ASSESSING HABITAT QUALITY OF AMERICAN WOODCOCK (*Scolopax minor*) AND VALIDATING A HANDHELD METER USING PLASMA METABOLITES

Christopher M. Roelandt

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ABSTRACT

ASSESSING HABITAT QUALITY OF AMERICAN WOODCOCK (*Scolopax minor*) AND VALIDATING A HANDHELD METER USING PLASMA METABOLITES

BY

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American woodcock (*Scolopax minor;* hereafter woodcock) is a small migratory upland bird found throughout eastern North America. The North American population has been declining at approximately 1% annually since the late 1960s. Declines in demand for forest products and changes in forest management practices have been major drivers in the decline of early successional forests, an ecosystem vital for woodcock. Reversing the decline in woodcock populations will require a better understanding of optimal habitat and methods for assessing habitat quality.

Improved methods for assessing habitat quality can aid in identifying areas best suited for management efforts while directing limited funding resources for the most beneficial conservation efforts. Current methods for assessing habitat quality to woodcock include singing-ground surveys, recruitment, and nesting success. Though these methods remain important, the body condition of individuals has been demonstrated to be effective as well.

Understanding the relationship between habitat characteristics and body condition is possible using blood plasma metabolites. Triglyceride (TRIG) and ß-hydroxybutyrate (BUTY) are two blood plasma metabolites effective in assessing habitat quality. These metabolites can be indicative of an individual's change in body mass. I researched the relationship between these metabolites and habitat characteristics surrounding the singing-grounds of male woodcock. I found a negative relationship with TRIG concentrations with coniferous forests and developed

land cover at the 50-meter buffer. The body condition of woodcock declined as the area of these landcover types increased. I expected additional habitat variables surrounding singing-grounds to relate to plasma metabolite indicators of body condition. Future research using plasma metabolites for assessing habitat quality would benefit from the use of telemetry to better estimate habitat use and a secondary method for measuring body condition.

I also evaluated a small handheld meter, CardioChek PA analyzer, for measuring TRIG concentrations in a field setting. The handheld meter's results were precise but did not produce accurate results in comparison with results produced in a laboratory setting. Future research utilizing this and other handheld meters for measuring plasma metabolite concentrations need to consider the effects of environmental conditions (light, temperature, and humidity) on functionality. I recommend future testing of this meter in a laboratory setting where environmental conditions are controlled and to identify the maximum time between blood collection and analysis needed to produce accurate and precise plasma metabolite concentration measurements.

DEDICATION

I would like to dedicate this thesis to my wife, Jenny, and my daughter, Annabelle.

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INTRODUCTION

The American woodcock (*Scolopax minor;* hereafter woodcock) is a small migratory woodland gamebird species found throughout eastern North America. Woodcock populations have been declining since the late 1960s at a rate of approximately 1% annually (Seamans & Rau 2018). Loss of early successional forest habitat throughout its range has been attributed to this decline in woodcock populations (Dessecker & McAuley 2001). Current knowledge of woodcock movements, migration, population trends, and habitat selection have all been or are currently being researched (Case & Sanders 2010; Moore & Krementz 2017; Brenner et al. 2019; Fish et al. 2019). Methods are lacking to differentiate and assess quality habitat (Guglielmo et al. 2005). Identifying methods for evaluating habitat quality and understanding the significance of the characteristics of high-quality habitat for woodcock will help in future management decisions (Case & Sanders 2010).

Starting in late fall and early winter, woodcock will begin their migration from their northern range, from western Great Lakes to Maine and across southeastern Canada, to their southern range in the southeastern United States, from Texas to Georgia. Woodcock will spend their winters in these southeastern states until late winter when they will begin to migrate back to their northern range. Once they have arrived at these northern areas, woodcock will establish territories for breeding. Male woodcock select openings within early successional forests, called singing-grounds, where they perform their crepuscular courtship displays. Male woodcock will use courtship displays in addition to vocalizations to attract nearby females and show dominance to other nearby male woodcock (Straw et al. 1994). Male woodcock use the forests surrounding these openings for diurnal feeding areas at a distance of between 50-964 meters, with an average

distance of 364 meters (Hudgins et al. 1985). Females will visit these males throughout the spring breeding season and nest in nearby early successional forest habitat.

Woodcock are an important species for various groups including hunters, bird watchers, and land managers. Woodcock are an important game bird species with over 200,000 birds harvested by hunters annually (Seamans & Rau 2018). The sale of hunting licenses, equipment, and other services make woodcock an important revenue contributor to state wildlife agencies and local economies. Efforts directed towards woodcock conservation benefit many other species; some of these species are also seeing a similar decline in populations. Species such as the golden-winged warbler (*Vermivora chrysoptera)* has experienced a declining population and benefit from conservation efforts directed toward woodcock, which makes the woodcock an important umbrella species (Sauer et al. 2017). Early successional forests managed for woodcock conservation have a higher bird diversity as well as a greater number of birds in comparison to random sites within forested ecosystems (Masse et al. 2015).

Early successional forests are forests dominated by pioneer plant species. Characteristics of these species allow them to quickly colonize an area following disturbance where the forest overstory was removed. Early successional forests are created from natural disturbances such as wildfire, windthrow, or insect outbreaks (Lorimer 2001). Currently, early successional forests are more frequently created through human-induced disturbances such as timber harvests. Forests with financially valuable timber are cut often removing the older and larger trees. If these harvests mimic natural disturbance regimes, it is referred to as ecological forestry and will allow early successional species to regenerate in the area (Warburton et al. 2011). Common tree species of early successional forests include aspen (*Populus* spp.*)*, alder (*Alnus* spp.*)*, and willow (*Salix* spp.). Their abundant seeds and ability to root sprout allow these species to quickly

regenerate in an area following a disturbance. Root sprouting or suckering occurs from growth from stumps and lateral roots from older trees that were removed during a disturbance event. Early successional forests play an important role at all stages of a woodcock's life. Assessing the quality of this habitat will be important for future management and reversing the decline in woodcock populations.

Common methods of measuring habitat quality of woodcock include the use of demographic characteristics as indicators. These characteristics include spring singing-ground surveys and estimates of recruitment and nesting success. While these characteristics and methods are important, understanding how the habitat affects individual body condition may give researchers and managers better insight into the qualities of used habitat.

Historically, researchers assessed body condition by relating habitat characteristics to a change in body mass (Biebach et al. 1986; Moore & Kerlinger 1987). Individuals using lower quality habitat exhibited a decrease in body mass between captures while individuals using higher quality habitat would exhibit increased body mass between captures (Cherry 1982; Moore & Kerlinge 1987). While this was a useful approach, there were some limitations to using this method. Estimating change in body mass requires researchers to capture individuals more than once leading to several problems. First, a recapture study requires capturing a larger number of individuals initially since not all will be recaptured. Second, recapturing animals also leads to capture bias where individuals with a poorer body condition are more likely recaptured, thereby inferring that the habitat is lower quality.

Another technique for assessing body condition is the use of plasma metabolites (Guglielmo et al. 2005; Jenni-Eiermann & Jenni 1994). Plasma metabolites provide insight into the energetics of the individual at the moment of capture. The use of plasma metabolites, unlike

the change in body mass method, only requires one capture thereby eliminating the problems associated with recapture. Triglyceride (TRIG) and β-hydroxybutyrate (BUTY) are two plasma metabolites shown to accurately indicate when an individual is in a state of storing fat (gaining body mass) or in a state of mobilizing stored fat (losing body mass; Cerasale & Guglielmo 2006; Jenni-Eiermann & Jenni 1994; Thomas & Swanson 2013). For instance, an elevated level of TRIG is indicative of individuals meeting their energetic needs and are storing body fats. While an individual with elevated BUTY level is not meeting its energetic needs and is mobilizing stored body fats to fulfill these needs.

