RELATIONSHIPS BETWEEN PLASMA METABOLITES AND MASS GAIN IN SEMIPALMATED SANDPIPERS DURING MIGRATORY STAGING IN THE NORTHUMBERLAND STRAIT

BY

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Abstract

Semipalmated Sandpipers are long distance migrants that depend upon staging sites to build reserves during their fall migration to non-breeding sites. Eastern breeding Sandpipers use sites in Atlantic Canada, such as Petit-Cap, NB, to prepare for migration to South America. Rates of mass gain are an important metric used to predict migratory success and assess site quality, although this is difficult to obtain given that it requires the recapture of individual birds, which is not feasible at many staging sites. Plasma metabolites are a useful tool for indicating these metabolic changes in mass over time, but these relationships have not been examined in free-living Semipalmated Sandpipers, despite their widespread use in the literature. At the study site, Petit-Cap, recaptures are possible, allowing us to test this relationship.

We examined plasma metabolites (plasma triglycerides, glycerol, and β-hydroxybutyrate) in relation to changes in mass in recaptured Semipalmated Sandpipers at Petit-Cap, NB. We failed to detect relationships between plasma triglycerides and β-hydroxybutyrate and mass changes in recaptured Semipalmated Sandpipers, suggesting that inferences of mass changes through plasma metabolites should not be integrated over long time intervals. We did find a significant positive relationship between glycerol and mass gain. As an extension of studying migratory refueling, we also assessed diets of the shorebirds while staging at this site. We found that bivalves were contributing largely to diets of both adult and juvenile Semipalmated Sandpipers, with no significant differences in diet breadth between the two age classes.

While collecting data for this study, we encountered a storm midway through the staging season. This provided an opportunity to examine the effects of short-term weather events upon body condition and refueling in staging Semipalmated Sandpipers. We found significant impacts upon migratory refueling in both recaptured and non-recaptured Semipalmated Sandpipers following a storm. Recaptured birds, on average, lost mass during the week following the storm. Similarly, non-recaptured birds had significantly lower size adjusted masses during versus before or after the week of the storm. These findings support previous research on the sensitivity of

staging shorebirds to environmental fluctuations and highlight the importance of considering weather events a threat to migratory refueling.

We emphasize the need for further investigation into the relationships between plasma metabolites, diet, handling, and weather upon rates of migratory refueling in Semipalmated Sandpipers.

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Chapter 1: Introduction

Migration

The benefits and risks of migration have been examined thoroughly in many studies (Alerstam et al., 2003; Avgar et al., 2014; Lincoln & Peterson, 1979; Somveille et al., 2015). Species migrate to take advantage of seasonal resources in different areas of the world; to avoid harsh winters, to breed, or avoid predators (Somveille et al., 2015). Such journeys directly affect the physiology, behaviors, survival, and other characteristics of migratory animals (Alerstam et al., 2003).

Semipalmated Sandpipers *(Calidris pusilla)* are a Nearctic breeding species, breeding in the Arctic before migrating to South America for the non-breeding season (Hicklin & Gratto-Trevor, 2020; Morrison, 1984). Adult and juvenile Semipalmated Sandpipers exhibit clear differences during migration. They migrate at slightly different times, with adults arriving about a month earlier than juveniles (Morrison, 1984). Adults are associated with high survival rates, higher fat loads, and shorter lengths of stay while staging (Dunn et al., 1988; Gratto-Trevor & Vacek, 2001; Mann et al., 2017).

As Semipalmated Sandpipers migrate southward following the breeding season, they make stops to rebuild energy reserves (Brown et al., 2017; Churchwell et al., 2018; Dunn et al., 1988). High quality staging sites must offer both adequate resources and safety (Hope et al., 2020; Sprague et al., 2008). Semipalmated Sandpipers make extensive use of coastal sites including mudflats, salt marshes, and beaches (Hicklin, 1987; Neima et al., 2020). Eastern breeding birds refuel in the Atlantic region, making use of sites such as the Bay of Fundy (Hicklin, 1987; Hicklin & Gratto-Trevor, 2020). Recent research has suggested coastal sites outside of the Bay of Fundy are also very important for staging (Doiron, 2021; Linhart, 2021).

Site condition is closely linked with success. In migratory American Redstarts, higher quality sites were associated with earlier arrival at breeding grounds, and subsequently, higher breeding success (Norris et al., 2004). Further studies have highlighted the effects of site quality degradation as a driver of shorebird declines (Li et al., 2019; Studds et al., 2017). At Semipalmated Sandpiper staging sites, extreme tidal events are known to affect behaviors and significantly increase length of stay in the Bay of Fundy (Mann et al., 2017). As such, environmental factors have the potential for significant effects upon the sandpipers. Weather events are associated with mass mortality as well as nonfatal migratory delays and changes in foraging behaviors (Beauchamp, 2006; Carneiro et al., 2020; Newton, 2007; Richardson, 1978). In the wake of global climate change, weather events are expected to increase in magnitude and number and should be examined as rising threats to Semipalmated Sandpipers (National Academies of Science, Engineering, and Medicine, 2016; Phelan, 2011).

Fuelling migration

Migrating birds accumulate most of their energy in the form of fat. Lipids are the most compact and efficient forms of energy storage, making it adaptive to store energy reserves as fats, though protein is also necessary to replenish flight muscles and therefore does make up a proportion of this added weight (Jenni & Jenni-Eiermann, 1998; Piersma, 1990). Overall, the majority of lipid stores in Semipalmated Sandpipers are triacylglycerols (Napolitano & Ackman, 1990).

Physiologically, fat deposition is complex. Birds take in food which, in the case of shorebird diets, are typically high in polyunsaturated fatty acids (Maillet & Weber, 2006; Quinn et al., 2017; Schnurr et al., 2020). As food is broken down, fatty acids are released, and then combine with glycerol molecules to form triacylglycerol via enzyme action (Allen, 1976). These triglycerides are then transported through the blood plasma, associated with lipoproteins due to their nonpolar nature (Cox & García-Palmieri, 1990). At deposition sites, these molecules are then hydrolyzed, allowing triglycerides to be stored in adipose tissue (Ramenofsky, 1990). This stored fuel is then used to power flight and metabolic functions during migration.

Optimizing fuel loads for migration is important; flight becomes costly when carrying too much weight and conversely, not enough fuel means that birds will not survive migration (Pennycuick, 1975, 1978). In addition to gaining fat in preparation for migration, migrants must also account for costs incurred after they reach their destination; the phenomenon of mass loss *after* reaching coastal staging areas is welldocumented in shorebirds and is attributed to factors like search, settling, and social costs (Alerstam & Lindström, 1990). The size of reserves and the rates at which they are gained are directly correlated with survival and migration speeds, and as such, are extremely important in understanding and predicting migratory success and survival (Harrington, 1998; Lindström, 1991).

Traditional methods of measuring mass gain rates are often inaccurate and difficult to obtain. Suggested techniques use average changes in mass in populations over time to measure fattening rates. However, this method does not account for the turnover of shorebirds, resulting in poor estimates. A more accurate method of estimating fat deposition rates for an individual bird from a single measurement would be invaluable in this field.

