relate to the rate of mass change in captive passerines (Jenni-Eiermann and Jenni 1994, Jenni and Schwilch 2001) and shorebirds (Williams et al. 1999, Jenni-Eiermann et al. 2002). Jenni-Eiermann et al. (2002) tested this relationship in Red Knots over the natural annual cycle on *ad. libitum* food and, as such, they evaluated seasonal metabolic variation rather than the effects of experimentally manipulated mass change. Williams et al. (1999) tested the relationship in Western Sandpipers for both natural (*ad. libitum* food) and experimentally manipulated mass change. However, the different phases of the mass cycle (i.e. mass loss or gain) did not occur concurrently and the relationship is tested for natural and experimental mass change with the same model. Here we test for differences within individual in metabolite concentrations between mass cycle phases (i.e. mass loss, mass gain, and stable mass). We further test the relationship between metabolites and experimentally induced mass change in a controlled experiment .

Diet type was determined to be an important factor influencing differential fuel utilization between fruit-eating omnivorous versus insectivorous species of passerine migrants (Gannes 2001). Furthermore, diet lipid and protein content has been shown to influence the rate of mass change in captive Garden Warblers (Bairlein, 1998). However, to our knowledge, the effect of diet composition on plasma metabolite concentrations has not yet been tested. Diet quality (e.g. lipid content) has been demonstrated to influence reproductive parameters and growth in some systems (Britz and Hecht 1997, Litzow and Piatt 2002, Williams and Miller 2003), suggesting that, in at least some species, diet composition is important to individual condition and performance. Lipid content of macrofaunal invertebrates has been shown in some systems to vary within taxa (Hartson et al. 2000), between taxa (Gardner et al. 1985), and seasonal variation in lipid content within taxa has been documented (Gardner et al. 1985, Hill 1992, Jana and Manna 1993). Therefore, migratory Western Sandpipers, which forage on a wide array of marine invertebrate taxa (see review in Wolf 2001), may be expected to experience differences in diet quality between stopover sites and seasonal variation in diet composition during migration. Here we test the effect of diet composition on metabolite levels of captive Western Sandpipers during different phases of the mass loss and gain cycle.

In this paper, therefore, we examine the effects of captivity, mass trajectory, and diet on plasma triglyceride, glycerol, and uric acid levels in Western Sandpipers. The specific objectives of the study were to determine whether a) there are differences within individual in metabolite levels between free-living (original time of capture) and captive states, b) there are differences in mass trajectory during mass loss and gain cycle between a low-fat and high-fat diet, c) there are differences in metabolite levels between mass cycle phases on the two different diets, and d) there is a significant relationship between metabolite levels and the rate of mass change and e) the relationship between metabolite levels and mass change is diet dependent.

METHODS

Animals and diets

Female juvenile Western Sandpipers were captured at two migratory stopover sites in the Fraser River Delta, Boundary Bay (49°04', 122° 58') and Robert's Banks (49° 03', 123° 08'), during the southward migration (August) of 2001 (n=10) and 2002 (n=36), using mist nets (Avinet, Dryden, NY, USA; see Chapter 1 for more detailed site descriptions). Guglielmo et al. (2002) detected no significant differences in metabolite levels between sexes; therefore, experiments were restricted to a single sex to increase sample size. When possible, individuals were blood sampled upon capture ("Wild" phase) to obtain free-living plasma metabolite levels (Chapter 3). Banding and all animal handling was carried out under Environment Canada (#10646, #59-02-0718) and Simon Fraser University Animal Care (#529B) permits. Birds were maintained in captivity at the Animal Care Facility at Simon Fraser University, in outdoor aviaries (3m x 6 m x 2m high) on a natural light cycle with a 24h per day infrared heat lamp and were provided water *ad libitum*. Birds were sustained on boiled egg (with shell) and mealworms the first few days of captivity and were transitioned slowly to Clarke's Fry (see below). After birds were successfully transitioned to the pellet food and mean body mass had stabilized, birds

were blood sampled to obtain pre-treatment metabolite values on *ad libitum* food consumption prior to diet manipulation ("Pre-treatment" phase).

Two different diets were used in this experiment. The standard diet fed to all captive Western Sandpipers at SFU, and the diet used as a control in previous diet manipulation experiments (Williams et al. 1999, Egeler et al. 2003; different brand of trout chow used by Jenni-Eiermann et al. 2002) is 1.5mm (width) Clark's Fry trout chow (Moore-Clark, Vancouver, Canada). This high-fat, high-protein diet, referred to hereafter as the high-fat diet, is primarily fish meal-based and consists of 47% protein, 18% fat, 2% fiber, 9% ash and 16.5% NFE and has a total energy content of 18,206 kJ/kg. Hikari Cichlid Baby 2.0mm (diameter) pellet (Hikari USA, Hayward, California) is a low-fat, high-protein diet, referred to hereafter as the low-fat diet, that is primarily fish meal-based and consists of 35% protein, 4% fat, 5% fiber, 9% ash, and 37% NFE and has a total energy content of 16,200 kJ/kg. During transition between diets, birds were left on the new diet for a minimum of two weeks with no disturbance except being fed daily and weighed weekly.

General experiment information

Daily food consumption (g/bird/day) was measured for the group as a whole throughout the experiment. Throughout their stay in captivity, the birds were weighed weekly to the nearest 0.1 g on an O'Haus digital balance. Body mass was measured weekly throughout the experiment and every other day during mass Loss and Gain phases; any individuals who lost mass precipitously were separated out from the rest of the group and excluded from the experiment. Once the individual recuperated to a mass above lean mass (22g), the individual was reintroduced to the group and used in successive experiments. During blood sampling, the birds were caught a few at a time and blood sampled indoors to allow the remaining individuals to return to normal foraging activity between disturbances. Birds returned to foraging within one minute of the researcher vacating the aviary. The time of day blood sampling occurred (0630, 1000, 1500, or 1900) did not affect metabolite concentrations in this system (repeated measures Wilks' Lambda $P > 0.1$; D. Seaman and T.D. Williams, unpubl. data). Therefore, all body mass measurements

and blood sampling occurred between 1000 and 1200 PST to allow birds sufficient time to feed before blood sampling and to allow adequate time before sunset to forage after blood sampling to prevent method-induced mass loss. Bleed time, the number of minutes between capture and blood sampling, averaged 7.6 ± 3.7 SD min, and 90% of the bleed times were less than 12 minutes. Total disturbance time, the number of minutes between the first disturbance and blood sampling averaged 23.7 ± 14.9 SD min. Birds were blood sampled via brachial venipuncture with a 26.5 gauge needle (Bencton-Dickson). Blood (up to 300 μL) was collected with heparinized capillary tubes and centrifuged at 5000 RPM for ten minutes, and the plasma was drawn off using heparanized capillary tubes and was stored at -20°C until assayed.

Diet Trials

This experiment was conducted over several months beginning in October, 2002. Throughout this experiment, *ad libitum* food was defined as 12.5g per bird per day. This represents a significant surplus of food, given that, in preliminary experiments, average food consumption was 6.6g ± 1.7g (SD) per bird per day. Birds were divided into two groups, equally divided in terms of age (juveniles (n=36) caught in August, 2002, versus the yearlings (n=4) caught in 2001) and capture site (Boundary Bay versus Robert's Bank). Half of the birds were left on the high-fat diet, while the other half was transitioned to the low-fat diet (see above for transition protocol). Each group was then cycled through mass loss (food restriction) and mass gain (refeeding) (Trial 1) with blood samples taken in the middle of the down ("Loss" phase) and up ("Gain" phase) curves, respectively. Mass loss was achieved by group food restriction, during which time they received 85% of the average food consumption (g/bird/day) for that group; to insure continued mass loss of the lighter birds, the food was further restricted to 80% during the final two days of the Loss phase. Once body mass stabilized, birds were blood sampled during *ad libitum* food consumption (stable mass; "AdLib" phase), and the birds were left on that diet for at least two weeks after the last blood sampling before being switched to the other diet. The same cycle was then repeated on the other diet (Trial 2) so that each individual served as its own control. This random order design accounts for possible time of

year effects, given that a minimum of one month would separate the two cycles due to recovery and transition periods. This design also controls for possible effects of experience (having been previously cycled through a loss-gain cycle), because half of the birds experienced diet manipulation for the first time on the high-fat diet and the other half experienced the manipulation for the first time on the low-fat diet. A portion (n = 7) of the first group of individuals transitioned to the low-fat diet was unable to stabilize body mass on that diet; they were isolated from the rest of the birds and, soon after, their body mass stabilized. Once stabilized, they were run through the mass cycle on the low-fat diet as a separate group referred to hereafter as the low-fat light diet group. Data was pooled between trials and analyzed for each diet treatment (low- or high fat). Eighteen individuals were cycled through loss-gain cycle on the low-fat diet and 28 individuals completed the cycle on the high-fat diet; 15 individuals completed the mass cycle on both diets.

Metabolite Assays

Free glycerol and triglyceride were assayed via sequential color endpoint assay (Trinder reagent A and B, respectively, Sigma-Aldrich Canada, Oakville, Ontario), using 5 μL of sample with 240 μL and 60 μL of reagents A and B respectively, with a reading taken at 540nm after 10 minutes of incubation at 37°C after the addition of each reagent. Triglyceride concentration (mmol L^{-1}) was calculated by subtracting free glycerol from total glycerol. Uric acid was assayed via color endpoint assay (WAKO USA, Richmond, Virginia), using 5 μL of sample with 300 μL of reagent, with a reading taken at 550nm after 10 minutes of incubation at 37°C. Assays were run in 400 μL flat-bottom 96-well microplates (NUNC, Denmark) and read with a microplate spectrophotometer (Biotec 340EL). Each plate was run with a standard curve based on a serial dilution of 2.54 mmol glycerol (Sigma-Aldrich) for the triglyceride-glycerol assay and 2.97 mmol uric acid (prepared in our laboratory) for the uric acid assays. Each plate also included a 19-day old hen plasma pool used to calculate inter-assay coefficient of variation. Inter-assay coefficients of variation were 3.1% (n=11), 7.0% (n=11), and 6.1% (n=4), and intra-assay coefficients of variation were 3.2% (n=6), 3.9% (n=6), and 3.1% (n=17) glycerol, triglyceride, and uric acid, respectively.