Although using plasma metabolites as an estimate of body condition may be an improvement to the change in body mass method, there are still limitations to be considered. Collecting, processing, storage, shipping, and wait time for results of plasma samples in a field setting can be difficult for researchers especially those with novice experience. The introduction of a handheld meter for measuring plasma metabolites concentrations in the field aims to reduce these limitations (Sommers et al. 2017). Originally designed for clinical use, the CardioChek PA analyzer has the potential to accurately measure TRIG concentrations in the field using only a small blood sample (PTS Diagnositics 2020). This analyzer potentially allows researchers to quickly assess body condition while eliminating limitations associated with collecting plasma samples in the field, storing samples, shipping samples to a lab, and waiting weeks to months for results.

This study will explore i) using body condition based on plasma metabolites to assess the quality of habitat surrounding male woodcock singing-grounds (Chapter 1); and ii) validate the use of a handheld meter for measuring a plasma metabolite, triglyceride, in the field (Chapter 2).

CHAPTER 1

USING BODY CONDITION TO ASSESS THE QUALITY OF HABITAT SURROUNDING MALE AMERICAN WOODCOCK (*SCOLOPAX MINOR)* SINGING-GROUNDS

Abstract

American woodcock (*Scolopax minor)* is a small migratory gamebird species found throughout eastern North America. The North American woodcock population has been declining at a rate of approximately 1% annually since the late 1960s. This decline has been attributed to a similar decline in early successional forests, a habitat vital to woodcock. The relationship between habitat quality and woodcock body condition is not well understood. Woodcock using higher quality habitat are expected to have a better body condition than birds using lower quality habitat. I assessed whether body condition, measured by plasma metabolites, relates to habitat types and characteristics as a potential indicator of habitat quality surrounding the singing-grounds of male woodcock. I collected a small blood sample from male woodcock captured in north-central Wisconsin during the spring breeding season. The concentration of two plasma metabolites, triglyceride (TRIG) and β-hydroxybutyrate (BUTY), was measured in the blood plasma as indicators of body condition. I used a geographic information system to quantify habitat characteristics within three buffered distances of each male woodcock singing-ground. I developed models to relate habitat variables with body condition of male woodcock. Based on the best-fit models, I found a negative relationship between TRIG concentrations and coniferous forest and developed cover types in close proximity to singing-grounds. This indicates that coniferous forest and developed cover reduce habitat quality for male woodcock. Future research should consider the rate of plasma metabolites fluctuations and consider other methods to better define male woodcock habitat use.

Introduction

For wildlife biologists and land managers, assessing wildlife habitat quality is an important task. Habitat provides resources that can affect fitness, survival, and reproductive success of individuals. High-quality habitat provides individuals with good protective cover, cover for breeding and raising young, and food to meet energetic needs (Johnson 2007). Individuals meeting their energetic needs have better fitness, which improves their ability to attract mates and defend territories (Bock & Jones 2004; Johnson 2007; Boyce et al. 2016).

Current methods for assessing habitat quality for wildlife often use demographic metrics, including population density, survival, and reproductive success (Bock & Jones 2004; Bieri et al. 2017). These metrics provide researchers with evidence that a species is benefiting from a specific habitat; however, this may not be telling the entire story (Chandler et al. 2009). While demographic metrics are important, understanding the effects of habitat characteristics on an individual's body condition is a useful tool for assessing habitat quality (Guglielmo et al. 2005; Beuth et al. 2016).

A common method of assessing body condition is to measure a change in body mass over time. Change in body mass can indicate whether an individual is obtaining adequate nutritional resources from its habitat (Biebach et al. 1986; Moore & Kerlinger 1987). This method presents several problems that stem from the need to recapture individuals. Recapturing individuals can be difficult because certain individuals may become trap shy. Another problem is capture bias which occurs when certain individuals are more easily captured. These more easily captured individuals may have poorer body condition which may affect their ability to evade capture, which may skew a researcher's ability to assess body condition of a population (Jenni-Eiermann & Jenni 1994; Guglielmo et al. 2002).

Another method for assessing body condition, which only requires one capture and is effective in various bird species, is the use of plasma metabolites (Jenni-Eiermann & Jenni 1994; Guglielmo et al. 2005; Williams et al. 2007). Plasma metabolites can reflect an individual's ability to acquire adequate food resources from its habitat. Two plasma metabolites shown to be effective at predicting change in body mass in bird species are triglyceride (TRIG) and β-hydroxybutyrate (BUTY) (Jenni-Eiermann & Jenni 1994; Seaman D. A. et al. 2006). Individuals with higher levels of TRIG and lower levels of BUTY are in a state of increasing body mass, or a good body condition. Alternatively, individuals with lower levels of TRIG and higher levels of BUTY are in a state of decreasing body mass, or a poor body condition (Williams T. D. et al. 1999; Seaman D. A. et al. 2006). Utilizing plasma metabolites to assess body condition could eliminate the difficulties associated with recapturing individuals, reduce stress on captured individuals, and reduce travel duration and associated resources needed by researchers in the field.

One species for which the use of plasma metabolites could be useful to assess habitat quality is the American woodcock (*Scolopax minor;* hereafter woodcock). The woodcock is a small migratory gamebird found throughout eastern North America. Populations of woodcock have been declining at approximately 1% annually since the late 1960s (Seamans & Rau 2018). Declining populations of woodcock have been attributed to a similar decline in early successional forests which are vital habitat (Dwyer et al. 1983; Gregg 1984; Sauer & Bortner 1991). A better understanding of the response of woodcock to varying habitat quality is needed (Case & Sanders 2010).

This study utilized plasma metabolites as an indicator of body condition in assessing habitat quality surrounding singing-grounds of male woodcock. Male woodcock exhibit strong

site fidelity around their singing-grounds, and remain close to their singing-grounds where they travel between 50 and 964 meters to their diurnal feeding cover (Hudgins et al. 1985). This behavior of using the same singing-ground throughout the spring breeding season will allow me to correlate body condition of male woodcock with habitat variables surrounding these sites. I hypothesize that male woodcock with good body condition will use different habitat than males with poor body condition which should indicate differences in habitat quality. My objective was to see if body condition, assessed by using plasma metabolites, indicated differences in quality of habitat surrounding singing-grounds of male woodcock.

Methods

Study Area

Singing-grounds are open areas in or near early successional forests used by male woodcock for their crepuscular courtship displays, often called the "sky dance," to attract females during the breeding season. I assessed the quality of habitat surrounding singing-grounds of male woodcock following spring migration at ten study sites located within two state-managed areas (Ackley State Wildlife Area and Northern Highland American Legion State Forest) in Langlade (45° 8' 31.9" N 89° 24' 1.2" W) and Oneida counties (45° 47' 32.6" N 89° 32' 55.6" W), Wisconsin (Figure 1.1). These two state-managed areas vary from one another in habitat composition. Study sites located in Northern Highland American Legion State Forest are mostly upland forests dominated by stands of aspen *(Populus* spp.*)* of various age classes (Figure 1.2). While study sites in Ackley State Wildlife Area are mostly dominated by lowland brush species, willow *(Salix* spp.*)* and alder *(Alnus* spp.; WiDNR Division of Forestry 2018; Figure 1.3).

Fieldwork was conducted during April, May, and the first 15 days of June during the years of 2018 and 2019. Climate data for long-term and field season means were collected from a weather station in Rhinelander, Wisconsin, a location centralized among study sites. The long-term means for April are a daily temperature of 5 °C, monthly total rainfall is 6.5 cm, and a monthly total snowfall is 15.5 cm. The long-term means for May are a daily temperature of 11.5 °C, a monthly total rainfall of 8.9 cm, and a monthly total snowfall of 1 cm. The long-term means for June are a daily temperature of 16.9 °C, a monthly total rainfall of 9.9 cm, and a monthly snowfall is 0.0 cm (NOAA 2020).

Singing-ground study sites were identified by selecting clearings within early successional forests where at least one male woodcock was observed. At each of these study sites, there was the possibility of subdominant males present that stayed near the periphery of the singing-ground, which allowed for the opportunity to sample more than just the dominant male. Once a male woodcock was identified to be present, field technicians and I returned the following evening to capture it during its crepuscular courtship display.