The relationship between fat deposition and plasma triglycerides may make it possible to estimate mass gain rates by measuring triglycerides in blood plasma (Jenni-Eiermann et al., 2002; Jenni-Eiermann & Jenni, 1994; Ramenofsky, 1990; Williams et al., 1999). Other metabolites in plasma can be similarly useful in explaining metabolic processes concerning migratory mass fluctuations. β-hydroxybutyrate is a ketone body that is synthesized by the body when carbohydrate levels are very low or during periods of fasting or intense energy expenditure (Newman & Verdin, 2017). Similarly, higher concentrations of glycerol are often associated with low rates of mass gain (Jenni-Eiermann et al., 2002; Lyons et al., 2008). In captive studies of both garden warblers and red knots, β-hydroxybutyrate levels were low during periods of mass gain but increased during fasting (Jenni-Eiermann et al., 2002; Jenni-Eiermann & Jenni, 1994). In similar studies on captive Western Sandpipers, metabolites were found to relate to mass changes over multiple days (Williams et al., 1999). However, this relationship between metabolites and mass gain has not been quantified for Semipalmated Sandpipers, even though it is used as a proxy within the field.

Diet Breadth

Resources at staging sites vary and have implications for migrants that depend on them. In the Bay of Fundy, Semipalmated Sandpipers feed extensively on the abundant *Corophium volutator* and a variety of polychaetes (Hicklin & Smith, 1979).

However, more recent studies have found that these shorebirds have relatively flexible diets and can depend upon a wide variety of prey, including other invertebrates (such as small molluscs, gastropods, and bivalves), and biofilm (Gerwing et al., 2016; Quinn & Hamilton, 2012; Skagen & Oman, 1996).

Since our objective is to examine mass gain rates of Semipalmated Sandpipers, which are influenced by diet, attention must be paid to the factors influencing diet. Recent studies found evidence of age-related spatial segregation in another shorebird species, *Calidris canutus canutus*. Red knot juveniles tended to forage in riskier, lower quality nearshore areas in comparison to older birds that preferred further offshore sites (van den Hout et al., 2017). In American Oystercatchers, juveniles were found to be 44% less efficient than their adult counterparts at foraging- eating smaller prey and eating more slowly (Goss-Custard & Durell, 1987). Doiron (2021) found that juvenile Semipalmated Sandpipers spend more time moving around and between staging sites. All of these suggest that differences in diet breadth may exist between adult and juvenile Semipalmated Sandpipers. Such differences can be assessed using stable isotopes ($\delta^{15}N$, $\delta^{13}C$) of blood plasma. This blood component has a rapid turnover, with a half-life of about three days, meaning that samples can be used to infer diet over approximately the previous week by comparing plasma signatures to those found in prey items (Hobson & Clark, 1993). Information provided from these stable isotope analyses will allow us to determine the trophic level of prey eaten and estimate diet breadth. Recent evidence suggests that diet may influence plasma metabolites, with certain nutritional prey compositions driving differences in metabolic profiles of consumer plasma (Cerasale & Guglielmo, 2006; Smith et al., 2007; Smith & McWilliams, 2009). Since adult and juvenile sandpipers behave differently while staging, assessing diet breadth may reveal drivers behind any variability in plasma metabolites between the age classes.

Study objectives

The primary goal of this study is to quantify the correlation between key plasma metabolites (plasma triglycerides, glycerol and β-hydroxybutyrate) and mass gain during migratory refueling. We also examined differences in diet breadth (carbon and nitrogen isotopes) between age classes, and how they relate to metabolites. Finally, we assessed the impact of a short-term weather event upon migratory refuelling.

Chapter 2: Methods

Shorebird Catching and Sampling

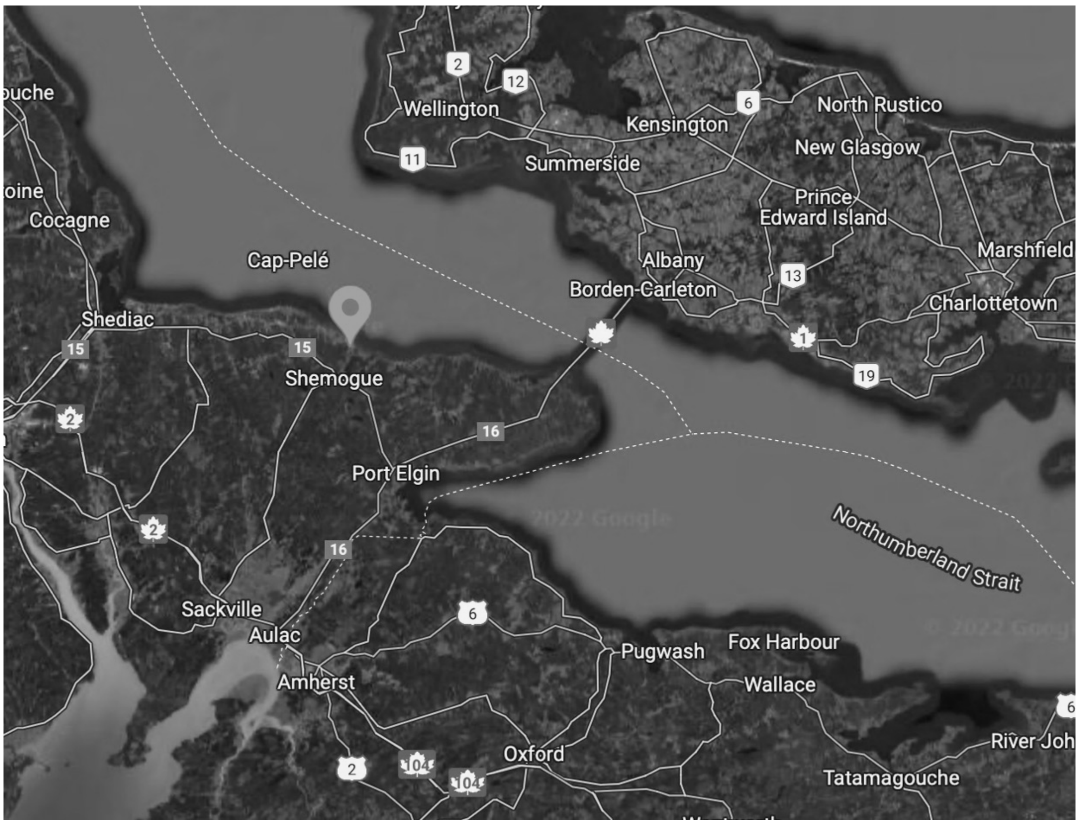

Figure 1.0. Study Site: Petit-Cap, NB. (46.19^{O}N, -64.15^{O}W). Study site is indicated with a pin. Map data from Google Earth, Maxar Technologies.

690 Semipalmated Sandpipers were captured at Petit-Cap, NB from July 28 to October 20, 2021 (Figure 1.0). Birds were captured using mist nets set up prior to sunset in areas of exposed tidal flat. Once captured, sandpipers were immediately weighed and flagged to prevent stress-related mass loss error (Castro et al., 1991; Davidson, 1984; Warnock et al., 1997). On their first capture, we took general morphometrics (bill culmen length, tarsus length, and flattened straightened wing

length) using digital calipers and wing bars. White, three-digit alphanumeric flags were placed on the upper right leg and glued with PVC glue. Unique USGS aluminum bands were secured on the upper left leg. Following this, we aged birds by assessing wear and patterns of feathers (Pyle, 1997). Juvenile birds had the 6th primary coverts pulled for isotopic analyses for another project and a portion of these birds had the brachial vein pricked with a 27-gauge sterile needle and had a drop of blood collected and preserved on an FTA card (Whatman Inc.) for later molecular sexing.