Statistical Analysis

All three metabolite levels were non-normally distributed, so we transformed the data using ln(metabolite+0.5) to make them approximate normal. There was no correlation between plasma triglyceride and glycerol levels ($P > 0.9$) or between glycerol and uric acid levels ($P > 0.3$), so these metabolites were analyzed separately. There was a positive correlation between triglyceride and uric acid levels ($r = 0.39$, $P < 0.0001$); however, the suspected non-linear relationship to mass (see Discussion) precluded the combination of these metabolites for analysis. Plasma triglyceride levels were positively related to bleed time (triglyceride: $F_{1,206} = 6.59$, $P < 0.015$, $m = 0.016$); glycerol and uric acid levels were independent of bleed time ($P > 0.2$, both metabolites). In addition, plasma triglyceride and uric acid levels were negatively related to total disturbance time (triglyceride: $F_{1,172} = 5.70$, $P < 0.02$, $m = -0.007$; uric acid: $F_{1,171} = 16.90$, $P < 0.0001$). Overall, plasma levels of triglyceride and uric acid were positively and marginally related to body mass, respectively (triglyceride: $F_{1,206} = 56.68$, $P < 0.0001$, $m = 0.069$; uric acid: $F_{1,203} = 3.26$, $P = 0.07$, $m = 0.018$). Glycerol was independent of all factors ($P > 0.2$, all factors), and the log transformed plasma glycerol concentrations will herein be referred to as glycerol levels. Plasma concentrations of triglyceride and uric acid were corrected for the particular factors that each metabolite was significantly related to, and the residual metabolite values will hereafter be referred to as triglyceride and uric acid levels, respectively. Mass trajectories during the mass loss and gain cycle were based on raw values. Differences between trials and diets in initial, lowest, and final body mass during the mass cycle were tested via paired *t*-tests. Comparisons within and between diets and phases are based on analyses of residual body mass values, corrected for size as indicated by tarsus length, that are referred to hereafter as size-corrected body mass.

Correlations between phases in size-corrected body mass and metabolite values were tested independently for each parameter using Pearson's correlations. Physiological effects of captivity were examined by testing, via paired t-test, differences between metabolite concentrations between birds during the Wild and Pre-treatment phases. To examine the effects of captivity during active fattening, the

differences in metabolite levels between Wild and Gain phases were tested, by diet, using paired *t*-tests. Phase and diet effects on metabolite values were tested separately due to diet*phase interactions within pairs of phases (see Figure 4.4). Differences in parameters between phases of the mass cycle (i.e. Loss, Gain, and AdLib) were tested via repeated measure ANOVA. Diet effects on metabolite values were tested within each phase of the mass cycle using paired t-tests.

The relationship between the rate of mass change and metabolite concentrations was tested for each metabolite independently on a pooled data set including individuals in all three mass cycle phases, using a linear mixed model with repeated measures to account for repetition of individuals, with diet as a covariate. Testing the mass change-metabolite relationship for all phases of the loss-gain cycle with a single model allowed for maximization of range of mass change. The dMass*diet interaction term was tested for each metabolite. For each metabolite, if the dMass*diet interaction term was significant, the relationship between dMass and metabolite concentration was tested separately by diet; else, the nonsignificant interaction term was dropped from the model. During the Loss phase, the rate of mass change (dMass; g/day) was calculated for the period between the initiation of food restriction and the first mass measurement after the Loss blood sample (median = 10 days; 10d). During the Gain phase, dMass was calculated for the period between the initiation of refeeding and the first mass measurement after the Gain blood sample (median = 4d). To explore the effect of the period of time over which dMass is calculated, values of dMass were also calculated over the shorter interval 3d prior and 2d after the Loss and Gain blood samples, respectively. The values of dMass calculated over the shorter versus longer intervals were highly correlated ($r = 0.81$, $P < 0.0001$); therefore the relationship of dMass and metabolite concentrations was tested using dMass values calculated over the longer period of time in order to maximize the length of time represented. Using the shorter time period does not change any of the results or conclusions of the subsequent analyses.

RESULTS

Mass trajectories on different diets

Within trial and diet groups, the overall pattern of mass loss and gain (i.e. the shape of the curve) is similar between individuals (Figure 4.1), although a wide range of individual masses are represented (Table 4.1). There was one outlier in the high-fat diet group during Trial 2 with an unusually high initial mass (~42g); the mean and range of body mass values for each trial-diet group in Table 4.1 includes the outlier to demonstrate total variability, but it was excluded from subsequent mass trajectory analyses. By standardizing the time scale by the number of days from the end of the experiment, it is evident that, within diet, the trajectories of mean mass are similar between Trials 1 and 2 (Figure 4.2a), although there are some differences in absolute mean values between trials (Table 4.1). The mean initial mass of birds in the low-fat light diet group was lighter than the low-fat group during Trial 1 ($F_{2,16} = 5.56$, $P < 0.02$; pairwise $P < 0.05$), but was not significantly different than that for the low-fat diet group during Trial 2 (pairwise $P > 0.05$). There was no variation in mean lowest or final mass between the three low-fat trial groups ($P > 0.1$, both parameters). Furthermore, the rate of mass change during Loss (i.e. slope of mass loss trajectory) did not vary between low-fat trial groups ($F_{2,19} = 0.26$, $P > 0.7$; Figure 4.2b). Pooled across trials, the minimum mass reached during Loss and the final mass reached during Gain by birds on the low-fat diet was lower than that for birds on the high-fat (minimum: $t_{43} = -3.40$, $P < 0.0015$; final: $t_{41} = -2.26$, $P < 0.03$); however, mean initial mass was independent of diet ($t_{42} = -1.11$, $P > 0.2$; Figure 4.2b). The slope of the mass loss trajectory was steeper (i.e. higher dMass) for birds on the low-fat diet compared with the high-fat diet ($t_{45} = -2.45$, $P < 0.02$); however, the rate of mass change was independent of diet during Gain ($t_{44} = -0.49$, $P > 0.6$; Figure 4.2b).

Comparison between free-living and captive birds

There was no correlation between size-corrected body mass or any metabolite values between birds at original capture (Wild phase) and the Pre-treatment phase ($P > 0.2$, all parameters; Table 4.2). Similarly, there was no correlation in

parameters between birds in the Wild and Gain phases on either diet ($P > 0.3$, all cases; Table 4.2).

Birds after transition into captivity, given an *ad libitum* high-fat diet, prior to diet manipulation (Pre-treatment) had lower uric acid levels and higher glycerol levels than they did at the time of original capture (glycerol: $t_{35} = 2.81$, $P < 0.01$; uric acid: $t_{31} = -4.48$, $P < 0.001$); triglyceride levels did not significantly differ between Pre-treatment and Wild phases ($t_{33} = -1.26$, $P > 0.2$; Figure 4.3). Birds actively gaining mass (Gain) on the same high-fat diet had lower values of all three metabolites than at the time of capture (triglyceride: $t_{25} = -5.68$, $P < 0.0001$; glycerol: $t_{26} = -2.80$, $P < 0.01$; uric acid: $t_{23} = -5.89$, $P < 0.0001$; Figure 4.3). By contrast, birds during the Gain phase on the low-fat diet did not differ from the original time of capture in any of the metabolite levels (triglyceride and uric acid: $P > 0.4$; glycerol: $P > 0.05$; Figure 4.3).

Effect of mass cycle phase and diet

Amounts of mass change induced during each of the mass cycle phases on each diet are detailed in Table 4.3. Within each diet, size-corrected body mass was positively correlated between all three mass cycle phases (low-fat: $r > 0.6$, $P < 0.007$; high-fat: $r > 0.4$, $P < 0.03$, all cases, Table 4.2) (e.g. the heaviest individual during Loss would also be the heaviest individual during Gain). By contrast, except for a marginal correlation between Loss and AdLib phases for uric acid ($r = 0.43$, $P = 0.04$), none of the three metabolite values were correlated between phases on either diet ($P > 0.1$, all cases; Table 4.2).

The significance values for variation between Loss, Gain, and AdLib mass cycle phases, as well as pairwise comparisons between phases, are detailed for each diet in Table 4.4. On the low-fat diet, there was a significant overall phase effect for all three metabolite values ($P < 0.02$; Figure 4.4). On the high-fat diet, there was a significant overall effect for glycerol and uric acid levels ($P < 0.002$), but triglyceride level was independent of phase ($P > 0.1$ Figure 4.4). On both diets, birds losing mass had higher glycerol levels than both Gain and AdLib phases ($P < 0.05$; Figure 4.4). On the low fat diet, birds losing mass had lower levels of uric acid and higher

levels of uric acid than both Gain and AdLib phases ($P < 0.005$, Figure 4.4). In contrast, birds losing mass on the low-fat diet had lower triglyceride levels than those in AdLib phase ($P < 0.003$); however, triglyceride levels did not differ between Loss and Gain phases ($P > 0.2$, Figure 4.4). On the high fat diet, birds losing mass had lower uric acid levels than the AdLib phase ($P < 0.01$), but uric acid levels did not differ between Loss and Gain phases ($P > 0.1$; Figure 4.4).