Male woodcock were captured from 20 April −4 June 2018, and 18 April − 30 May 2019 (IACUC protocol PRO00008018). Male woodcock have strong site fidelity to their singing-grounds and will return to these singing-grounds throughout the spring breeding season. Male woodcock will defend these singing-grounds from other nearby males. I used this site fidelity and territorial behavior to capture male woodcock using mist nets and audio lures (McAuley & Longcore 1993).

Before the evening displays, I set up mist nets within the singing-grounds. Mist nets made of fine nylon thread with a mesh size of 60mm to capture woodcock were spread between two poles made of electrical conduit. As evening approached, technicians and I placed an MP3

player with speakers to project woodcock calls and displays near the mist nets to act as an audio lure to induce a defensive territorial response from the dominant male woodcock. When responding to the audio lure, the male would become entangled in the mist nets (McAuley & Longcore 1993).

Morphometric measurement of American woodcock

I promptly removed each captured woodcock from the mist net to reduce stress and risk of injury. Both female and male woodcock were fitted with a federal bird band. Each band is stamped with a unique number and is used for future identification if the bird is recaptured or harvested during the hunting season. I recorded the sex of each woodcock using morphometric measurements and plumage characteristics (Sepik 1994; Pyle 2008). Female woodcock were promptly released after banding, as only male woodcock were used in this study. Male woodcock were processed to collect additional morphometric measurements and blood samples. The morphometric measurements recorded included wing length, tarsus length, bill length, and body mass. These measurements were measured to the nearest tenths of a millimeter and body mass was measured to the nearest tenth of a gram. I aged male woodcock with the use of plumage characteristics and molt patterns to assign them to one of two age categories, second-year (SY) and after-second-year (ASY). Woodcock hatched in the previous spring were classified as SY birds, while those hatched in prior years were classified as ASY (Pyle 2008).

Blood and plasma sample collection and handling

I collected blood samples from each male woodcock following the *Guidelines to the Use of Wild Birds in Research* (Fair et al. 2010). I collected a maximum of 600 µL blood sample from the brachial artery in the wing of each male woodcock using a 25 ga needle and a 1000 µL syringe. The sample was separated into two 300 µL heparinized blood centrifuge tubes (Owen

2011; Sheldon et al. 2008). The blood collection site was treated with a clotting substance (Blood Stop Powder part number 79204, Neogen Animal Safety, Lexington, KY) and a cotton ball to prevent further bleeding. Each woodcock was held in a breathable cloth bag to limit movement for 5 minutes following the blood draw. Following the 5-minute holding period, each woodcock was reevaluated for any health or injury concerns before being released at the capture location (Fair et al. 2010).

One of the 300 µL tubes from each blood sample was centrifuged to separate plasma from the whole blood (4,800 x g for 10 mins on an HWLAB min centrifuge). The other 300 µL tube was used in a separate study to validate a handheld meter (see Chapter 2). Plasma was pipetted from the centrifuge tube and transferred to a cryotube (Owen 2011). Each cryotube was labeled and placed in a cooler with ice packs for transport back to the location with equipment for extended storage. Each sample was placed into a frost-free deep freezer at -20 ºC for storage until it could be sent to a lab for analysis (Williams et al. 2007; Sommers et al. 2017).

Plasma samples were stored in the freezer until the completion of the field season (< 7 weeks). Following the field season, plasma samples were packed into coolers with 7 kg of dry ice and shipped overnight to the Scott McWilliams Bird Lab at the University of Rhode Island. Colorimetric assay kits were used to analyze TRIG (Triglyceride Reagent part number T2449, Sigma-Aldrich Inc., St. Louis, MO) and BUTY (β-hydroxybutyrate (Ketone Body) Colorimetric Assay Kit part number 700190, Ann Arbor, MI) concentrations from the plasma samples.

GIS Habitat Data

I selected habitat variables *a priori* as variables found to be biologically important as diurnal habitat for woodcock. Habitat variables included landcover type (e.g. lowland brush),

landscape metrics (e.g. fractal dimension index), and the topography characteristics (e.g. elevation) within the buffered distances surrounding the point of capture.

I quantified landcover at three buffered distances, 50 m, 364 m, and 964 m to represent the range and mean distance male woodcock travel between singing-grounds and diurnal feeding habitat (Hudgins et al. 1985). Landcover data and attributes were retrieved from the Wisconsin Department of Natural Resources' (WIDNR) Wisconsin Forest Inventory and Reporting System (WisFIRS) at a 1-meter square resolution. WisFIRS data included landcover type (e.g. lowland brush), year of origin (i.e. year of the last harvest), and dominant overstory species (e.g. aspen). WisFIRS data within buffered distances around capture points were validated by a field technician and me for size class of dominant overstory tree species and landcover type (WiDNR Division of Forestry 2018). Corrections to WisFIRS attributes or classification of landcover types on private lands, which were not available within WisFIRS, were updated in the appropriate shapefiles using ArcGIS.

Data Analysis

Each captured woodcock was placed in one of 2 groups for the county of capture (Langlade or Oneida, WI), year of capture (2018 or 2019), and age (SY or ASY). TRIG and BUTY concentrations were log-transformed to satisfy normality assumptions. I used Welch's t-test to compare mean body mass, mean TRIG concentrations, and mean BUTY concentrations between each of these groups. To correct for multiple comparisons a Bonferonni correction was applied and significant differences between groups were determined at $\alpha < 0.017$.

Using ArcGIS Pro 2.5.0 and the package *landscapemetrics,* I quantified the landcover within each of the three buffered distances around each singing-ground (Hesselbarth et al. 2019; ERSI 2019; R Core Team 2019). WisFIRS data were transformed from shapefile to raster data

files and used in the program *landscapemetrics* where the raster data were extracted and quantified at the three buffered distances.

I used principal component analysis (PCA) to reduce the dimensionality of landcover data at each buffered distance (Vaughan & Omerod 2005). Each of the approximately 30 landcover types found within the buffered areas was used to create principle components. Landcover types data were standardized and centered before analysis (James et al. 2013). Selected principle components were included in modeling when eigenvalues were greater than 1 since eigenvalues less than 1 would explain less than 10% of the variation among the habitat variables (Ecke et al. 2002; Yata & Aoshima 2010).

To account for the spatial correlation between male woodcock captured from the same sites, I used generalized estimating equations (GEE) to determine which habitat variables predicted either TRIG or BUTY concentrations (Storch 2002) in program R using the packages *geepack* and *MESS* (R Core Team 2019; Højsgaard et al. 2019; Ekstrom 2019). Independent variables were added to the models using forward stepwise selection (James et al. 2013). Best-fit model selection was completed by selecting the model with the lowest quasilikelihood under the independence model criterion (QIC; Pan 2001; Cui 2007; Barnett et al. 2010). The capture site location was used in the models as a random effect since multiple male woodcock were captured at each site and likely had overlapping habitat use. All habitat variables and temporal variables (Table 1.1) were compared to both TRIG and BUTY concentrations at the three buffered distances and were selected for significance when $P<0.05$.

Results

Woodcock Morphometrics and Blood Plasma Metabolites

During the 2018 and 2019 spring field seasons, I captured and sampled 69 individual male woodcock from two counties in north-central Wisconsin. During the 2018 and 2019 spring field seasons, 35 and 34 male woodcock were captured and sampled, respectively. Male woodcock sampled in Oneida County had a mean body mass of 135.41 g (Range = 121.9-162.9 g, SE = 1.23, N = 49), mean TRIG concentration of 1.41 mmol/L (Range = 0.18-2.86 mmol/L, SE = 0.10, n =46), and a mean BUTY concentration of 0.10 mmol/L (Range = 0.06-0.28 mmol/L, SE = 0.01, n =43). Male woodcock sampled in Langlade County had a mean body mass of 132.78 g (Range = 121.9-142.4g, SE = 1.41, n =20), mean TRIG concentration of 1.49 mmol/L (Range = 0.40-2.78 mmol/L, SE = 0.13, n =20), and a mean BUTY concentration of 0.09 mmol/L (Range = 0.06-0.18 mmol/L, SE = 0.01, n =19) (Table 1.3). There was no difference in body mass (T (40) = -1.09, P = 0.28), TRIG concentrations (T (37) = 0.81, P = 0.42), or BUTY concentrations (T (56) = -1.47, P = 0.15) between birds captured in each county.