When birds were recaptured, they were immediately re-weighed once more. These masses were corrected prior to analyses by subtracting the average weight of a flag and a band to be consistent with the first mass collected. Only birds that were caught at least three days after their initial capture were sampled for plasma metabolites, to minimize the effects of handling on subsequent measurements (Atkinson et al., 2007; Davidson, 1984). Since the purpose of the experiment was to examine the relationship between metabolites and mass gain rates, this three-day buffer was used to ensure that the birds were gaining mass normally after exposure to stress. Then, 140 uL blood samples for plasma metabolites were taken from the brachial vein, using heparinized, 70 uL capillary tubes, Eppendorf tubes, and 27.5-gauge needles within 20 minutes of capture. Small pieces of paper towel and pressure were applied to the bleed site until wounds had clotted. Following this, birds were allowed to rest in dark enclosed pens, and then released. Blood samples were immediately placed on ice, and plasma was separated in lab by spinning samples at 10 000 rpm for one minute (mySPIN12 Mini Centrifuge, Thermo Scientific), and stored at -20°C prior to metabolite analyses. In total, 33 birds were recaptured, and 23 had plasma samples collected. Blood samples were taken in the same way for isotopic diet analyses- though birds were only sampled if their mass was greater than 32 g, and these birds did not need to be recaptured. In total, 36 samples were taken for isotope analysis, 8 of which were recaptured birds.

Plasma metabolite analyses

Levels of total plasma triglycerides present in samples were determined using two colorimetric assays: one for free glycerol (Cayman Item No. 10010755, Ann Arbor, MI, USA), and one for triglycerides (Cayman Item No. 10010303, Ann Arbor, MI, USA). All colorimetric assays were read using a Spectramax Plus 348 spectrophotometer (Molecular Devices LLC, Sand Jose, CA).

The free glycerol assay works by converting free glycerol into quinoneimine dye and occurs through enzymatic action as follows.

$$Glycerol + ATP \xrightarrow{Glycerol\ Kinase} Glycerol - 3 - Phosphate + ADP$$

$$Glycerol - 3 - Phosphate + O2 \xrightarrow{Glycerol\ Phosphate\ Oxidase} Dihydroxyacetone\ Phosphate + H2O2$$

$$2H2O2 + 4 - AAP + ESPA \xrightarrow{Peroxidase} Quinoneimine\ dye + 4H2O$$

The assay was prepared by diluting 10 uL plasma samples 1:5 with the provided standard diluent. Duplicates of 10 uL were pipetted into a 96 well plate, then 150 uL of the diluted Enzyme Buffer solution (glycerol kinase, glycerol phosphate oxidase, and peroxidase) was added. The plate was shaken and then incubated at room temperature for 15 minutes prior to reading at 540 nm. Amounts of glycerol were then determined using a standard curve, using the equation below.

$$Glycerol\ \left(\frac{mg}{L}\right) = \left[\frac{Corrected\ absorbance - y\ intercept}{slope}\right] * Dilution\ factor$$

The triglyceride assay works in the same way, by converting glycerol to dye, but with an extra step, lipoprotein lipase, to free glycerol from triglycerides.

$$Triglycerides \xrightarrow{Lipoprotein\ Lipase} Glycerol + Fatty\ acids$$

$$Glycerol + ATP \xrightarrow{Glycerol\ Kinase} Glycerol - 3 - Phosphate + ADP$$

$$Glycerol - 3 - Phosphate + O2 \xrightarrow{Glycerol\ Phosphate\ Oxidase} Dihydroxyacetone\ Phosphate + H2O2$$

$$2H2O2 + 4 - AAP + ESPA \xrightarrow{Peroxidase} Quinoneimine\ dye + 4H2O$$

This was prepared by placing duplicates of the diluted (1:5) samples into a 96 well plate, followed by 150 uL of the Triglyceride Enzyme Mixture (lipoprotein lipase, glycerol kinase, glycerol phosphate oxidase, and peroxidase). The plate was shaken and then incubated at room temperature for one hour. Absorbances were read at 540 nm, and triglyceride levels were calculated using a standard curve determined from provided standards.

$$Triglycerides \left(\frac{mg}{dL}\right) = \left[\frac{Corrected\ absorbance - y\ intercept}{slope}\right] * Dilution\ factor$$

The total triglycerides in the blood were then calculated by subtracting the value from the first assay from the second- therefore accounting for any free glycerol in the blood. Total plasma triglyceride values were converted from mg/dL to more comparable units of mmol/L by multiplying by a factor of 0.01129 (Haney et al., 2007).

Concentrations of β-hydroxybutyrate were measured using colorimetric assays (Cayman Item No. 700190, Ann Arbor, MI, USA). This assay works through the production of formazan dye, which occurs when NADH reduced through the action of β-hydroxybutyrate dehydrogenase reacts with the included detector. The samples were diluted 1:9, 1:14, 1:19,1:21 with the standard diluent, depending on the available volume of plasma. 50 uL of sample were pipetted into 96 well plates, followed by 50 uL of the Developer Solution (WST-1). Samples were then shaken and incubated at room temperature in the dark for 30 minutes. The assays were measured at 450 nm. Concentrations were determined using a relationship derived from a standard curve.

$$\beta - hydroxybutyrate \xrightarrow{\beta - hydroxybutyrate\ dehydrogenase} Acetoacetate + NADH + H$$

$$NADH + WST1 \xrightarrow{Diaphorase} WST1\ Formazan$$

$$\beta - hydroxybutyrate\left(\frac{mmol}{L}\right) = \left[\frac{Corrected\ absorbance - y\ intercept}{slope}\right] * Dilution\ factor$$

Diet Analyses

The diet of Semipalmated Sandpipers at Petit-Cap was estimated using stable isotope analyses. Only blood samples from birds heavier than 32 g were used. Since plasma in sandpipers has a turnover rate of about 3 days, this limit ensures the birds (which arrive at ~20 g) have been feeding on prey items in the area for long enough to reflect their local diet (Hobson & Clark, 1993; Quinn & Hamilton, 2012). Therefore, the stable isotopes in the plasma can be compared to those found in local prey items. Large amounts of sediment were sampled in late August from areas where the shorebirds were seen foraging. Biofilm samples were also taken at this time, using a spatula to scrape the visible layer of biofilm from the top of sediments into scintillation vials. Samples were taken back to lab to be live sorted, where they were separated by taxa (Predatory polychaetes, tube-forming polychaetes (i.e. *Spionidae*), *Gemma gemma, Macoma*, *Mya arenaria, Hydrobia,* and amphipods). All samples were stored at -20°C. Biofilm samples intended for carbon analyses were acid-treated to react with and remove inorganic carbon (i.e. carbonate) (Kennedy et al., 2005). A weak solution of hydrochloric acid (0.5 M) was added by dropper and allowed to react with the sample. This was removed and repeated until no bubbles were observed in solution. Bivalves (*Mya arenaria* and *Gemma gemma*) and gastropods (*Hydrobia*) were dissected from their carbonate shells. 15 uL plasma samples were pipetted into small, pre-weighed tin boats, with a minimum sample weight of 0.5 mg. Following this, all samples were dried in an oven at 70°C for 24 hours, then ground to a fine powder (plasma samples were not ground). Target weights for invertebrates were 1.00 mg, and