Within the Loss phase, birds on the low-fat diet had marginally higher triglyceride levels than those on the high-fat diet ($P = 0.06$; Figure 4.4); glycerol and uric acid levels did not differ on the two diets ($P > 0.1$, both metabolites). Within both the Gain and AdLib phases, birds on the low-fat diet had higher triglyceride and uric acid levels than those on the high-fat diet(triglyceride: $P < 0.0005$, both phases; uric acid: $P < 0.007$, both phases); glycerol levels were independent of diet ($P > 0.4$, both phases; Figure 4.4).

Relationship between metabolites and rate of mass change

For both triglyceride and glycerol levels, the dMass*diet interaction was nonsignificant, so it was dropped from each model. Triglyceride levels were independent of the rate of mass change ($F_{1,47} = 0.65$, $P > 0.4$). However, there was a significant diet effect on the relationship with birds on the low-fat diet having higher triglyceride levels ($F_{1,27} = 86.49$, $P < 0.0001$; Figure 4.5); this is consistent with the analysis of means for each phase. Glycerol levels were negatively related to the rate of mass change ($F_{1,44} = 44.50$, $P < 0.0001$, $m = -0.06$) but were independent of diet ($P > 0.2$; Figure 4.5). For uric acid levels, there was a significant dMass*diet interaction term ($F_{1,38} = 7.52$, $P < 0.01$); therefore we analyzed each diet separately. For birds on the low-fat diet, uric acid levels were positively related to the rate of mass change ($F_{1,22} = 26.13$, $P < 0.0001$, $m = 0.12$); however, uric acid levels were independent of the rate of mass change on the high-fat diet ($F_{1,33} = 0.95$, $P > 0.3$; Figure 4.5). After taking the dMass*diet interaction into account (i.e. leaving it in the model), there was a significant diet effect on the relationship between uric acid and the rate of mass change, with birds on the low fat diet having higher uric acid levels ($F_{1,19} = 6.22$, $P < 0.0001$, Figure 4.5).

DISCUSSION

Our most important findings were that 1) the mass trajectories during the mass loss and gain cycle were different on the two diets, 2) birds gaining mass on the low-fat diet had similar metabolite profiles as at the time of capture (free-living), but metabolite levels of birds gaining mass on the high-fat diet differed from the time of capture, 3) metabolite levels reflected the different phases of the mass cycle, 4) metabolite levels differed between diets within mass cycle phase, and 5) glycerol was negatively related and uric acid was positively related to the rate of mass change, but triglyceride levels were independent of mass change.

Detecting metabolite differences: possible confounding factors

Time of year

Jenni-Eiermann et al. (2002) showed that in a congeneric shorebird, the Red Knot, there is a naturally occurring cycle in fattening in captivity which is reflected in changes in plasma metabolite concentrations (Jenni-Eiermann et al. 2002). This endogenous cycle in metabolites throughout the year could be a confounding variable in an experimental study design which has different treatments occurring at different times of year, as is the case in our study. To correct for this, birds experienced the mass cycle on the two different diets in random order. Furthermore, our entire experiment took place between October and January which falls within a stable mass phase in the natural cycle reported in the study of Jenni-Eiermann et al. (2002). We are, therefore, confident that time of year is not confounding our results.

Molt

Plasma triglyceride levels have been shown to covary with the timing of molt (Jenni-Eiermann and Jenni 1996, Totzke and Bairlein 1998, Jenni-Eiermann et al. 2002. Our study (October-January) occurred almost exclusively outside of the periods of body molt for the Western Sandpiper. No individuals in our study experienced wing molt; body molt was observed in a few of the individuals, however only toward the end of the second trial (January). We are, therefore, confident that molt is not confounding our results.

Effects of mass cycle phase on plasma metabolite concentrations

Several previous studies have suggested that plasma metabolite concentrations track mass gain over the annual cycle in captive passerines (DeGraw et al. 1979, Totzke and Bairlein 1998) and shorebirds (Jenni-Eiermann et al. 2002). Jenni-Eiermann and Jenni (1994) detected differences in metabolite concentrations between phases of mass loss and gain in the passerine Garden Warbler; however, the effect of mass cycle phases on metabolite levels has not, to our knowledge, been tested in shorebirds. Western sandpipers losing mass had high glycerol and lower uric acid levels than birds gaining or with stable mass. Jenni-Eiermann and Jenni (1994) similarly concluded that high levels of glycerol reflect the mass losing phase and that high levels of uric acid reflect the mass gaining phase in the Garden Warbler. In our study, the phase effect on triglyceride levels was diet-dependent. Triglyceride levels were independent of phase on the high fat diet; there was an overall phase effect on triglyceride levels on the low-fat diet, but no difference was detected between the Loss and Gain phases. Jenni-Eiermann and Jenni (1994) also detected an overall phase effect on triglyceride levels, but they do not report the pairwise comparison results that test for differences between individual pairs of phases. Furthermore, individual warblers were taken through the entire mass cycle such that each individual experiences stable, losing and gaining mass conditions; this is the same design used in our study. Jenni-Eiermann and Jenni (1994), however, do not correct their model for repetition of individuals which might affect the comparability of the two studies.

Diet effects on metabolite levels

The trajectory of the mass loss and gain cycle induced by food restriction and refeeding were different for birds fed a low- versus high-fat diet. The comparison of metabolite levels between birds during the free-living state of migratory hyperphagia (fattening) and those gaining mass in captivity differed between the two diets. Captive birds being fed high-fat (18%) trout chow, the diet used for previous captive shorebird metabolite experiments (Williams et al. 1999, Jenni-Eiermann et al. 2002), had lower triglyceride and uric acid levels than when they were captured on the

mudflat. However, birds being fed the low-fat (4%) diet, which more closely represents the average lipid content of marine invertebrate prey targeted by Western Sandpipers on mudflats (2%, Egeler and Williams 2000), had similar metabolite profiles as at the time of first capture. Therefore, the disparity between metabolic responses to mass change in free-living versus captive individuals may be, in part, due to differences between the previously used captive diet and natural prey items. Furthermore, birds on the low-fat diet had higher triglyceride levels within all three mass cycle phases and higher uric acid levels during Gain and AdLib phases than those eating the high-fat diet; glycerol was independent of diet.

The effect of diet composition on plasma metabolite concentrations of avian migrants has not yet, to our knowledge, been tested; however, Bairlein (1998) tested for effects of diet lipid and protein content on migratory fattening, measured as the rate of mass change (g/day), in the passerine Garden Warbler. Bairlein (1998) measured fattening on a more exhaustive suite of 13 diet compositions varying in protein fat and carbohydrate fractions, and the results may offer insight into our study. In our study, the diets vary dramatically in lipid content (18% versus 4%); the protein levels are similar but not equal (47% versus 35%). Bairlein (1998) demonstrated that the fattening rate achieved by the warblers depended on the percent protein and the relative proportion of protein to fat as well as the percent lipid contained by the diet. The warblers achieved the highest rate of fattening on a diet consisting 5% protein and 10% fat (5:10), which is lower in protein but similar in fat to their natural diet (15:10). Furthermore, the warblers achieved a higher rate of fattening on the 5:10 diet than on a diet of identical protein content but higher fat content (5:20). Similarly, in our study, the birds on the diet lower in both fat and protein (~45:20 versus ~35:5) had plasma metabolite concentrations indicative of higher fattening. The plasma metabolite concentrations in our study indicate similar effects of diet composition on the rate of fattening to those previously demonstrated (Bairlein 1998). The influence of protein as well as lipid content on the metabolite levels achieved by an individual is consistent with the diet effect on both triglyceride and uric acid levels of the birds in our study.

The relationship between the rate of mass change and the plasma concentrations of two of the three metabolites tested (triglyceride and uric acid) was dependent on diet composition, demonstrated by the diet effect on the dMass-

metabolite relationship for each metabolite as well as the significant dMass*diet interaction term for uric acid. While the mass cycle phase (i.e. mass loss or gain) was reflected by metabolite concentrations on both diets, the actual triglyceride or uric acid concentration for any given rate of mass change was higher on the low-fat diet. This suggests that inter-site variation in metabolite levels should be interpreted with caution if the diet composition of the prey consumed varies between stopover sites.

Relationship between metabolite levels and rate of mass change

We detected a significant negative relationship between the rate of mass change and plasma glycerol in captive Western Sandpipers over a period of 10d for Loss and 4d for Gain (median values); this is consistent with the relationship previously demonstrated over the period of 1, 2, and 7d in this species by Williams et al. (1999). The relationship between the rate of mass change and uric acid levels has not been previously tested in the Western Sandpiper. In our study, plasma uric acid levels related positively to the rate of mass change on the low-fat diet but were independent of mass change on the high-fat diet. This result is in contrast to the study by Jenni-Eiermann et al. (2002) in which the uric acid levels of Red Knots over a period of 5-7d related negatively to the rate of mass change. Although birds in our study and that by Jenni-Eiermann et al. (2002) were fed trout chows, the nutritional information is not reported for the diet fed to the knots. The difference in the relationship between the rate of mass change and uric acid level on the two different diets as well as the disparity in results between the Jenni-Eiermann et al. (2002) study and our study may be caused by the differential protein content of the diets, which is theoretically known to affect uric acid levels (Jenni-Eiermann and Jenni 1998). Uric acid levels are, furthermore, known to physiologically represent both protein catabolism and high turnover (e.g. fattening) (Jenni-Eiermann and Jenni 1994, Guyton and Hall 1996, Jenni-Eiermann and Jenni 1998); therefore, differential representation of range of mass change (i.e. loss or gain) is a possible explanation for the positive versus negative relationship between uric acid and rate of mass change detected by our study versus the Jenni-Eiermann et al. (2002) study.