When male woodcock were grouped by age class, SY male woodcock had a mean body mass of 134.65 g (Range = 121.9-162.9 g, SE = 1.27, n =44), mean TRIG concentration of 1.41 mmol/L (Range = 0.27-2.78 mmol/L, SE = 0.10, n =41), and a mean BUTY concentration of 0.09 mmol/L (Range = 0.06-0.26 mmol/L, SE = 0.01, n =38). After-second-year male woodcock had a mean body mass of 134.65 g (Range = 121.9-152 g, SE = 1.51, n =25), a mean TRIG concentration of 1.48 mmol/L (Range = 0.18-2.86 mmol/L, SE = 0.12, n =25), and a mean BUTY concentration of 0.10 mmol/L (Range = 0.06-0.28 mmol/L, SE = 0.01, n =24). I found no difference in body mass (T (44) = 0.46, P = 0.65), TRIG concentrations (T (48) = 0.80, P = 0.43), or BUTY concentrations (T (40) = 0.88, P = 0.39) between age classes.

I found a difference in BUTY concentrations between capture years (2018 vs. 2019; $T_{(41)} = 3.29$, p = 0.002; Figure 1.4), but no differences were observed for body mass ($T_{(56)} = 1.31$, p = 0.20) or TRIG concentrations ($T_{(58)} = -0.99$, p = 0.33). Male woodcock captured in 2018 had a mean body mass of 136.21 g (Range = 121.9-162.9 g, SE = 1.69, n =35), a mean TRIG concentration of 1.40 mmol/L (Range = 0.18-2.86 mmol/L, SE = 0.13, n =33), and a mean BUTY concentration of 0.11 mmol/L (Range = 0.06-0.28 mmol/L, SE = 0.01, n =29). Male woodcock captured in 2019 had a mean body mass of 133.04 g (Range = 123.7 g, SE = 0.88, n =34), a mean TRIG concentration of 1.47 mmol/L (Range = 0.74-2.78 mmol/L, SE = 0.09, n =33), and a lower mean BUTY concentration of 0.08 mmol/L (Range = 0.06-0.15 mmol/L, SE = <0.01, n =33).

Weather Conditions Between Field Seasons

Weather conditions during the 2018 field season were abnormal and more severe in comparison to long-term averages and the 2019 field season (Table 1.2). During the 2018 field season, snowfall totaled 75.2 cm in April and was 0 cm in May and June. I continued to observe snow cover in forested land covers into early May although this was not recorded by the National Oceanic and Atmospheric Administration (NOAA). Snow cover for the 2019 field season lasted until April 19th with a total snowfall for April of 20.3 cm (NOAA 2020), a total snowfall of 0.3 cm in May, and total snowfall of 0 cm in June; these were similar to long-term averages (Table 1.2). Mean temperature and rainfall for April, May, and June for both the 2018 and 2019 field seasons were consistent with the long-term averages.

Reduction of Dimensionality of Landcover Type Variables

I used principal component analysis (PCA) to reduce the dimensionality of independent landcover variables into 1-5 continuous explanatory variables for my models at each of the three

buffered distances. At the 50 m buffered distance, five principal components explained more than 10% of the variance (Table 1.4). The first principle component at the 50 m buffer, which explained 35% of the variance, was positively influenced by the amount of aspen forests and negatively influenced by the amount of grasslands. The second principle component, which showed a negative relationship in predicting TRIG concentrations at the 50 m buffer, explained 17% of the variance and was positively influenced by the amount of coniferous forest and developed landcover.

For the 364 m buffer, the assumed mean distance of travel between singing-grounds and diurnal feeding sites for male woodcock had two principal components that explained more than 10% of the variance in landcover variables (Table 1.5). The first principle component, which explained 46% of the variance, was positively influenced by aspen and negatively influenced by lowland brush. The second principle component, which explained 21% of the variance, was negatively influenced by coniferous and developed landcover.

At the 964 m buffered distance, three principal components explained more than 10% of the variance in landcover type variables (Table 1.6). The first principle component, which explained 48% of the variance, was positively influenced by the amount of aspen and deciduous forests while negatively influenced by the amount of lowland herbaceous vegetation. The second principle component, which explained 19% of the variance, was negatively influenced by the amount of coniferous forest and wetlands while being positively influenced by deciduous forest landcover. The third principle component, which explained 12% of the variance, was negatively influenced by lowland and upland brush while positively influenced by grassland landcover.

Modeling of Habitat Variables for Predicting Body Condition

The best fit GEE model in predicting the effects of habitat variables on TRIG and BUTY was a model at the 50 m buffered distance. This model was negatively influenced by principal component 2 (PC2) in predicting TRIG concentrations (GEE, X = -0.109, P = 0.007). At the 50 m buffered distance, PC2 was weighted for coniferous forest and developed landcover types with a factor loading of 0.698 and 0.679, respectively. No other variables significantly explained TRIG or BUTY concentrations for any of the buffered distances (Table 1.7).

Discussion

Woodcock body condition differences between capture years

BUTY concentration differed between capture years. During the spring field season of 2018, the mean BUTY concentration was significantly higher than in Spring 2019, indicating that male woodcock were less able to acquire food and/or under higher energetic stress. Male woodcock captured during the 2018 field season were likely mobilizing fat reserves to meet their energetic needs. The weather between these years varied greatly (NOAA 2020). During the field season of 2018, snow cover lasted into early May, due to a mid-April snowstorm, which dropped approximately 50 cm of snow. This late snowstorm occurred following the first documented arrival, March 28th, of male woodcock to the area (Sullivan et al. 2009; eBird 2020). The monthly total snowfall for April 2018, in particular, was nearly 60 cm more than the long-term average, and it was more than 50 cm more than observed in April of 2019. The mean daily temperature of 5 °C for April 2018 was also lower than 9 °C for April of 2019 (NOAA 2020). This late snowstorm, snow cover, and reduced mean daily temperature likely played a role in limiting the ability of male woodcock to find food, and it likely increased the energetic stress to birds, which would result in the metabolism of fat reserves. Body fat reserve has been found to

decline in times of poor environmental conditions in many species including song sparrows (*Melospiza melodia*), white-tailed ptarmigan (*Lagopus leucurus*), and willow ptarmigan (*L. lagopus*; O'Connor 1978; Wingfield 1985; Sandberg & Moore 1996; Martin & Wiebe 2004). The following spring field season of 2019 was a more typical spring with no late snowstorms and monthly snowfall similar to that of the long-term average. This more typical spring season likely allowed male woodcock the ability to find necessary food, which resulted in lower mean BUTY concentrations (NOAA 2020).

Woodcock Body Condition as an Indicator of Habitat Quality

The best-fit model for predicting TRIG concentrations identified a negative relationship with principle component two (PC2) at the 50 m buffered distance. PC2 was positively influenced by the amount of both coniferous and developed landcover (Table 1.4). Both coniferous forests (e.g. red pine) and developed landcover (e.g. roadways) are not typically associated with woodcock diurnal habitat (Dwyer et al. 1983; Masse et al 2014). Increased proportion of coniferous and developed landcover types likely reduced the amount of desirable habitat proximal to singing-grounds which may increase the travel distance for male woodcock between food and singing-grounds. An increase in traveling distances to acquire food has been shown to negatively influence body condition (Catry et al. 2013; Hinsley et al. 2008; Ropert-Coudert et al 2004).