40.00 and 70.00 mg for biofilm, depending on particle size. Stable isotopes were analyzed using an elemental analyzer (Elementar Pyrocube Elemental Analyzer) and spectrometer (Isoprime Precision Isotope Ratio Mass Spectrometer), at the Stable Isotope Laboratory, Mount Allison University. The relative ratio of sample isotopic signature to standards was calculated using the following equation.

$$\delta^a X_{(Sample)} = [(\frac{R(sample)}{R(standard)}) - 1] * 1000$$

Fractional coefficients derived from Dunlin (*Calidris alpina*), a closely related sandpiper species, were used to adjust isotope signatures, as the processes of the body involved with nutrient uptake can skew values and make them incomparable to source signatures (Ogden et al., 2004; Quinn & Hamilton, 2012). Applying these coefficients places the consumer within the range of prey items in the mixing models.

Statistical Analyses

Overview

Analyses were performed using R statistical programming (version 1.2.1335) with R Studio interface (RStudio Team 2018). Some data were processed using tidyverse functions (Wickham et al., 2019). Assumptions of homogeneity of variance were tested using NCV (non-constant variance) tests, Levene's tests, and residual plots. Normality was also visually assessed, and formally tested using Shapiro-Wilk normality tests. When data failed to pass parametric assumptions, transformations were applied. If data still did not comply with necessary assumptions, non-parametric testing was implemented. Data were plotted using the 'ggplot2' package (Wickham, 2016), 'simmr' package, (version 0.4.5) (Parnell, 2021), and 'SIBER' package (Jackson et al., 2011).

Plasma triglycerides and mass gain

Plasma glycerol and triglyceride samples with variability between the sample duplicates greater than 15% were not used in analyses. For recaptured birds, this left sixteen samples- but four of these birds experienced a weather event that affected their fattening rate and were therefore excluded from these mass gain analyses.

The relationship between plasma metabolites and changes in mass was investigated using multiple regressions of mass change per day against the metabolites (plasma triglycerides, glycerol, and β-hydroxybutyrate) and mass at recapture. Simple linear regressions were also used to assess some relationships separately. Change in mass was calculated by subtracting the mass at recapture from the mass at first capture, divided by the time elapsed between capture events. A regression was also run to test the relationship between days elapsed between capture events and plasma triglycerides.

In other metabolite analyses, relationships were tested against size adjusted mass, calculated as follows. This adjustment controls for the effect of the size of the bird upon fat levels (Davidson, 1983; Piersma & Davidson, 1991; Winker et al., 1992).

$$Size\ adjusted\ mass\ (g) = \frac{Mass}{Wing\ chord\ length^3} * 10000$$

These relationships were investigated first using linear regressions. Data from recaptured birds passed assumptions, and the effect of size adjusted mass upon plasma triglycerides was visualized and tested. Data from non-recaptured birds (n=32) failed assumptions, so a square root transformation was applied prior to analysis. Next, an ANCOVA was used to examine the effects of independent factors of age, recapture status, and adjusted mass on a dependent variable of plasma triglyceride values.

Effects of weather events on refueling

Analysis of effects of a severe storm on recaptured birds were performed using two Welch's T-tests, with adjusted mass as the dependent variable, and then with mass gain rates. Data for non-recaptured birds failed assumptions even after transformations, so a non-parametric Kruskal-Wallis test was used to test storm effects on adjusted mass.

Diet

Diets of Semipalmated Sandpipers were estimated through Bayesian mixing models, using isotopic signatures from plasma and local prey samples, using the 'simmr' package, (version 0.4.5) (Parnell, 2021). Potential prey items were sampled from areas of known foraging and their isotopic signatures added into models. General diet niches were estimated using these signatures with the'SIBER' package (Jackson et al., 2011). A non-parametric permutational multivariate analysis of variance (PERMANOVA) was used to assess differences in isotopic signatures between hatch year and after hatch year Semipalmated Sandpipers, since data failed all univariate and multivariate assumptions. This was performed with the 'adonis' function in the 'vegan' package (version 2.5-7) (permutations = 999, dissimilarity matrix = Bray-Curtis) (Oksanen et al., 2020). A test of differences of dispersions was also completed, using the betadisper function in 'vegan'.

Chapter 3: Results

Plasma Triglycerides and mass gain

The relationship between plasma triglyceride values and size adjusted mass varied between birds captured for the first time and recaptures (Table 1.1, Figure 1.1). Although fatter birds had higher plasma triglyceride levels overall, the slope was much steeper for birds captured for the first time and did not differ from zero for recaptured birds (Table 1.1, Figure 1.1). These relationships were consistent among ages (Table 1.1). Furthermore, we found no evidence of a significant relationship between plasma triglycerides or β-hydroxybutyrate and rates of mass gain (g/day) in recaptured Semipalmated Sandpipers (Table 1.2, Table 1.3, Figure 1.2). We did find a borderline significant relationship between rates of mass gain and glycerol in a multiple regression, and a significant relationship in a simple linear regression (Table 1.2, Table 1.3, Figure 1.2). Time of recovery following the first capture (days elapsed between capture events) had no significant relationship with plasma triglycerides (Table 1.3).

Table 1.1. Results from an analysis of covariance of the effect of recapture status, age, and size adjusted mass on plasma triglycerides in Semipalmated Sandpipers captured at Petit-Cap, NB. Significant and interpretable values are bolded.

Variable	M.S.	df	F value	*p* value
mass	49.73	1	45.79	<0.0001
status	0.66	1	0.61	0.44
age	0.17	2	0.16	0.85
mass *status	4.54	1	4.18	**0.049**
mass* age	0.35	1	0.33	0.57
status*age	0.026	1	0.02	0.88
mass*status*age	0.006	1	0.01	0.94
Residuals	1.086	35		