A relationship between mass change and triglyceride levels in captive Western Sandpipers was not detected in our study; a similar lack of relationship between this metabolite and mass change was reported in Red Knots (Jenni-Eiermann et al. 2002). While, Williams et al. (1999) similarly failed to detect a relationship over 7d in Western Sandpipers, a significant positive relationship between plasma metabolite concentrations and the rate of mass change was detected over 1 and 2d. Similarly, Jenni-Eiermann and Jenni (1994) detected a positive relationship between triglyceride levels and mass change in the passerine Garden Warbler, over a period of 5h but did not detect a relationship over a longer 24h period; periods in excess of 1d were not tested in that system. The sampling periods of 7d that were tested by Williams et al. (1999) were under natural mass change conditions and reflected less extreme mean rates of mass change (g/day ± SE; Gain: 0.13 ± 0.08; loss: -0.36 ± 0.08) than those experienced by the birds on the experimentally-induced mass cycle (Loss 1d: -2.41 ± 0.27; Gain 2d: 1.31 ± 0.24; Gain 1d: 2.13 ± 0.20). The rate of mass gain experienced by the 2d natural mass change group (0.31 ± 0.11) was also low and was similar to the 7d natural mass change group. However, the rate of mass loss experienced by the 2d natural mass change group (-2.16 ± 0.32) was much steeper than those experienced during the Loss phase in our study, and there was no sample of low rates of mass loss over 2d. The rates of mass gain tested by Williams et al. (1999) are similar to those experienced by the birds in our study (see Table 4.3). However, the mean mass losses experienced over the short periods of time in the study by Williams et al. (1999) are well below mean mass loss experienced in our study. Because of the large disparity between mass loss values, it was not possible to test this relationship on a subset of individuals that experienced similar levels of mass loss. The shallow mass loss induced in our study was a logistical necessity in order to space out bleed times since Loss and Gain samples occurred consecutively in the same individual. The less extreme rates of mass change in our study may have restricted the range of mass change sufficiently to lose the power necessary to detect the relationship reported by Williams et al. (1999); a similar effect could be influencing our ability to detect differences in triglyceride levels between Loss and Gain phases. Regardless, our study and that of Williams et al. (1999) suggest that the relationship between triglyceride concentration and mass change is much weaker than that for glycerol and uric acid in captive Western Sandpipers.

Mass fluctuations throughout experiments

Throughout the experiment, initial body mass varied between individuals (range = 22g-42g), and 25% of the birds (n=10) had an initial mass below 25g. There are two possible hypotheses to explain the lightness of these individuals even though they receive *ad libitum* food: a)there is a social hierarchy preventing them from gaining extensively so they eat only what they need physiologically (food limited hypothesis) or b) they are maintaining their body mass at a lower level (intrinsic mass differences hypothesis). If the former is true, one would expect the lighter birds to lose mass more precipitously than the heavier birds as their food availability would be even more limited once restricted. If the latter is true, one would expect the low body mass to be maintained once food is restricted. The fact that the lighter individuals loss significantly less mass during food restriction (8% versus 14% over ~10 day Loss phase; $P < 0.01$) does not support the food limited hypothesis and suggests that different individuals have different intrinsic mass thresholds. Initially, it may seem that the lack of correlation within individual body mass at the time of original capture and in captivity is inconsistent with this hypothesis. However, once in captivity, there is a positive correlation of body mass within individual between mass cycle phases. This indicates that the body mass at stopover sites during migration does not predict the response of the individual to captivity, but once in captivity individuals maintain rank order of body mass within groups throughout the mass cycle phase. Migratory hyperphagia is thought to be controlled by an internal circannual clock (Gwinner 1986, Cadee et al. 1996), and annual cycles of mass gain on *ad. libitum* food corresponding to migratory fattening has been demonstrated in a congeneric migrant shorebird, the Red Knot (Jenni-Eiermann et al. 2002). Our experiment occurred under natural light during a time of year (October-January) corresponding to periods of non-fattening (Jenni-Eiermann et al. 2002). While the majority of individuals continued to experience the large mass fluctuations experienced by free-living migrants (Lindstrom et al. 1999), the lighter birds may be a group of individuals that did not maintain their hyperphagic condition in captivity.

CONCLUSIONS

Glycerol and uric acid levels in captive Western Sandpipers reflected mass cycle phase and relate negatively and positively to the rate of mass change, respectively. However, triglyceride levels were less reflective of mass phase and not related the rate of mass change; the power to detect this relationship may be increased if birds experienced steeper rates of mass loss. Glycerol and uric acid seemed to reflect changes in body mass over a smaller range of mass change values than triglyceride. This is consistent with the conclusion by Williams et al. (1999) that glycerol may prove particularly useful at predicting values of mass change in free-living individuals. However, the ability to apply glycerol values in this manner has been impeded by our apparent lack of understanding of the biological significance of glycerol in free-living migrant systems (see Chapter 3). Furthermore, this study illuminated several effects of diet composition on plasma metabolite levels. Future studies of free-living individuals should take the effects of potentially different diet compositions into account.

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FIGURE LEGEND

Figure 4.1 Body mass of individual captive Western Sandpipers during mass loss and gain cycles during Trials 1 and 2 for low-fat and high-fat diet groups. A group of light birds on low-fat diet were run through the mass cycle separately.

Figure 4.2 Mean body mass of captive Western Sandpipers during mass loss and gain cycles during two separate trials on different diets, collapsed onto the same axis, depicting a) low-fat (closed squares), low-fat light (open squares) and high-fat (circles) diet groups during trials one (thin line) and two (thick line), and b) low-fat (squares) and high-fat (circles) diets, pooled between trials. Days of experiment have been standardized from the end of the experiment.

Figure 4.3 Variation in mean triglyceride (top), glycerol (middle) and uric acid (bottom) levels in captive Western Sandpipers at time of capture (Wild), in captivity prior to diet manipulation (Pre-treatment), and during Gain phase of mass cycle on a high-fat and low-fat diet.

Figure 4.4 Inter-phase variation during mass loss and gain cycle in mean triglyceride (top) glycerol (middle), and uric acid (bottom) levels in captive Western Sandpipers on low-fat (closed circles) and high-fat (open circles) diet.

Figure 4.5. The relationship between the rate of mass change (dMass) and triglyceride (top), glycerol (middle) and uric acid (bottom) levels of captive Western Sandpipers during mass loss and gain cycle on a low-fat diet (closed circles, thick line) and a high-fat diet (open circles, thin line).

Table 4.1 Body mass statistics of captive Western Sandpipers during mass loss and gain cycle during Trials 1 and 2 for low-fat and high-fat diet groups. A group of light birds on the low-fat diet (low-fat$_L$) were run through the mass cycle separately. Sample sizes are annotated in parentheses after each diet group (Trial 1, Trial 2).

Diet	Parameter	Statistic	Trial 1			Trial 2			*P*
High-fat	Initial Mass (g)	Range	24.30	-	32.70	22.24	-	42.28	
(n=15,13)	Initial Mass (g)	Mean ± SD	29.12	±	2.42	27.67	±	5.33	0.1636
	Lowest Mass (g)	Range	22.84	-	28.17	19.91	-	28.98	
	Lowest Mass (g)	Mean ± SD	25.61	±	1.90	23.87	±	2.46	0.0369
	Final Mass (g)	Range	26.03	-	36.15	22.74	-	35.70	
	Final Mass (g)	Mean ± SD	30.76	±	3.18	29.33	±	5.02	0.5722
Low-fat	Initial Mass (g)	Range	25.30	-	35.00	23.05	-	27.15	
(n=7,5)	Initial Mass (g)	Mean ± SD	30.10	±	3.78	25.19	±	1.61	0.0167
	Lowest Mass (g)	Range	22.45	-	24.92	20.62	-	22.97	
	Lowest Mass (g)	Mean ± SD	23.67	±	0.84	21.97	±	0.89	0.1636
	Final Mass (g)	Range	26.19	-	31.88	24.82	-	32.61	
	Final Mass (g)	Mean ± SD	28.83	±	2.18	27.54	±	3.02	0.6073
Low-fat$_L$	Initial Mass (g)	Range	22.51	-	30.83				
(n=8)	Initial Mass (g)	Mean ± SD	24.78	±	3.08				
	Lowest Mass (g)	Range	18.11	-	26.05				
	Lowest Mass (g)	Mean ± SD	22.09	±	2.33				
	Final Mass (g)	Range	22.69	-	35.69				
	Final Mass (g)	Mean ± SD	26.74	±	4.90				

Table 4.2 Statistical significance values for correlations between size-corrected body mass and metabolite values in captive Western Sandpipers between a)birds at original capture (Wild) and birds in Pre-treatment (PreTx) and Gain phases on a low-fat and high-fat diet and b)mass cycle phases on both diets. Coefficients of correlation (r) are included for statistically significant ($P < 0.05$, bold type) correlations.