Other than the negative relationship between TRIG concentrations and PC2 at the 50 m buffered distance, no other habitat variable was found to have a significant relationship with either metabolite at any other buffered distance. I expected to find more relationships between *a prior* selected habitat variables and body condition. An example of an expected outcome would have been a positive relationship between woodcock with higher TRIG and lower BUTY

concentrations with increased area of deciduous forests within a buffered distance surrounding singing grounds (Straw et al. 1994; Masse et al 2014). This lack of relationship between habitat variables and body condition has helped to identify areas of improvement for future research using plasma metabolites for assessing habitat quality.

Management Recommendations

My models indicated that male woodcock using singing-grounds in closer proximity to coniferous forests and developed land covers, such as housing and roadways, are in a poorer body condition. My recommendations for future management of woodcock habitat would be to create singing-grounds at a distance greater than 50 m away from coniferous forests and developed land covers. Creating singing-grounds away from these land cover types will help to ensure that occupying male woodcock will be in a better body condition.

Improvements for Future Research

This study's methodological approach could be improved by refining the scale of inference. The area of the 50 m, 364 m, and 964 m buffered distance were 0.78 ha, 42 ha, and 292 ha, respectively. These buffered distances were selected for use as the distance of travel between singing-grounds and diurnal feeding habitat, but these distances do not take into consideration the small area of habitat that woodcock are selecting for their home range and feeding. The mean home range for male woodcock was found to be much smaller (< 6 ha) than the area of the 364 m and 964 m buffered distances (Godfrey 1974; Hudgins et al. 1985). My study used buffered distances to estimate habitat availability, as opposed to habitat use or selected by male woodcock.

The use of transmitters and radio telemetry would produce an estimate of home range area and the distance birds travel between feeding and display areas. Additionally, habitat

selection can be modeled by comparing habitat use to availability. Telemetry locations and home range estimates would have created additional opportunities for the collection of habitat variables in the field for use in habitat selection modeling and may include stem density, mean basal area, canopy height, soil characteristics, soil invertebrate population density, and other breeding habitat variables known to be important for woodcock (Godfrey 1974; Masse et al 2014). Some of these habitat variables were difficult or impossible to estimate in the field using buffered areas since the amount of area to be assessed was too large and time constraints prevented this from being completed during the field season.

Another study improvement would be the addition of another method for assessing body condition. Both TRIG and BUTY concentrations are known to fluctuate over short periods (<1 hour; Jenni-Eiermann & Jenni 1994; Zajac et al 2006). This short timeframe likely only relates to the most recent landcover area used which may not be representative of all available landcover types and area in the buffered distances. Adding another method indicative of a longer period would increase the size of the area of habitat that would influence body condition; one such method is the use of deuterium dilution.

Deuterium dilution uses a hydrogen isotope-labeled water which is first injected into an individual and, later, a blood sample is collected from the same individual to measure the isotopic enrichment of the blood sample (Speakman et al. 2001). Deuterium dilution is effective in estimating fat composition in many bird species, making it a useful indicator of body condition (Eichhorn & Visser 2008; McWilliams & Whitman 2013). Unlike TRIG and BUTY, which are measurements of body fat storage trajectory, deuterium dilution estimates the actual composition of body fat on an individual, making it an indicator for a longer period. This method would likely be better correlated with the habitat quality of large foraging areas due to the

extended amount of time available for woodcock to interact or utilize more of the available habitat. Future research utilizing plasma metabolites for assessing habitat quality needs to take into consideration the short period in which they can fluctuate. This short period does not allow for the assessment of habitat at large scales.

Table 1.1: Habitat variables used in generalized estimating equation to predict β-hydroxybutyrate and triglyceride concentrations in male American woodcock (*Scolopax minor*) in north-central Wisconsin during the springs of 2018 and 2019. Habitat variables are grouped into four groups: landcover types, size class of aspen stands, topography, and landscape metrics. Also included are temporal variables year of capture, Julian date, and time between capture and blood collection.

Variables	Description
Landcover types*	
UPB	Upland brush within buffered area (ha)
ASP	Apen forestwithin buffered area (ha)
WET	Wetlandswithin buffered area (ha)
DCFOR	Decideous forestwithin buffered area (ha)
GRASS	Grasslandswithin buffered area (ha)
LWHB	Lowland herbaceous vegetationwithin buffered area (ha)
DVLP	Developed within buffered area (ha)
LWB	Lowland brushwithin buffered area (ha)
CNFOR	Conifereous forestswithin buffered area (ha)
Size class of aspen stands	
Large pole aspen	Mean dbh ≤2.5 cm
Large sapling aspen	Mean dbh ≥2.6 cm & ≤6 cm
Pole aspen	Mean dbh ≥6.1 cm & ≤11.5 cm
Seedling aspen	Mean dbh ≥11.6 cm & ≤17 cm
Small sapling aspen	Mean dbh ≥17.1 cm
Topography	
Elevation	Mean elevation within buffered area (m)
Slope	Mean slope within buffered area (degrees)
Northness	Mean north/south aspect of topography (degrees)
Eastness	Mean east/west aspect of topography (degrees)
Landscape metrics	
CONTAG	Contagion index = exten to which patchs of cover types are aggregated or clumped within the buffered area
FRAC	Fractal dimension index = shape index based on permiter to area relationships of cover types within buffered area
SHDI	Shannon's diversity index = the amount of patches of various cover types within buffered area
TE	Total edge = total length (m) of edge of all cover types within buffered area
MPA	Mean patch area = mean patch area of all cover types within buffered area
Temporal variables	
BLD	Amount of time between capture and blood collection
YR	Year of capture (2018 or 2019)
JUL	Julian date of capture

*Landcover types were combined using principle componenent analysis (PCA). Loadings for landcover variables can be found in Tables 3-5.

Table 1.2: Climate and weather data for long-term and field season means, respectively, collected at a weather station in Rhinelander, Wisconsin, representing a centralized location for sites in a study assessing habitat quality of American woodcock (*Scolopax minor*). Field seasons occurred during the spring of 2018 and 2019. Ranges for each weather variable are the values within the parenthesis.

	1908-2020 Averages	2018	2019
April			
Mean Daily Temperture (° C)	5.0	5.0 (-5.0-20.3)	9.0 (-1.7-19.4)
Total Rainfall (c)	6.5	5.9 (0.0-1.8)	9.0 (0.0-2.9)
Total Snowfall (c)	15.5	*75.2 (0.0-25.4)	20.3 (0.0-5.1)
May			
Mean Daily Temperture (° C)	11.5	23.9 (12.2-32.8)	15.5 (3.9-25.6)
Total Rainfall (c)	8.9	4.5 (0.0-2.0)	13.6 (0.0-4.0)
Total Snowfall (c)	1.0	0.0 (0.0-0.0)	0.3 (0.0-0.1)
June			
Mean Daily Temperture (° C)	16.9	22.5 (14.4-26.7)	22.8 (15.6-30.6)
Total Rainfall (c)	9.9	5.9 (0.0-3.3)	3.7 (0.0-6.6)
Total Snowfall (c)	0.0	0.0 (0.0-0.0)	0.0 (0.0-0.0)

*Snow cover was obsered at study sites into the beginning of May 2018

Table 1.3: Summary of data for triglyceride (mmol/L), β-hydroxybutyrate (mmol/L), and body-mass (g) by age class, county of capture, and year in north-central Wisconsin of male American woodcock (*Scolopax minor*) sampled during the springs of 2018 and 2019.