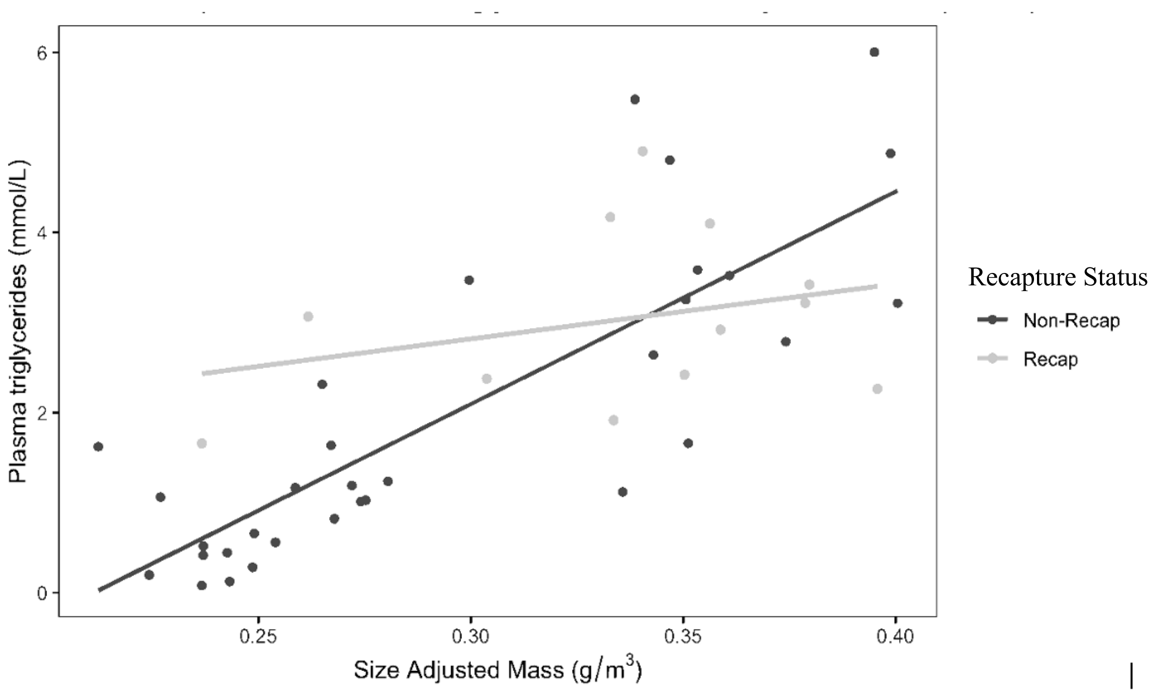

Figure 1.1. Plotted interaction from an ANCOVA between plasma triglyceride levels (mmol/L) and size adjusted mass (g/mm^3) and recapture status. N_{Recap}=12 and $N_{Non-Recaps}$=32. Results from statistical analyses are provided in Table 1.1.

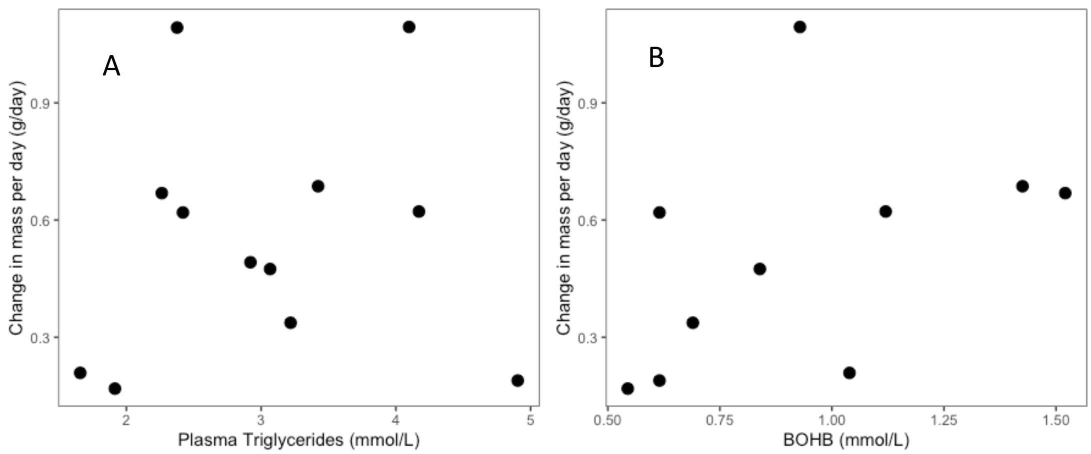

Figure 1.2 Relationship between plasma triglycerides (mmol/L), A, and β-hydroxybutyrate (BOHB) (mmol/L), B, and change in mass per day (g/day). Results of statistical analyses are provided in Table 1.3.

Table 1.2 Results from multiple regression of plasma triglycerides, glycerol, and mass at capture upon mass gain rates (g/day). ($F_{3,8}$=1.58, p=0.30., R^2 = 0.37).

Variable	Std. Beta Coefficient	t value	*p* value
Plasma T	-0.20	-0.61	0.56
Gly	0.64	1.98	0.08
Mass	0.093	0.31	0.77

Table 1.3. Results from univariate linear regressions of the relationships between plasma metabolites and differing measurements of body condition in Semipalmated Sandpipers or time between captures. Mass gain rates were the difference in mass from first and second capture divided by days elapsed between captures. Significant values are bolded. Plasma T refers to plasma triglycerides, BOHB to β-hydroxybutyrate, Gly to plasma glycerol. The data for the non-recapture regression were square root transformed to meet assumptions.

Recapture status	IV	DV	Std. Beta coefficient	R^2	df	F statistic	t value	*p* value
Recaptured	Plasma T	Mass gain	0.12	0.015	1,10	0.15	0.38	0.71
Recaptured	BOHB	Mass gain	0.31	0.21	1,8	2.14	1.46	0.18
Recaptured	Gly	Mass gain	0.58	0.34	1,10	5.13	2.26	**0.047**
Recaptured	Adj. mass	Plasma T	0.29	0.087	1,10	0.95	0.98	0.35
Non-recaptured	Adj. mass	Plasma T	0.83	0.68	1,30	64.27	8.02	**<0.0001**
Recaptured	Days between captures	Plasma T	-0.071	0.0047	1,10	0.05	-0.22	0.83

Diet

Estimates from stable isotope data of Semipalmated Sandpiper blood plasma and potential prey items at Petit-Cap, NB, suggest that bivalves make up a substantive portion of diet (Figure 2.1, Figure 2.2). No significant difference was detected in isotopes between age classes (Fig 2.1. PERMANOVA; $F_{1,17}$= 1.18, p=0.34). Similarly, no significant differences in dispersion were detected between age classes, though there was a tendency to greater dispersion in juvenile birds (Fig 2.3. Multivariate dispersion; $F_{1,17}$ =2.98, p=0.10.). In general, hatch year birds tend towards a slightly broader diet niche than after hatch year birds. No significant effect of isotopic signature was detected upon plasma triglycerides (Table 2.1).