	Parameter	Diet		Mass	Trig	Glycerol	Uric acid
(a)	Wild - PreTx	High-fat	P	0.8034	0.8777	0.8609	0.4489
	Wild - Gain	High-fat	P	0.3256	0.7233	0.4478	0.9791
	Wild - Gain	Low-fat	P	0.3753	0.8511	0.5769	0.9526
(b)	Loss - Gain	High-fat	P	**0.0299**	0.8102	0.3606	0.4637
			r	0.4531			
	Gain - AdLib	High-fat	P	**0.0002**	0.2966	0.7575	0.1721
			r	0.7028			
	Loss - AdLib	High-fat	P	**0.0026**	0.1914	0.7247	**0.0405**
			r	0.5979			0.4301
	Loss - Gain	Low-fat	P	**0.0014**	0.2356	0.7250	0.4674
			r	0.7102			
	Gain - AdLib	Low-fat	P	**0.0012**	0.3890	0.2534	0.5574
			r	0.7169			
	Loss - AdLib	Low-fat	P	**0.0061**	0.1308	0.9862	0.7681
			r	0.6355			

Table 4.3. Range and mean (±95% CI) rates of mass change (g/day) for captive Western Sandpipers during different phases of an experimentally induced mass loss and gain cycle on low- and high-fat diets.

Phase	Diet	n	Min dMass	Max dMass	Mean ± CI dMass	
Loss	Low	19	-2.17	-0.06	-0.56	± 0.24
Loss	High	27	-1.69	-0.04	-0.35	± 0.12
Gain	Low	19	0.28	2.41	1.21	± 0.24
Gain	High	28	0.49	1.43	1.43	± 0.30
AdLib	Low	18	-0.44	0.92	0.05	± 0.14
AdLib	High	24	-0.74	0.73	-0.07	± 0.12

Table 4.4 Statistical significance values for repeated measure ANOVA testing for differences in metabolite values of captive Western Sandpipers between Loss, Gain, and AdLib mass cycle phases on a low-fat and high-fat diet. Significant pairwise comparisons ($P < 0.05$) are in bold type; minus sign indicates the value of first phase (1st-2nd) is lower and plus sign indicates the second phase value is higher.

Parameter	Comparison	P_{LOW}		P_{HIGH}	
Mass Change	Overall	0.0001		0.0001	
	Loss-Gain	**0.0001**	-	**0.0001**	-
	Gain-AdLib	**0.0003**	+	**0.0065**	+
	Loss-AdLib	**0.0001**	-	**0.0001**	-
Triglyceride	Overall	0.0074		0.1460	
	Loss-Gain	0.2652			
	Gain-AdLib	**0.0473**	-		
	Loss-AdLib	**0.0026**	-		
Glycerol	Overall	0.0026		0.0025	
	Loss-Gain	**0.0012**	+	**0.0006**	+
	Gain-AdLib	0.8457		0.0782	
	Loss-AdLib	**0.0020**	+	**0.0349**	+
Uric acid	Overall	0.0001		0.0030	
	Loss-Gain	**0.0003**	-	0.1447	
	Gain-AdLib	0.2729		**0.0283**	-
	Loss-AdLib	**0.0001**	-	**0.0006**	-

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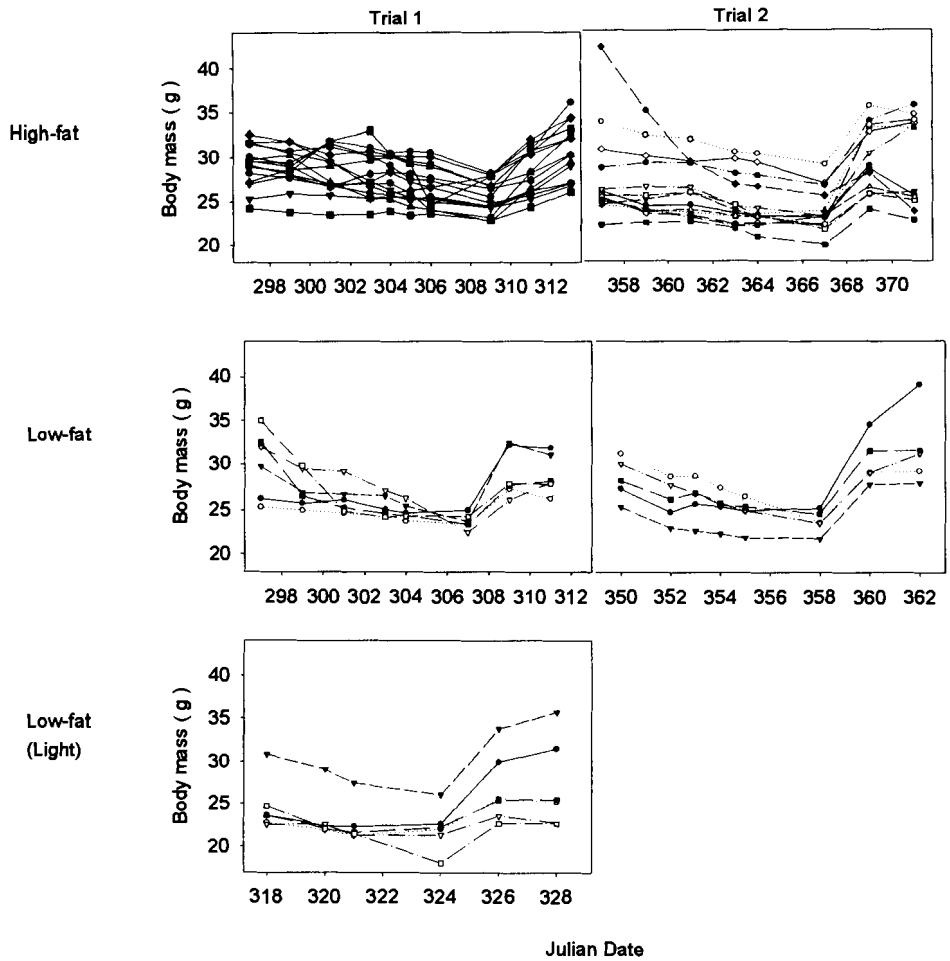

Trial 1
Trial 2
High-fat
Low-fat
Low-fat
(Light)
Body mass (g)
40
35
30
25
20
298 300 302 304 306 308 310 312
358 360 362 364 366 368 370
350 352 354 356 358 360 362
318 320 322 324 326 328
Julian Date

Figure 4.1

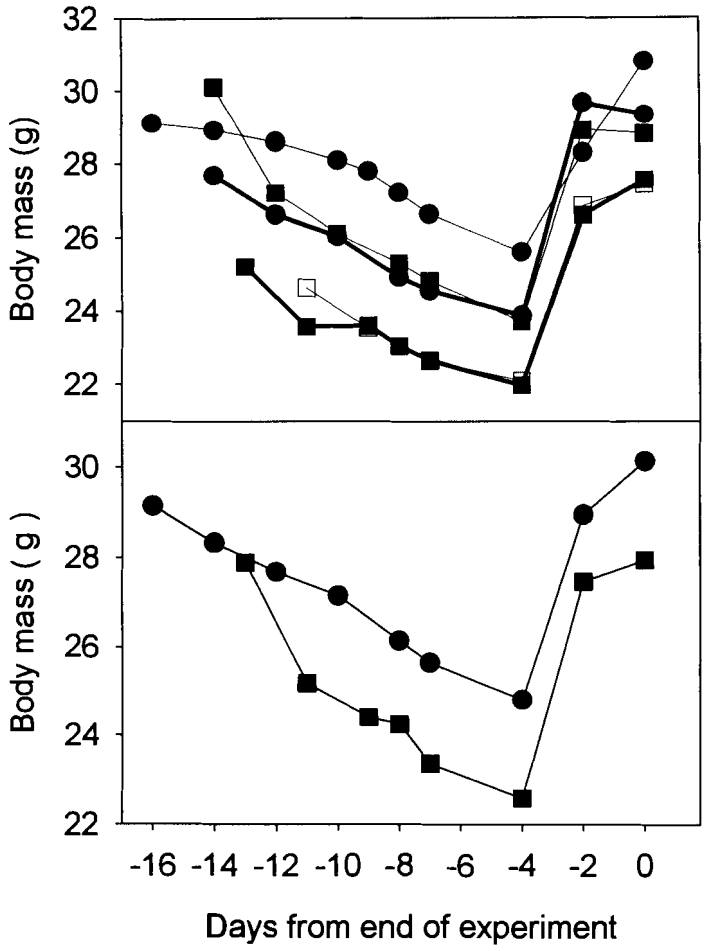

Body mass (g)
32
30
28
26
24
22
Body mass (g)
30
28
26
24
22
-16 -14 -12 -10 -8 -6 -4 -2 0
Days from end of experiment