	Triglyceride			β-hydroxybuturate			Body Mass		
	N	Mean (mmol/L)	SE	N	Mean (mmol/L)	SE	N	Mean (g)	SE
Age class									
Second-Year (SY)	41	1.41	0.10	38	0.09	0.01	44	134.65	1.27
After-Second-Year (ASY)	25	1.48	0.12	24	0.10	0.01	25	134.65	1.51
County of capture									
Oneida County	46	1.41	0.10	43	0.10	0.01	49	135.41	1.23
Langlade County	20	1.49	0.13	19	0.09	0.01	20	132.78	1.41
Year of capture									
2018 Season	33	1.40	0.13	29	0.11	0.01	35	136.21	1.69
2019 Season	33	1.47	0.09	33	0.08	< 0.01	34	133.04	0.88
All Birds	66	1.44	0.08	62	0.10	0.01	69	134.65	0.97

Table 1.4: Principal component loadings of landcover types within a 50 m buffer around capture points of male American woodcock (*Scolopax minor*) during the springs of 2018 and 2019. Values of principal components 1-5 are shown for each landcover type included in the principal component analysis. Eigenvalues, percent of the variance, and cumulative percentage increase in the variance explained by the five principal components. Principle components were used in reducing the dimensionality of landcover type variables before modeling using a generalized estimating equation to predict the body condition of male American woodcock.

	Principal Components				
Elements	PC1	PC2	PC3	PC4	PC5
Aspen (ASP)	0.59	-0.04	0.04	0.03	0.05
Wetlands (WET)	-0.18	-0.09	-0.88	0.14	-0.17
Deciduous forests (DCFOR)	0.59	-0.03	0.03	0.03	0.05
Grasslands (GRASS)	-0.46	-0.15	0.44	0.38	-0.07
Lowland herbaceous vegetation (LWHB)	0.02	0.13	0.13	-0.44	-0.84
Developed (DVLP)	-0.04	0.68	-0.04	0.13	0.23
Lowland brush (LWB)	-0.23	-0.06	-0.01	-0.79	0.45
Coniferous forest (CNFOR)	-0.04	0.70	0.00	-0.01	-0.06
Eigenvalue	2.82	1.34	1.09	1.04	1.03
% of variance	35%	17%	14%	13%	13%
Cumulative %	35%	52%	66%	79%	92%

Table 1.5: Principal component loadings of landcover types within a 364 m buffer around capture points of male American woodcock (*Scolopax minor*) during the springs of 2018 and 2019. Values of principle components one and two are shown for each landcover type included in the principal component analysis. Eigenvalues, percent of the variance, and cumulative percentage increase in the variance explained by the two principal components. Principle components were used in reducing the dimensionality of landcover type variables before modeling using a generalized estimating equation to predict the body condition of male American woodcock.

	Principal Components	
Elements	PC1	PC2
Aspen (ASP)	0.489	0.139
Wetlands (WET)	0.321	0.198
Deciduous forests (DCFOR)	0.485	0.086
Grasslands (GRASS)	-0.320	0.266
Lowland herbaceous vegetation (LWHB)	-0.342	0.283
Developed (DVLP)	-0.142	-0.586
Lowland brush (LWB)	-0.427	0.133
Coniferous forest (CNFOR)	0.028	-0.649
Eigenvalue	3.719	1.695
% of variance	46%	21%
Cumulative %	46%	68%

Table 1.6: Principal component loadings of landcover types within a 964 m buffer around capture points of male American woodcock (*Scolopax minor*) during the springs of 2018 and 2019. Values of principal components 1-3 are shown for each landcover type included in the principal component analysis. Eigenvalues, percent of the variance, and cumulative percentage increase in the variance explained by the 3 principal components. Principle components were used in reducing the dimensionality of landcover type variables before modeling using a generalized estimating equation to predict the body condition of male American woodcock.

	Principal Components		
Elements	PC1	PC2	PC3
Upland brush (UPB)	0.015	-0.375	-0.436
Aspen (ASP)	0.393	0.266	-0.125
Wetlands (WET)	0.312	-0.378	0.344
Deciduous forests (DCFOR)	0.395	0.393	0.080
Grasslands (GRASS)	-0.370	0.011	0.528
Lowland herbaceous vegetation (I	-0.449	0.038	0.217
Developed (DVLP)	-0.253	-0.357	-0.064
Lowland brush (LWB)	-0.340	0.134	-0.582
Coniferous forest (CNFOR)	0.273	-0.587	-0.016
Eigenvalue	4.293	1.706	1.070
% of variance	48%	19%	12%
Cumulative %	48%	67%	79%

Table 1.7: Generalized estimating equation models for triglyceride and β-hydroxybutyrate at three different buffered distances surrounding singing-grounds of male American woodcock (*Scolopax minor*) in north-central Wisconsin during the springs of 2018 and 2019. Model variables and descriptions can be found in Table 1.1. Landcover type variable dimensionality was reduced using principal component analysis, loadings for principal components can be found in Tables 1.4-1.6. The best fit model was selected with the lowest quasilikelihood under the independence model criterion.

Model	**Intercept**	*Std.Err*	**PC2**	*Std.Err*	**EAST**	*Std.Err*	**QIC**
TRIG							
50m Buffer n = 53 (Oneida n = 45, Langlade n = 7)							
Intercept-only Model	**1.232**	*0.105*					24.83
Best Model	**1.313**	*0.106*	**-0.109**	*0.040*	**0.351**	*0.231*	**20.00**
364m Buffer n = 60 (Oneida n = 40, Langlade n = 20)							
Intercept-only Model	**1.434**	*0.069*					**21.65**
Best Model	-	-					-
964m Buffer n = 65 (Oneida n = 45, Langlade n = 20)							
Intercept-only Model	**1.405**	*0.071*					23.28
Best Model	-	-					-
BUTY							
50m Buffer n = 49 (Oneida n = 42, Langlade n = 7)							
Intercept-only Model	**0.100**	*0.006*					**1.95**
Best Model	-	-					-
364m Buffer n = 57 (Oneida n = 38, Langlade n = 19)							
Intercept-only Model	**0.097**	*0.005*					**1.99**
Best Model	-	-					-
964m Buffer n = 61 (Oneida n = 42, Langlade n = 19)							
Intercept-only Model	**0.097**	*0.005*					**1.95**
Best Model	-	-					-

PC2 = principle component two, created with habitat landcover types
EAST = the proportion of east facing slope
QIC = quasilikelihood under the indepence model criterion