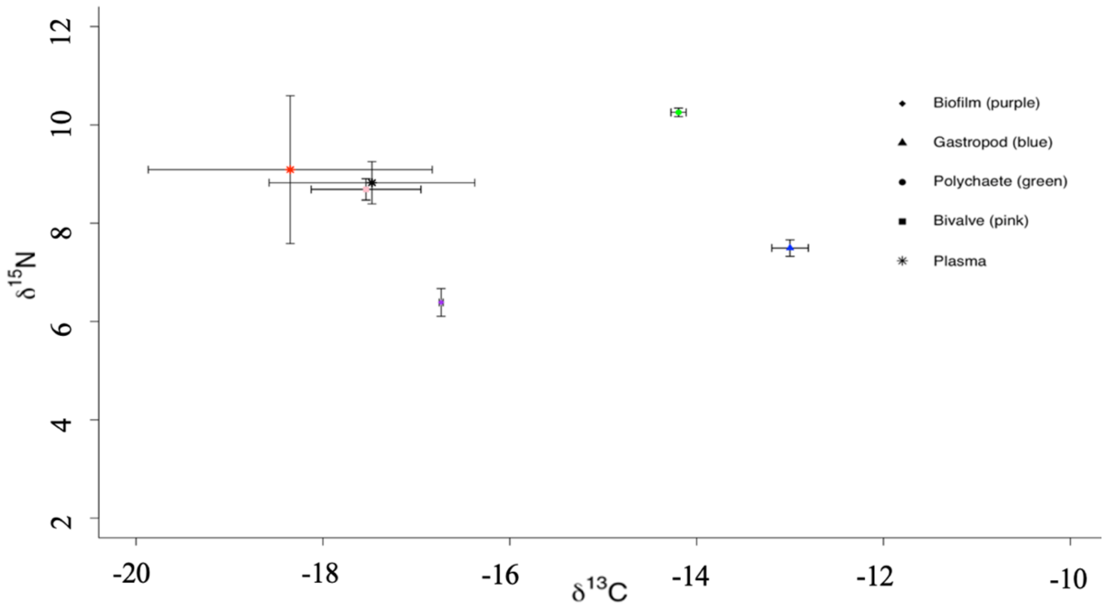

Figure 2.1. Average $\delta^{15}N$ and $\delta^{13}C$ isotope signatures of prey and plasma from Semipalmated Sandpipers captured at Petit-Cap. HY Semipalmated Sandpipers are denoted by black, AHY by red. N_{HY}=10, N_{AHY}=8. A fractionation coefficient of -3.3 was applied to $\delta^{13}C$ and a coefficient of -0.5 was applied to $\delta^{15}N$ signatures. Grey error bars represent standard deviation.

Table 2.1 Results of a multiple regression of plasma triglycerides against isotopic signatures of after hatch year and hatch year Semipalmated Sandpipers captured at Petit-Cap, NB. ($F_{2,7}$=0.015, p=0.99, R^2=0.0041).

Factor	Std. Beta coefficient	t value	*p* value
$\delta^{15}N$	0.025	0.53	0.95
$\delta^{13}C$	0.070	0.17	0.87

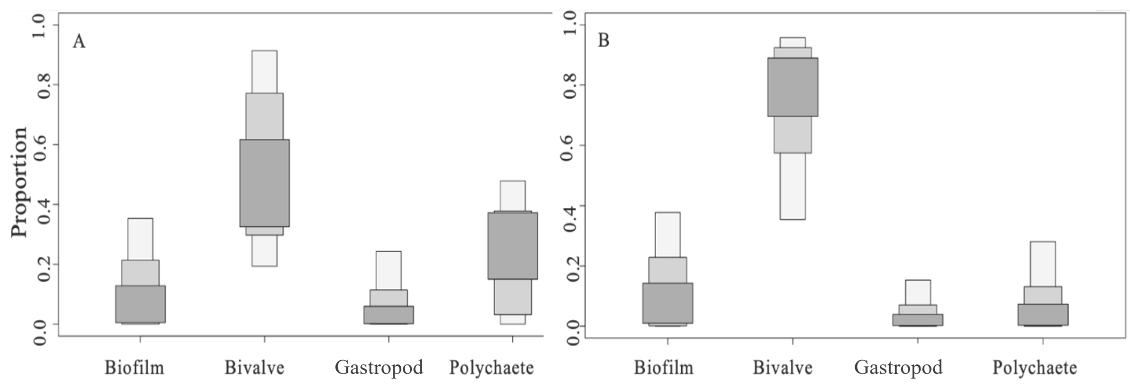

Figure 2.2. Estimated proportion of potential prey items in the diets of hatch year (A) and after hatch year (B) Semipalmated Sandpipers captured at Petit-Cap, NB from isotopic mixing models. Proportions are shown with 95, 75, and 25% credibility levels, denoted by lightening shades, respectively.

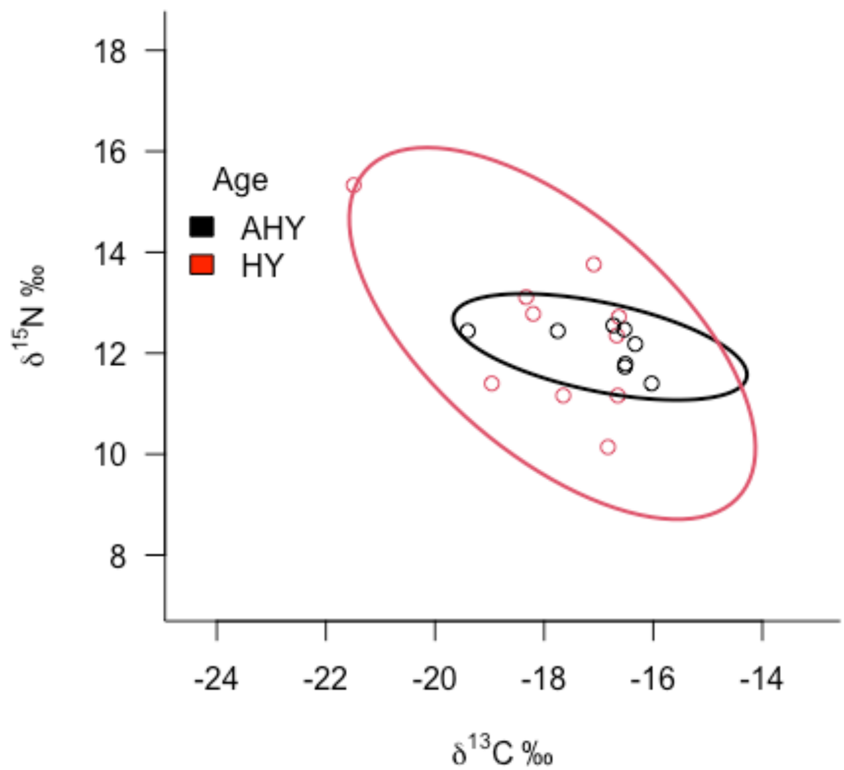

Figure 2.3 Isotopic diet niches of hatch year (HY) and after hatch year (AHY) Semipalmated Sandpipers captured at Petit-Cap, NB, estimated using δ^{15}N and δ^{13}C isotope signatures of blood plasma in parts per thousand. $N_{HY} = 10$, $N_{AHY} = 8$.

Effects of weather events on refueling

A severe weather event occurring on September 2, 2021, had significant effects upon body condition of Semipalmated Sandpipers at Petit-Cap, NB. Only hatch year birds were included in analyses, since no adults were caught following the storm. Size adjusted masses and mass gain rates of recaptured hatch year Semipalmated Sandpipers that were recaptured within seven days of the weather event were significantly lower than those recaptured before or after this period (Table 3.1, Figure 3.1). Size adjusted masses were also significantly lower in non-recaptured birds captured within the seven-day window (Table 3.1, Figure 3.2).

Table 3.1. Results from analyses of effects of storm exposure on recaptured and non-recaptured hatch year Semipalmated Sandpipers captured at Petit-Cap, NB. Dependent variable is denoted by DV. Significant values are bolded.