Figure 4.2

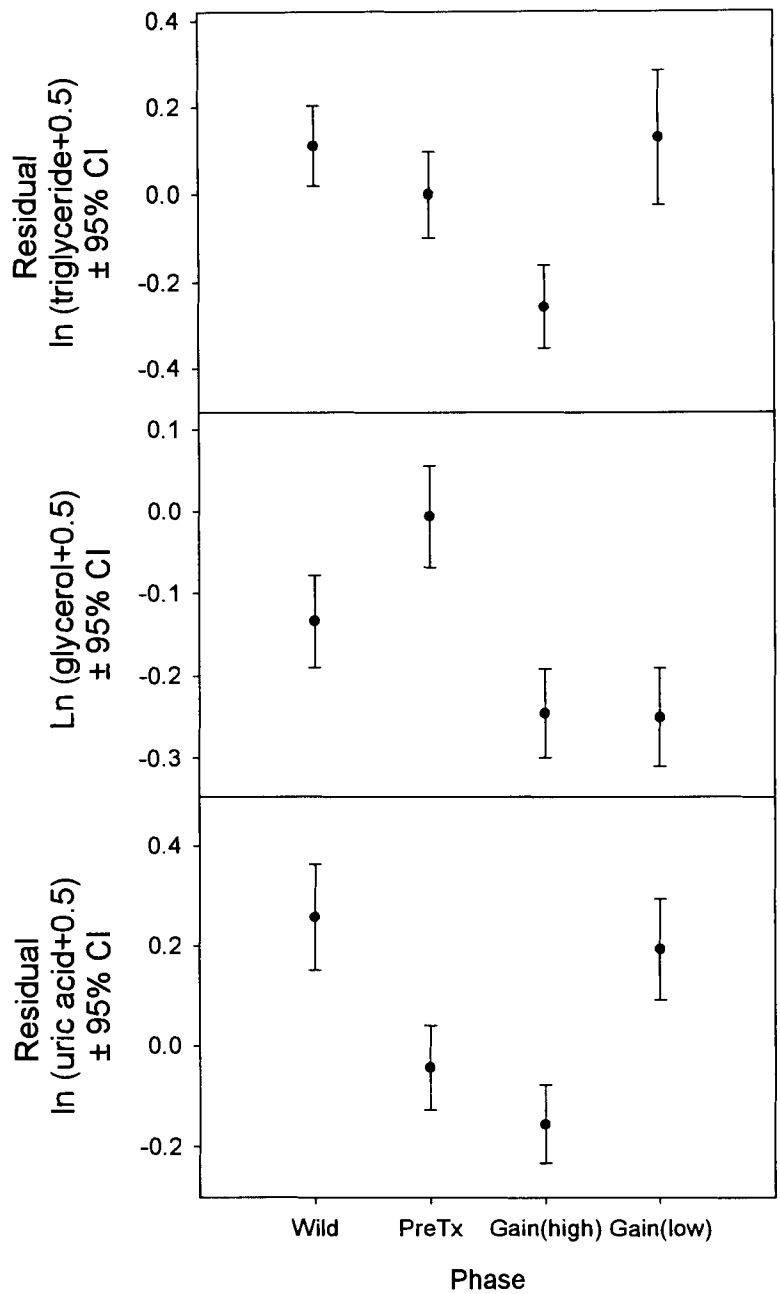

Residual
ln (triglyceride+0.5)
± 95% CI
0.4
0.2
0.0
-0.2
-0.4
Ln (glycerol+0.5)
± 95% CI
0.1
0.0
-0.1
-0.2
-0.3
Residual
ln (uric acid+0.5)
± 95% CI
0.4
0.2
0.0
-0.2
Wild
PreTx
Gain(high)
Gain(low)
Phase

Figure 4.3

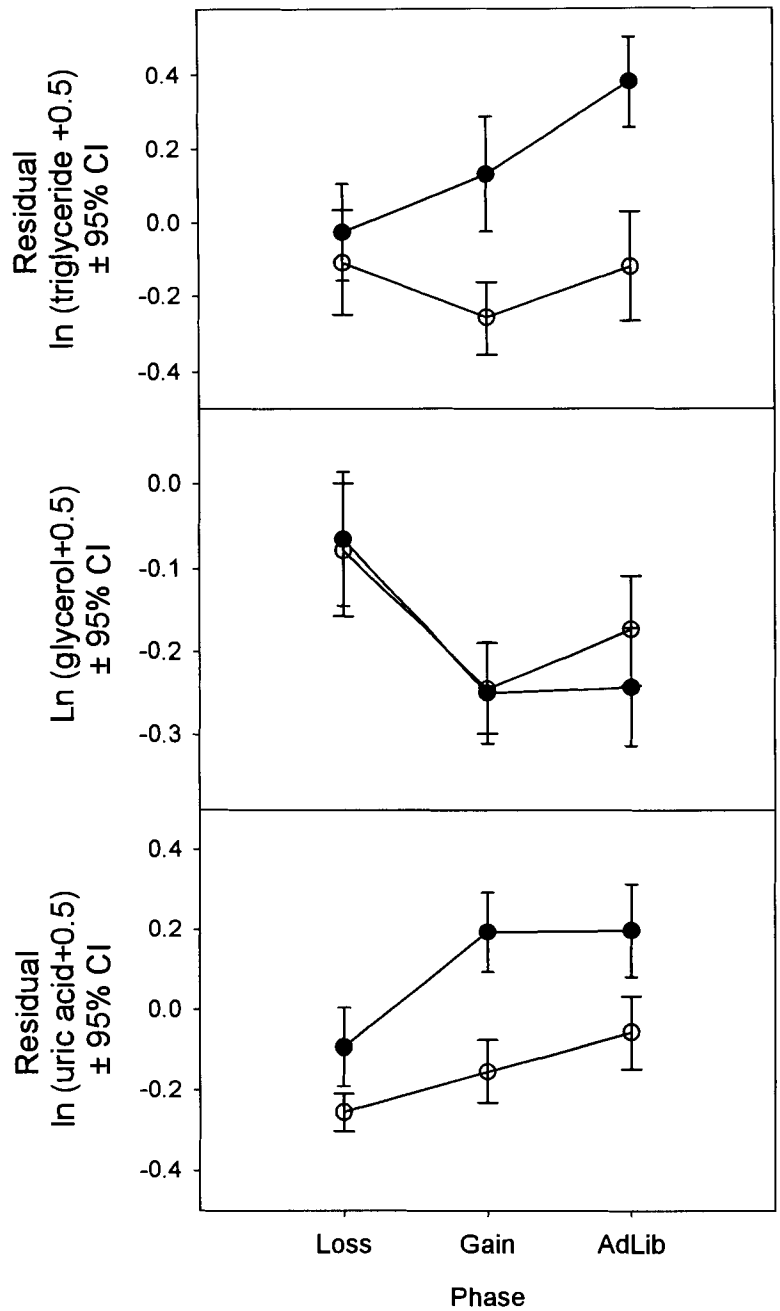
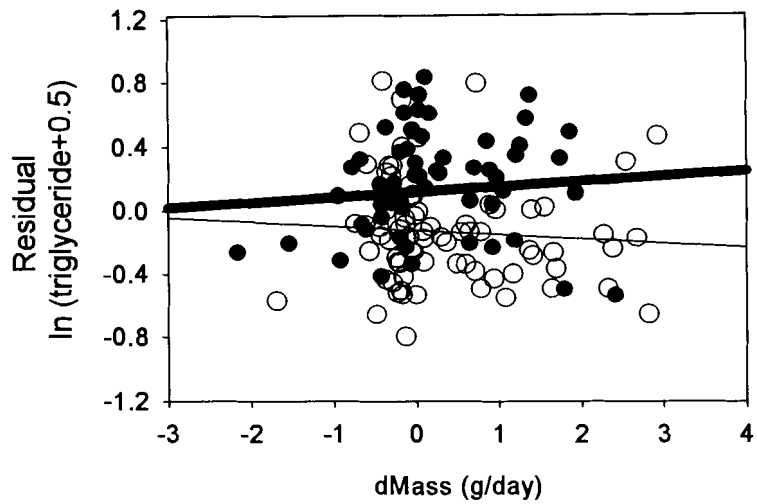
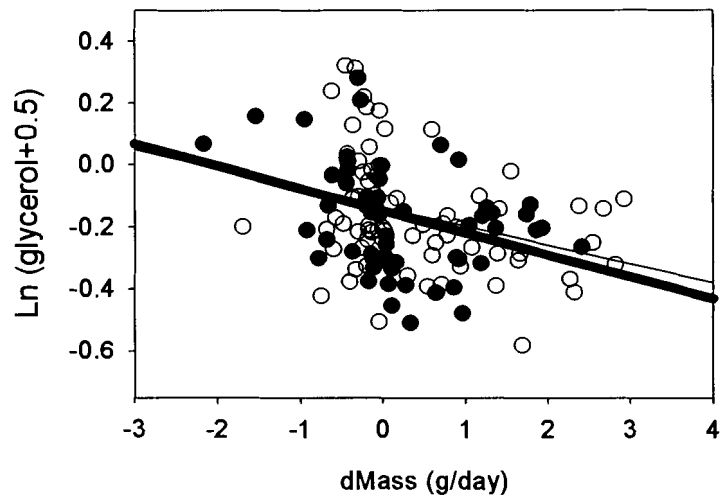

Residual
ln (triglyceride +0.5)
± 95% CI
0.4
0.2
0.0
-0.2
-0.4
Ln (glycerol+0.5)
± 95% CI
0.0
-0.1
-0.2
-0.3
Residual
ln (uric acid+0.5)
± 95% CI
0.4
0.2
0.0
-0.2
-0.4
Loss
Gain
AdLib
Phase

Figure 4.4


Residual
ln (triglyceride+0.5)
1.2
0.8
0.4
0.0
-0.4
-0.8
-1.2
-3
-2
-1
0
1
2
3
4
dMass (g/day)


Ln (glycerol+0.5)
0.4
0.2
0.0
-0.2
-0.4
-0.6
-3
-2
-1
0
1
2
3
4
dMass (g/day)

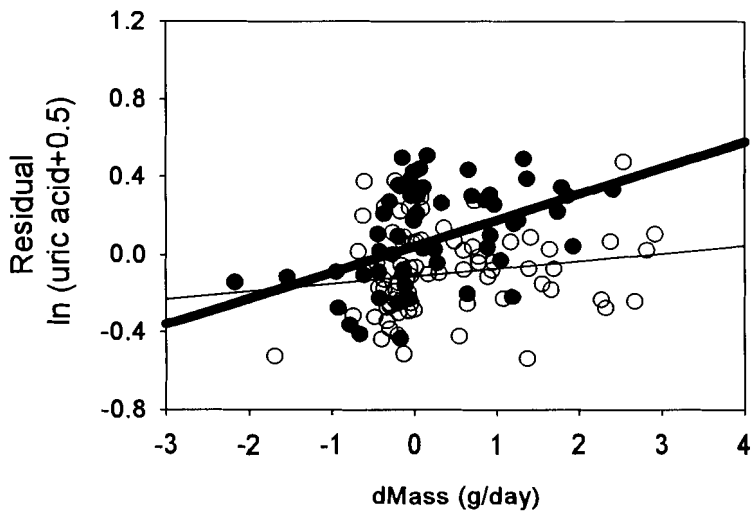

Residual
ln (uric acid+0.5)
1.2
0.8
0.4
0.0
-0.4
-0.8
-3
-2
-1
0
1
2
3
4
dMass (g/day)

Figure 4.5

CHAPTER FIVE:
GENERAL SYNTHESIS AND
FUTURE DIRECTIONS

COMING TO AN END....