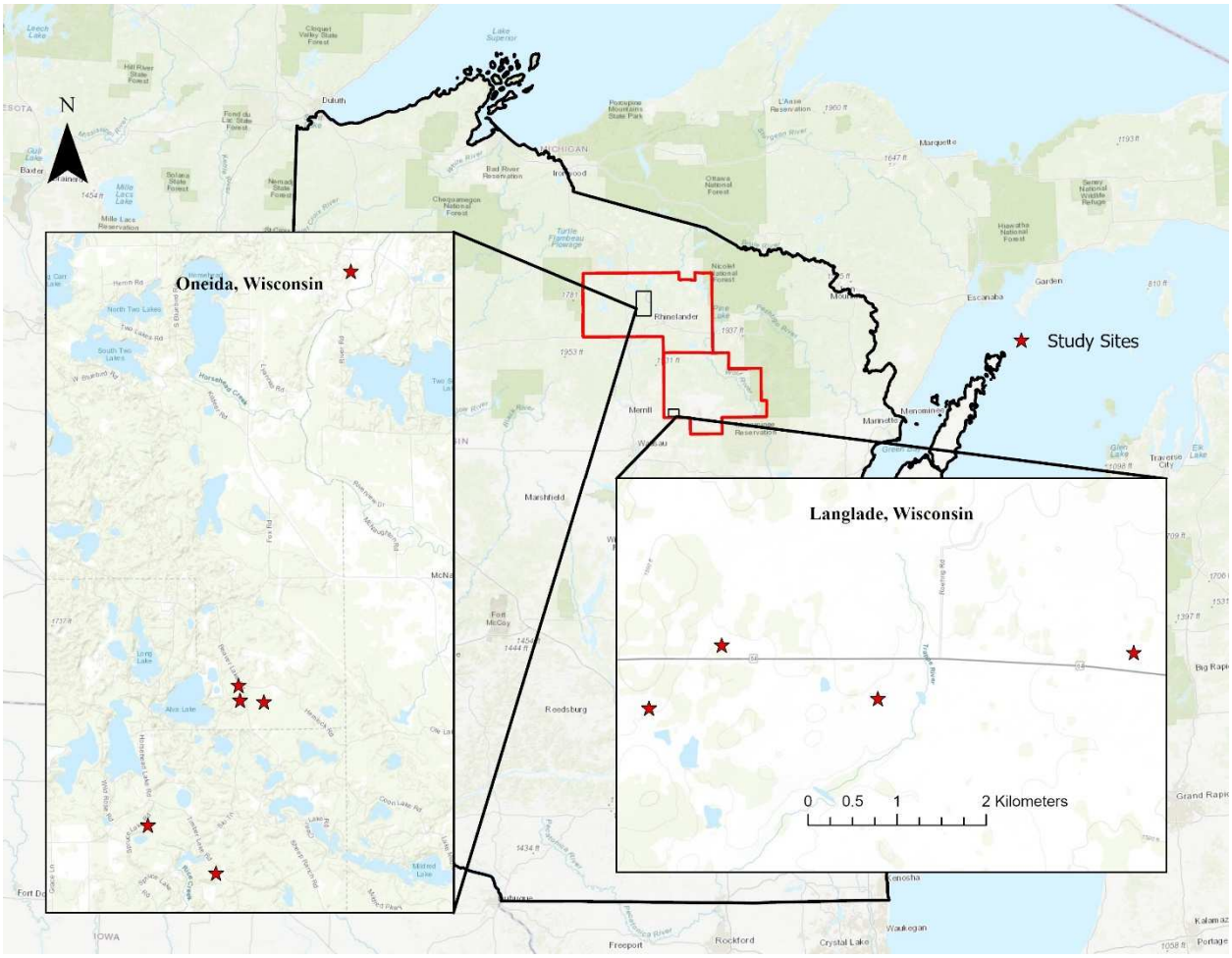

Figure 1.1: Study sites for assessing habitat quality of male American woodcock (*Scolopax minor*) using body condition were located in two counties (Oneida and Langlade counties) in north-central Wisconsin during the springs of 2018 and 2019.

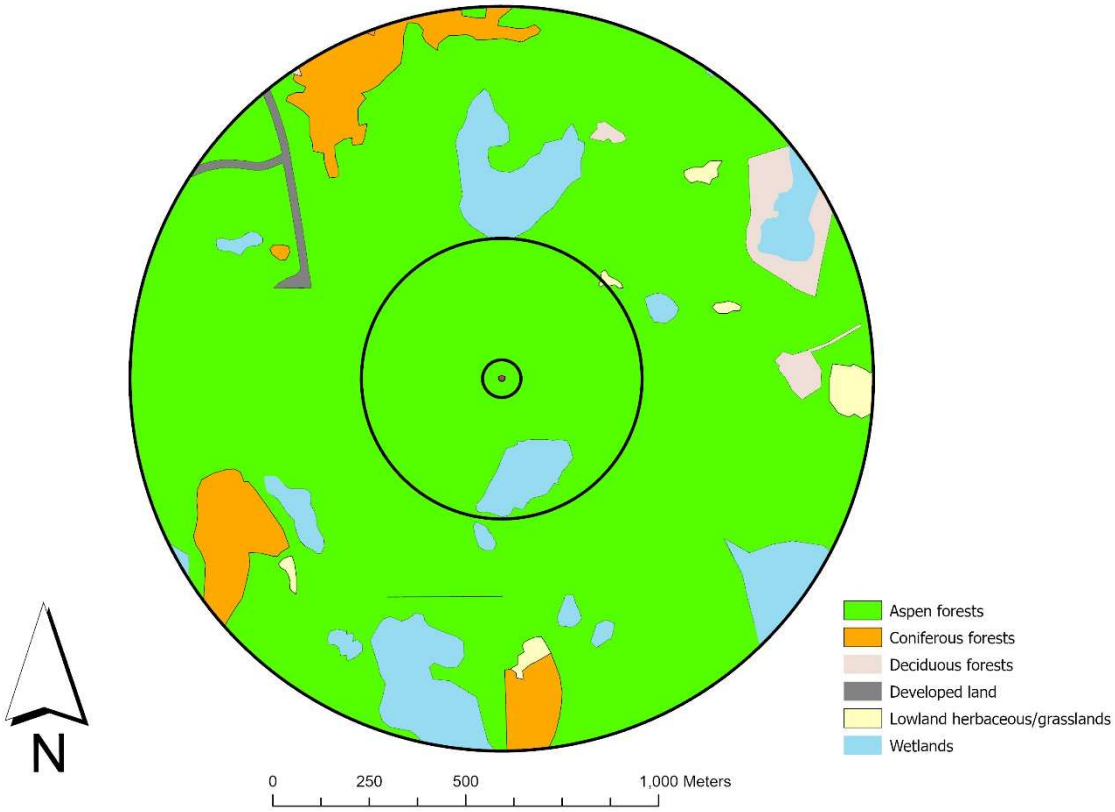

Figure 1.2: A representative plot showing landcover types surround one capture point in Oneida County, Wisconsin of male American woodcock (*Scolopax minor*). The dominant landcover type surrounding capture points in Oneida County was aspen forests. A point in the center is the location of the capture of the male American woodcock, the inner circle is the 50 m buffer, the next circle indicates the 364 m buffer, and the extent of the map is the 964 m buffer. Three buffered distances were selected for the range and mean distance a male American woodcock will travel between singing-grounds and diurnal feeding sites.

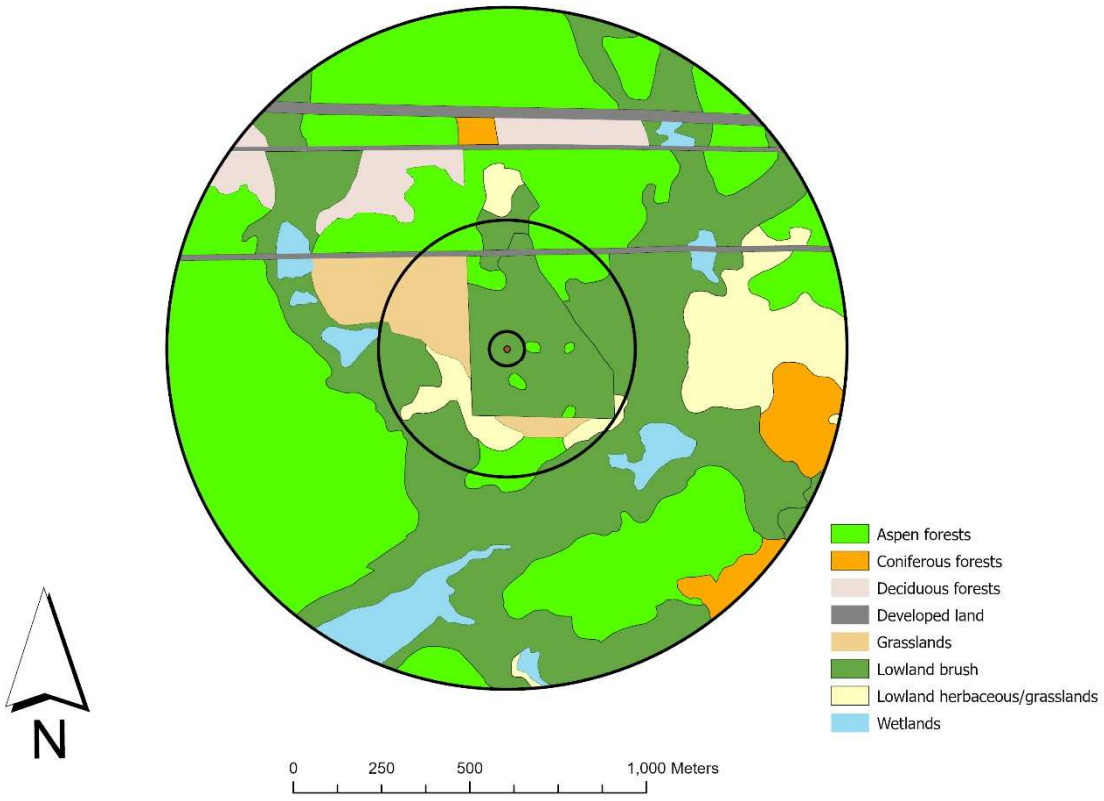

Figure 1.3: A representative plot showing landcover types surround one capture point in Langlade County, Wisconsin of male American woodcock (*Scolopax minor*). Landcover types in Langlade County, Wisconsin were more dominated by both lowland brush. A point in the center is the location of the capture of the male American woodcock, the inner circle is the 50 m buffer, the next circle indicates the 364 m buffer, and the extent of the map is the 964 m buffer. Three buffered distances were selected for the range and mean distance a male American woodcock will travel between singing-grounds and diurnal feeding sites.