Recapture status	Analysis	DV	df	t value	Chi squared	*p* value
Recaptured	Welch's T-test	Adj. mass	8.12	2.96	**-**	**0.018**
Recaptured	Welch's T-test	Mass gain	6.96	4.39	**-**	**0.0032**
Non-recaptured	Kruskal-Wallis	Adj. mass	2	-	59.04	**<0.0001**

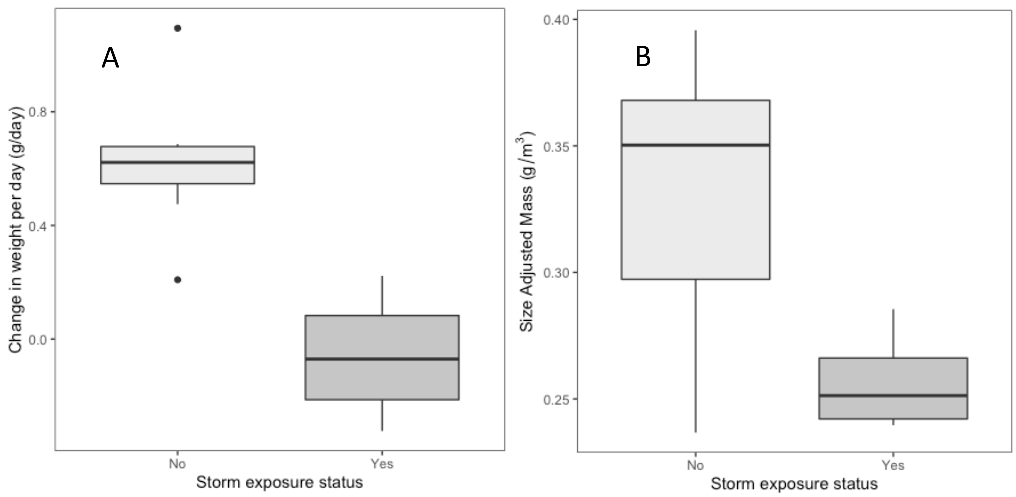

Figure 3.1. Boxplots of size mass gain per day (A) and adjusted mass (B) and storm exposure status of recaptured hatch year Semipalmated Sandpipers captured at Petit-Cap, NB. n=7 for no, n=4 for yes. Boxes represent first and third quartiles around the median line. Whiskers represent interquartile range, and points represent outliers. Birds were considered "Yes" to storm if the time elapsed between capture and recapture fell inside of the week of the storm event. Results of statistical analyses are provided in Table 3.1.

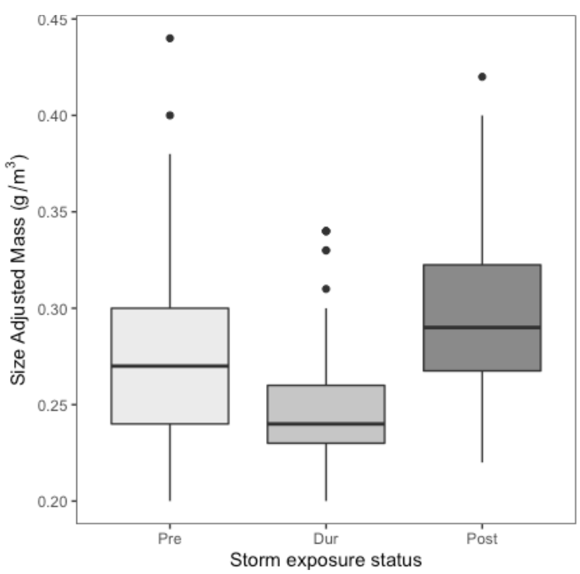

Figure 3.2. Boxplots of size adjusted mass and storm exposure status of hatch-year Semipalmated Sandpipers caught at Petit-Cap, NB. $n_{Pre\text{-}Storm}$=213, $n_{During\text{-}Storm}$=79, and $n_{Post\text{-}Storm}$= 100. Boxes represent first and third quartiles around the median line. Whiskers represent interquartile range, and points represent outliers. Birds were considered "pre-storm" if they were captured before the storm event, "during-storm" if they were captured in the week following the storm (September 2-9), and "post-storm" if caught after September 9. Results of statistical analyses are provided in Table 3.1.

Chapter 4: Discussion

Eastern breeding Semipalmated Sandpipers depend upon staging sites in Maritime Canada to fuel their southbound migration (Hicklin, 1987; Hicklin & Gratto-Trevor, 2020). These sites are dynamic and although they provide birds with an opportunity to refuel, they can experience environmental fluctuations and variation that affect refueling in shorebirds (Beauchamp, 2006; Gerwing et al., 2015; Mann et al., 2017; Newton, 2007). Assessing rates of mass gain is critical in understanding how birds are being affected and predicting migratory success (Alerstam & Lindström, 1990). Mass gain rates can also be used to assess site quality (Bayly et al., 2016; Bayly et al., 2012; Dunn, 2001; Weber et al., 1998). However, this is a difficult metric to obtain, especially at sites in the Bay of Fundy where shorebird numbers are high and recapturing birds within a season is virtually impossible. Plasma metabolites are widely used as a proxy for mass gain, though studies have only confirmed relationships in captive birds. Little work has been done to confirm the existence of these relationships in free-living Semipalmated Sandpipers. Therefore, working at a site where within-season recaptures were possible, we examined the relationship between plasma metabolites as a proxy for rates of mass gain, assessed variability in diets, and examined influences of a storm event in Semipalmated Sandpipers at Petit-Cap, NB.

Plasma metabolites and rates of mass gain

Surprisingly, we did detect a significant positive relationship between glycerol and mass gain but found no significant relationship between rates of mass gain and plasma triglycerides, or β-hydroxybutyrate concentrations in recaptured Semipalmated Sandpipers. Many previous studies have suggested a strong correlation does indeed exist (Jenni-Eiermann et al., 2002; Jenni-Eiermann & Jenni, 1994; Williams et al., 1999). Jenni-Eiermann and Jenni (1994) reported that metabolites reflect body mass changes over a few hours in Garden warblers (*Sylvia borin*). Williams et al. (1999) found that this relationship can be extended over a slightly longer period in captive Western Sandpipers (*Calidris mauri*). However, the relationship with triglycerides in Western Sandpipers was only positively correlated to rates of mass change one to two days prior to blood sampling (Williams et al. 1999). Our recaptured birds were sampled after considerably longer periods of time (4-20 days after their initial capture), suggesting that under field conditions the same constraints apply; metabolites are unable to reflect mass changes for more than a short interval. Williams et al. (1999) found that other metabolites (glycerol and β-hydroxybutyrate) continued to be correlated with mass changes over longer time periods. However, this relationship became more inconsistent as the period increased, which may explain the lack of relationships in our recaptured birds. Importantly, there is evidence of a U-shaped relationship between glycerol and triglycerides, which may explain the high glycerol levels we detected in both birds that were gaining or losing mass, and resulted in a significant, positive relationship (Guglielmo et al., 2005). Our findings thus suggest that relationships between plasma metabolites in free-living Semipalmated Sandpipers and rates of mass gain should only be interpreted in shorter time frames. Unfortunately, the relationship observed by Williams et al. (1999) is untestable under field conditions because the initial capture of birds introduces a handling stress that causes birds to lose mass following capture; thereby skewing the relationship between metabolites, time, and mass gain (Davidson, 1984; Pienkowski et al., 1979; Warnock et al., 1997). Although our findings did not reflect our initial predictions, this information is important in understanding how best to interpret metabolite data relative to mass change in free living migratory shorebirds.