This study has elucidated several key points regarding fattening and habitat quality for migratory Western Sandpipers. The key points from each chapter have, in turn, generated unanswered questions which merit further research. The major synthesizing topics of future research illuminated by this work are 1)variation in metabolite levels between northward and southward migrations, 2) evaluation of other aspects of the prey-base, such as vertical prey distribution and prey quality, which may further explain inter-site variation in metabolite levels, 3) the development of individual-based correlations to evaluate the factors determining habitat quality, and 4) the development of metabolite-based predictive equations for the rate of mass change in free-living individuals.

Seasonal differences in metabolite levels

Plasma metabolite analysis detected stronger inter-site variation in metabolite levels during northward than southward migrations; this may be suggestive of an inherent seasonal (northward versus southward migration) difference in migration strategy employed by Western Sandpipers during migratory stopover. General differences between northward and southward migrations in this and other shorebird species have been reported (O'Reilly and Wingfield 1995, Lank et al. 2003); possible origins of this difference within the context of metabolite levels are discussed in Chapter 3. Schaub and Jenni (2001) sampled five sites during both northward and southward migrations, but did not analyze the data for differences in metabolite levels between migration stages. Guglielmo et al. (2002) sampled two sites exclusively during southward juvenile migration, therefore, precluding analysis of seasonal variation. While studies have reported temporal variation throughout the year in birds (DeGraw et al. 1979, Jenni-Eiermann et al. 2002), no study to date has tested for variation in metabolite levels between northward and southward migration. Our study had an unbalanced design in that different sites were sampled during each of the migration stages (northward, southward adult, southward juvenile); only two of the nine sites were sampled during multiple migration stages. This design was a logistical

necessity in order to maximize the total number of sites sampled; at many of the sites, this design was further dictated by the seasonally-dependent use of the sites by Western Sandpipers. This study was able to test for seasonal variation in metabolite levels on a small scale. Within both of the reference sites, Boundary Bay and Robert's Bank, triglyceride levels were independent of migration stage while both glycerol levels and body mass were higher during northward than southward migration. The fact that glycerol levels, which should indicate fat utilization be negatively related to body mass, did not appear to covary with triglyceride and varied between migration stage in the same direction as mass is further suggestive of our lack of understanding of the biological significance of glycerol in free-living individuals. Future studies that sample multiple sites spanning the entire stopover region during both northward and southward migrations would more thoroughly address the issue of seasonal variation in metabolite levels. To further understand the differential inter-site variation during northward versus southward migration, collaborative studies concurrently sampling multiple sites from various migratory stopover regions are necessary.

Factors determining habitat quality

This study clearly establishes that there are differences in metabolite levels between sites, and this is the first study to report a positive correlation between plasma metabolite levels and any measure of food availability in free-living birds. However, macrofaunal prey abundance only represents a portion of the available prey base at these sites. To further understand what factors determine habitat quality, future investigations should focus on exploring inter-site variation in multiple prey-related factors at these stopover sites. The importance of meiofaunal prey in the diet of Western Sandpipers, as evidenced by consumption, has been demonstrated (Sutherland et al. 2000, Wolf 2001). Techniques to adequately evaluate this portion of the prey base on a landscape scale need to be developed. Furthermore, in light of possibilities of individual morphometric-based foraging specializations (Elner and Seaman 2003), more in-depth quantification of prey abundance along a vertical distribution rather than on a per-core basis could further enhance our understanding of the relationship between the prey that is in the mud and the fat that is on the bird.

In addition to prey abundance, which seems to influence the capacity of a stopover site to support hyperphagia and high rates of fattening, prey biomass and quality (i.e. lipid and/or protein composition and total energy content) could account for some of the unexplained variation in metabolite levels between sites. Diet quality information is even more important in light of the results of the captive diet manipulation experiments that suggest that the relationship between the rate of mass change and metabolite levels is diet dependent.

Individual variation in morphology and behavior has rarely been considered in migratory Western Sandpipers. Due to the landscape scale of this study, individual variation was not assessed and analyses were restricted to detecting differences between sites, treated as an individual unit. While differences on a larger scale were the focus of this study, criticisms that this approach ignores individual variation merits consideration. The evaluation of the relationship between metabolite levels and biological and physical site characteristics as well as the foraging behavior of the birds using the sites on the individual rather than site level could add great insight into the suggestion of morphometric-based individual specialization (Elner and Seaman 2003). Furthermore, individual-based correlation analyses would dramatically increase the statistical power of detection in order to more thoroughly investigate the factors determining habitat quality. There are several aspects of this study that would have benefited from an individual-based approach and, while they are not without logistical difficulty, each aspect is discussed in detail below.

This study provided evidence for birds achieving higher triglyceride levels doing so at sites with more abundant macrofaunal prey. However, as discussed in Chapter 3, the statistical analysis used to detect this trend had low power. Multi-researcher collaboration allowing for an increase in the number of sites along the Pacific coast sampled concurrently would potentially allow for a statistically more robust test of this relationship. A logistically difficult but enhanced approach would be to adapt the probe-mark method (Sutherland et al. 2000, Wolf 2001); the control cores taken by that method would provide a value of prey abundance close to where a particular individual was foraging. However, for this method to be useful to this study, it would have to accompany the capture of the individual in order to have a value of prey abundance and metabolite levels for each individual. Although it is logistically difficult to target particular individuals for capture and to locate the core

location, the total number of cores to be analyzed would be comparable to that undertaken for this study. Alternatively, emphasis on quantitative fecal analysis may be more logistically feasible. It would not provide individual-based samples of prey abundance but would provide individual-based information not available in the present study. While it requires significant knowledge of invertebrate taxa (and their parts), quantitative analysis of fecal samples in the Western Sandpiper has been accomplished (Wolf 2001). Quantitative fecal analysis would allow for the correlation between metabolite levels and consumed prey rather than available prey. In addition, in contrast to the logistical difficulty of obtaining prey abundance and metabolite values for the same individual, fecal samples are easily collected from captured individuals, allowing for a logistically feasible method of obtaining a prey-related and metabolite value for each individual. The inclusion of individual data points in the correlation analysis would increase the sample size (from 7 to ~100), which would substantially increase the statistical power of the analysis. The determination of the prey consumption value would require a technique by which to collect the first fecal sample produced after capture (i.e. often in the net) and standardization to account for time since capture (i.e. fasting) as well as quantity of fecal sample.

This study found no evidence of a correlation between foraging mode and metabolite levels (D. Seaman and T. Williams, unpubl. data); however, confidence in the lack of relationship is weak given the low statistical power of detection. The achievement of similar metabolite levels at sites where birds employ different foraging modes indicates some level of global plasticity in foraging and suggests that the Western Sandpiper, on the species level, can achieve fat deposition on both surface-dwelling and burrow-dwelling prey. However, these results do not address individual plasticity or the possibility of individual specialization of foraging mode (Elner and Seaman 2003). The ability to test the correlation between individual foraging mode and individual fattening rate would be the optimal empirical approach to testing the ideas put forth by Elner and Seaman (2003) as well as increasing the statistical power of the analysis. Because it is not practical to target the capture of a particular individual (e.g. after conducting foraging observations on that individual) nor is it likely to resight the individual after release at large sites, it has to date been practically impossible to have foraging behavior and fattening rate data for the same

individual; therefore, the analyses have been necessarily restricted to the site level. A technique by which to capture, and blood sample, a particular individual after conducting foraging observations on it would be invaluable.

The influence of physical site parameters on fattening rates is particularly difficult to assess because the majority of them (e.g. size) do not vary seasonally or annually. Substrate penetrability (i.e. softness), however is a physical site characteristic that would be expected to be variable. There was inter-annual variation in substrate softness within site in our study (Chapter 2 this volume). The analyses used in this study were based on site level means; however, the substrate softness is undoubtedly spatially variable within a site. Wolf (2001) demonstrated that foraging mode correlated to substrate softness and hypothesized that the correlated foraging modes differed in energetic cost which, in turn, influenced the realized fattening rates at two different sites. Wolf (2001) was able to isolate individual peck marks and probe holes made by individuals on whom foraging observations were conducted. The combination of the technique employed by Wolf (2001) with the (idealistic) suggestion of having fattening rate data for individuals of known foraging behavior would allow one to test individual-based correlations between substrate softness, foraging mode, and fattening rate. This would allow a direct test of the hypothesis proposed by Wolf (2001) of fattening rates mediated by differential energetic requirements of different foraging mode.

Predicting rate of mass change from metabolite levels

Several studies have focused on testing the relationship between the rate of mass change and metabolite levels (Jenni-Eiermann and Jenni 1994, Williams et al. 1999, Jenni and Schwilch 2001); a primary goal of these studies has been to validate the technique in order to be able to predict the rate of mass change of a free-living individual based on the metabolite concentrations of a single blood sample. These studies often graph the metabolite value as the independent variable (x-axis) in order to emphasize the predictive potential of the analysis. In our study, metabolite levels were necessarily the dependent variable (y-axis in Figure 4.5) in order to correct for repetition of individuals as well as to test for the diet effect. Furthermore, this graphic

and analytical approach emphasizes the fact that while plasma metabolite analysis may eventually be useful for providing predictive equations for free-living birds, there is not yet sufficient information to apply this technique to our system in that manner; these concerns are described in detail below. Should sufficient data be obtained to support predictive equations, it would, however, be possible to use the model used in our study (metabolite value as the dependent variable) to predict the rate of mass change via inverse prediction.

The application of a predictive equation for rate of mass change in free-living individuals would require the decision of which metabolite or combination of metabolites to use. We have been unable in this study to combine metabolites (e.g. principal component analysis) due to the lack of correlation between glycerol and triglyceride levels (see Chapter 3) and the fact that high uric acid levels can be indicative of either fasting or fattening (see Chapter 1 for further detail). Glycerol had the strongest relationship with the rate of mass change in two independent studies of captive Western Sandpipers (Williams et al. 1999, Chapter 4 this volume), suggesting that it might have the greatest potential for predictive capacity. Furthermore, there was no diet effect on the relationship between glycerol and mass change, making it potentially reliable regardless of diet composition. However, while we seem able to interpret glycerol levels in captive individuals, the biological significance of variation in glycerol levels in free-living individuals is much less clear and may be due to the non-foraging factor of exercise on plasma glycerol levels (see Chapter 3 for discussion). This ambiguous representation of physiological state complicates the use of glycerol to predict mass change in free-living individuals. On the low-fat diet which more closely approximates the Western Sandpiper's natural diet, uric acid was significantly related to the rate of mass change. However, high levels of uric acid can be indicative of either protein catabolism or high turnover (i.e. fattening) (Guyton and Hall 1996, Jenni-Eiermann and Jenni 1994); this duality in the interpretation of uric acid is further evidenced by the conflicting results of a positive (Chapter 4 this volume) versus negative (Jenni-Eiermann et al. 2002) relationship to the rate of mass change. In the context of biological interpretation in free-living individuals, triglyceride still seems to have the most predictive potential of the metabolites analyzed in this study. While a significant relationship between the rate of mass change and triglyceride levels was not detected in this study, the lack of

detection is likely due to the narrow range of mass change induced in the captive individuals. The detection of the relationship was further complicated by low power due to the experimental design controlling for time effects and having individuals as their own control; an experiment targeted at testing for this relationship alone instead of in conjunction with diet effect would have more power of detection. If a study with more power was able to detect a relationship between triglyceride levels and the rate of mass change over a wide range of mass changes, that equation should be valid for the narrower range of mass change experienced in free-living individuals.

While there is potential for the predictive value of metabolite concentrations as indicators of mass change from one-time captures of free-living individuals, the relationship between the rate of mass change and the plasma concentrations of both triglyceride and uric acid was dependent on diet composition (i.e. protein and lipid content). If a single predictive equation (i.e. irrespective of diet) was employed, the rate of mass change that would be predicted for any given concentration of triglyceride or uric acid would be higher on the low-fat diet than the high-fat diet. Predictive equations using uric acid would be further complicated by the significant diet*rate of mass change interaction term. The diet-dependent nature of the mass change-metabolite relationship illuminated in this study suggests that differences in diet quality should be addressed prior to the application of predictive equations (e.g. Jenni-Eiermann and Jenni 1994, Jenni and Schwilch 2001) that were developed in captivity on a single diet. In addition, results of studies on captive individuals should be evaluated with caution if the diet composition of the diet provided in captivity differs from that available to free-living individuals in that system (e.g. trout chow used in shorebird studies; Williams et al. 1999, Jenni-Eiermann et al. 2002, Chapter 4 this volume). Furthermore, differences in fattening rates detected between sites (e.g. Schaub and Jenni 2001, Guglielmo et al. 2002, Chapter 3 this volume) should be interpreted cautiously if the lipid and/or protein content of the prey in the system of interest varies between stopover sites. While the prey base in our system has been demonstrated to vary between sites within the GB/PS stopover region (Chapter 2, this volume) and the lipid content of some invertebrates represented in the Western Sandpiper diet has been measured (Egeler and Williams 2000), a detailed study of the lipid and protein content (and total energy content) of the prey available to and consumed by Western Sandpipers has not been conducted. This study

clearly demonstrated differences in metabolite levels between stopover sites on a landscape scale. The results of the captive diet manipulation experiments in this study (Chapter 4) suggest that the fundamental step of testing the relationship between metabolite levels and mass change may be problematic if the diet employed during such experiments is not of similar diet composition to natural prey items. Furthermore, the biological interpretation of inter-site variation in metabolite levels should take diet composition into consideration. In the event of variable diet quality between sites, metabolite analysis may actually serve as an index of diet quality rather than the rate of fattening; in either case, metabolite concentrations are representative of habitat quality. Nevertheless, information on diet quality in this system is lacking and would enhance both the evaluation of the plausibility of predictive equations for mass change based on metabolite levels of free-living individuals as well as the evaluation of the factors determining habitat quality in this system, and should be the focus of future research.

Conservation and management implications

This study has demonstrated that migratory stopover sites within the GB/PS are heterogeneous and differ in several ecological and physical site characteristics. We further demonstrated that Western Sandpipers using these variable stopover sites achieved different metabolite levels and that plasma metabolite analysis was a useful measure of habitat quality. Furthermore, this study has provided evidence that this inter-site variation in metabolite levels may be correlated to at least one aspect of the available prey base. This study has elucidated several concerns that must be addressed prior to using metabolite concentrations to predict an absolute rate of mass change in free-living Western Sandpipers at migratory stopover sites. We are, however, confident that high plasma triglyceride levels are indicative of high levels of fattening and that comparison between sites of triglyceride levels provides useful information about the capacity of one site to support fattening relative to another site.

This study has demonstrated the dynamic nature of migratory stopover sites. The variation in habitat quality between sites as well as the temporal (seasonal and annual) heterogeneity of microhabitats and their use by shorebirds, which was

demonstrated by Jensen Access, should be of interest to managers. As stopover sites change over time, site selection choices and decisions made by shorebirds may also change within this changing landscape. The inter-site variation in metabolite levels detected during northward migration was related to a biological and physical site characteristic, suggesting that stopover site characteristics are reflected in the metabolite profiles of migratory shorebirds using those sites during stopover. While the correlation analyses to identify the primary factors determining habitat quality (i.e. a stopover site's capacity to support fattening) are in their infancy, this type of information may allow managers to prevent development or land management practices that would make a site less able to support hyperphagia by shorebirds during migration.

Land conservation, including acquisition, is a part of most management plans; in order for managers to set aside the optimal combination of stopover sites that will best prevent population-level declines, more information on site use and selection by shorebirds is necessary. The majority of efforts to protect wetlands for migratory shorebirds have mainly targeted stopover sites used by the most shorebirds. Currently, the only major effort in shorebird habitat conservation is protection of stopover sites within the Western Hemisphere Shorebird Reserve Network (WHSRN) (Bildstein et al. 1991). Inclusion criteria for the network are based on the numbers of shorebirds using the site and, as such, sites of regional and hemispheric importance are defined as those being used by at least 20,000 and 500,000 individuals, respectively (Bildstein et al. 1991). However, this management strategy is only optimal if all mud is treated equal in the eyes of individual sandpipers and if stopover site selection is flexible on the individual level. There is evidence that some small stopover sites during southward migration are used by lighter individuals (Ydenberg et al. 2002); suggesting that birds of different condition may have different stopover site requirements or preferences. Furthermore, the example in our study of individuals using the small Totten Inlet, which supports extremely high densities of shorebirds during northward migration, regardless of the lower triglyceride levels they achieved there may be suggestive of inflexible site selection. Site fidelity even after habitat deterioration has been demonstrated in some systems (Ganter and Cooke 1998; Esler et al. 2002); it is possible that site selection decisions in our system are based on factors other than habitat quality (i.e. the fattening rate achievable at that

site). While inter-annual stopover site fidelity is not known in the Western Sandpiper, Butler et al. (2002) found that once individuals settle within the Fraser River Delta, they tend to stay on the same or adjacent beach throughout their stay in the delta; this suggests once an individual chooses a particular stopover site, it may stay in the vicinity throughout the stopover duration even if subsequent levels of fattening achieved at that site are low. Site selection independent of habitat quality, even if only by a portion of the population, suggests that all stopover sites that are used by shorebirds during migration may be important on the population level.

There seem to be two major findings from this study that suggest a two-objective management strategy. First, metabolite levels are variable between stopover sites and are reflective of biological and physical site characteristics, suggesting that land management decisions should strive to limit activities that would make sites less capable of supporting high rates of fattening, and land acquisition decisions should pay particular attention to those sites supporting high rates of fattening. However, this study also demonstrated that, at least in this system, bird abundance at stopover sites is not an adequate index for habitat quality, as indicated by the fattening rates supported by the site. There is evidence that some sites that support high densities of shorebirds during migration do not support high triglyceride levels. While there is limited data for the Western Sandpiper on site selection and fidelity, there is some evidence that site selection is not based on habitat quality and that sites that support lower rates of fattening may still be of importance to the birds that use them. Therefore, management strategies should not be based exclusively on the capacity to support fattening, and the possibility of individual specialization and requirements (Elner and Seaman 2003) should be taken into consideration.

This study has elucidated several key points regarding fattening rates and habitat quality for migratory Western Sandpipers. The findings within, in conjunction with a further understanding of the primary factors determining habitat quality and individual needs, could enhance the ability of managers to make sound management decisions concerning migratory stopover sites for the Western Sandpiper and other shorebirds.

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