Plasma metabolites, adjusted mass, and recapture status

We noted a strong positive relationship between plasma triglycerides and size adjusted mass in birds that were sampled on their first capture. This is consistent with previous work on this species and other similar shorebird species (Churchwell et al., 2018; Guglielmo et al., 2002; Lyons et al., 2008; Seaman, 2003). However, we unexpectedly failed to detect such a relationship in recaptured Semipalmated Sandpipers; the slope of the line for recaptured birds was significantly shallower and not different from zero.

The relationship seen in non-recaptured birds was more variable in fatter birds; triglycerides of birds with lower adjusted masses fit the relationship considerably better (Figure 1.1). These fatter birds are at a mass that is sufficient to complete migration (Maillet & Weber, 2006). If these birds reach this mass a few days prior to sampling, fueling may no longer be their priority, but rather maintenance of fat stores, so plasma triglycerides could decrease. We speculate that refueling rates may asymptote at this point, since birds must achieve optimal fat stores that still allow efficient flight (Pennycuick, 1975, 1978). If these birds reach the target mass nearer the time of sampling, their triglycerides likely still reflect a higher refueling rate. This might explain the non-significant relationship in recaptured birds, which were generally recaptured in this range of fatter body condition rather than at lighter masses, therefore introducing more variability into the model.

The differences in the relationship between metabolites and body condition between recaptured birds and those captured for the first time may also be partially driven by handling effects, and this needs additional consideration. Our current understanding of handling effects on mass in shorebirds suggest that birds typically lose mass following a capture event, with significant fat loss after 24 hours post capture (Davidson, 1984; C. Gratto-Trevor, 2018; Pienkowski et al., 1979; Warnock et al., 1997). Some of our recaptured birds with low fat loads had high triglyceride levels. Given that we know that this reflects metabolic state in just the previous two days, it is possible that this might be indicative of birds feeding heavily to make up for mass lost due to capture events, thereby generating a shallower slope- even in lighter birds. Since recaptures are rare (even at our site), sample size was small, so further work must be done to examine responses to handling- particularly in the context of metabolite profiles. Since relationships between body condition and metabolites differed in relation to recapture status, we also suggest that inferences of mass gain rates from recaptured birds may be rendered less accurate due to these handling effects.

Diet

Isotopic analyses suggest that bivalves contribute the most to Semipalmated Sandpipers diets whilst staging at Petit-Cap. This result is consistent with previous findings at this site (Bellefontaine, 2020). The similarity between juvenile and adult diet breadth is slightly unexpected, as many studies have shown the inexperience and inefficiency of younger shorebirds affects many foraging decisions and behaviors (Doiron, 2021; Goss-Custard & Durell, 1987; van den Hout et al., 2017). However, prey availability at Petit-Cap may explain this result; if alternative prey items are less abundant than bivalves at the site, both age classes may be restricted in their foraging decisions.

Although a multiple regression of isotopic signatures upon plasma triglycerides did not suggest any relationship, the sample size was small, and we cannot rule out the possibility that diet affects plasma metabolites. In general terms, bivalves are high in fatty acids (Joseph, 1982). Studies on captive White throated Sparrows (*Zonotrichia albicollis*) found that diet composition influence plasma metabolite levels and body composition; in particular, that certain diets (i.e. high glucose) result in markedly higher plasma lipids (Smith et al., 2007; Smith & McWilliams, 2009). However, Cerasale & Guglielmo (2006) found that diet influenced some plasma metabolites- but not plasma triglycerides. This might explain why we saw no relationship between isotopic signature and triglycerides. Still, this has implications in the interpretation of our findings; the deviations in other metabolites from expected relationships in coastal staging shorebirds in response to both adjusted mass and mass changes may have been partially affected by their diet. Further, Hobson & Clark (1993) found that isotopic analyses reflect diet over six days. Plasma triglycerides appear to reflect mass gain over half of that time, which may explain this lack of a relationship between the two. Other methods to examine diet isotopes may provide a more appropriate assessment of this relationship, such as breath isotopes, as these measures provide information on diets over a shorter time period than blood plasma (Hatch et al., 2002; Hobson et al., 2022; Podlesak et al., 2005). The influence of nutritional composition of prey should be further investigated in relation to metabolite levels in staging shorebirds.

Effects of weather events on refueling

Migratory shorebirds are vulnerable to changes at staging sites (Li et al., 2019; Studds et al., 2017). The effects of weather on migratory timing are well understood, with events such as high winds or rains known to delay migratory movements, but impacts upon refueling and body condition have been less studied (Carneiro et al., 2020; Richardson, 1978). Although extreme weather events are already linked with mass mortality of migratory birds (Newton, 2007), these non-fatal effects less unclear. The storm on September 2nd at Petit-Cap provided an opportunity to examine such impacts upon both recaptured and non-recaptured Semipalmated Sandpipers. The habitat drastically changed following the storm. Thick mats of algae were covering typical foraging grounds, and the shoreline was washed up. Therefore, we suspect that prey items were rendered inaccessible to the small shorebirds. As predicted, recaptured birds that were caught for the second time within a week after the storm showed significantly lower size adjusted masses and, on average, negative rates of mass gain. Similar effects were seen in non-recaptured birds that experienced the storm. Although masses of birds captured generally increase as the season progresses, there was a drop in size adjusted mass during the post storm period, followed by a rebound later in the season. Previous studies show that weather affects foraging strategies of Semipalmated Sandpipers in the Bay of Fundy (Beauchamp, 2006). Other studies suggest that the aftermath of weather events may be the driver behind negative effects; when foraging areas are impacted, birds may struggle to find appropriate resources (Wiley & Wunderle, 1993). This may explain some of the differences seen in storm versus non-storm birds in our study. If birds were foraging less efficiently, or prey was suddenly less available, the refueling rates, and associated size adjusted masses, likely dropped. We suggest that the effects of weather events should be examined in more detail as we have found evidence that even small storms have detrimental effects.

Conclusions

Semipalmated Sandpipers are in decline (Andres et al., 2012; BirdLife International, 2016; Morrison et al., 2001, 2012). These migrants make use of some of the most dynamic ecosystems in the world as staging locations, and their ability to continue to use these sites is threatened by anthropogenic and environmental influences. Understanding relationships between migratory refueling and the many factors that influence success is crucial in the conservation of this species.

Our study examined the relationship between plasma metabolites and rates of mass gain in staging Semipalmated Sandpipers. We found that this relationship in free living birds does reflect findings in captive studies- that the relationships are strong short term but become non-significant as time between initial mass and blood sampling increases. This underlines the limitations of using metabolites to infer changes in mass over extended time intervals. This may change the way that we use these proxies in the field, by restricting estimates to shorter time frames.

We found significant negative effects of weather events at this staging location upon body condition and refueling rates. Although we saw a recovery later in the stopover season, this has addressed a gap in the literature, wherein most effects of weather upon these migrants focus on length of stay, associated with more severe events. Understanding the impacts that these events have upon Semipalmated Sandpipers is very important, particularly as weather events are projected to increase with climate change (National Academies of Science, Engineering, and Medicine, 2016; Phelan, 2011).

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