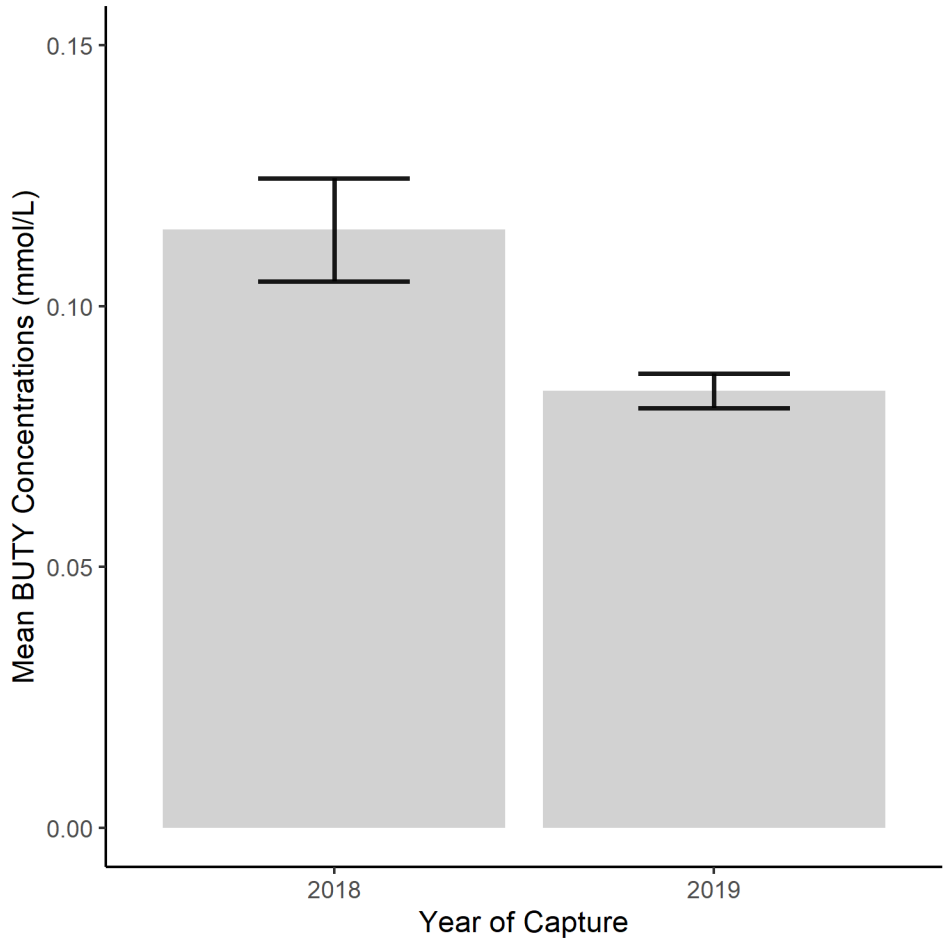

Figure 1.4: Mean (± SE) β-hydroxybutyrate concentration in male American woodcock (*Scolopax minor*) captured during the 2018 field season had a higher mean concentration 0.11 mmol/L (Range = 0.06-0.28 mmol/L, SE = 0.01, n =29) than male woodcock captured during the 2019 field season 0.08 mmol/L (Range = 0.06-0.15 mmol/L, SE = <0.01, n =33; T (41) = 3.29, P = 0.002).

Chapter 2

VALIDATING THE USE OF A HANDHELD METER FOR MEASURING A PLASMA METABOLITE, TRIGLYCERIDE, IN THE FIELD

Abstract

Plasma metabolite concentration analyses are effective in predicting change in body mass and are useful as an indicator of body condition in birds. Triglyceride (TRIG) is a plasma metabolite demonstrated to be effective for indicating when birds are either in a state of fattening or fasting. TRIG can be a useful indicator of food resources consumed by individual birds using a habitat and aid land managers interested in assessing the quality of that habitat. Blood plasma sample collection in a field setting can be challenging and methods aimed at reducing these challenges may help to expand the measurement of plasma metabolites like TRIG in the field. I evaluated the use of a small handheld meter, CardioChek PA analyzer, for measuring TRIG concentrations as a means to reduce these challenges. I compared the TRIG concentrations from the handheld meter to the results of the same plasma sample analyzed in a laboratory to determine the validity of using a handheld meter in the field. The handheld meter's results were precise but not accurate in the field, possibly due to the effects of environmental conditions on the meter's function. I recommend further research into the limitations of this handheld meter and developing methods to reduce the effects of light, temperature, and humidity on its function in the field.

Introduction

Body condition of bird species can be used by researchers and wildlife managers to assess the quality of food resources consumed by individuals in a habitat. Historically, researchers measured the change in body mass over time. The technique for measuring a change in body mass over time requires the capture and recapture of an individual. Multiple captures can lead to trap shyness and capture bias (Guglielmo et al. 2002).

A more recent technique assesses body condition by measuring plasma metabolite concentrations. Plasma metabolites have been shown to accurately predict a change in body mass of individuals while eliminating the need for recaptures (Jenni-Eiermann & Jenni 1994; Williams T. D. et al. 1999; Seaman et al. 2005). Triglyceride (TRIG) is one plasma metabolite that is effective in predicting change in body mass (Williams T. D. et al. 1999; Cerasale & Guglielmo 2006; Anteau & Afton 2008). TRIG alone has been found to predict 44% of the variation in the change of body mass (Jenni-Eiermann & Jenni 1994). TRIG is produced when lipids are transported to peripheral adipose tissue. Increased TRIG concentrations in blood plasma indicate that individuals are meeting their dietary needs and are in a state of fattening (Williams et al. 2007; Smith et al. 2007). TRIG, therefore, is a useful indicator of body condition in birds because it requires only one capture and blood sample to determine if dietary needs are being met. Using TRIG as a tool for assessing body condition can give wildlife managers insight into the quality of food resources individuals are receiving from their habitat.

Plasma metabolite concentrations are measured by collecting blood samples from birds in the field and transporting these samples to a lab for analysis. The collection of blood samples in the field can be difficult, especially for those with novice experience. Birds do not hold still and have small veins, making the blood collection process especially difficult in a field environment

where conditions are not ideal. Once a blood sample is collected it must be handled appropriately to reduce degradation of the sample which could influence the accuracy of the plasma metabolite analysis (Seaman et al. 2005; Owen 2011). Samples must be placed into blood tubes containing an anticoagulant such as heparin to prevent clotting, which can influence the accuracy of a blood sample and the ability to complete plasma metabolite analysis. Samples must then be centrifuged to separate plasma from the whole blood (Owen 2011). Centrifuging in the field requires transporting a portable centrifuge with a power source and lab supplies such as centrifuge tubes. Once centrifuged, the plasma is pipetted from the whole blood, placed into a cryotube for storage, and stored in deep freeze (-20º - -80º C) until samples can be analyzed in a laboratory (Guglielmo et al. 2002; Williams et al. 2007; Sommers et al. 2017). A handheld meter, as a new method for measuring plasma metabolites, would possibly eliminate some of the challenges in the sample preparation steps and plasma metabolite analysis.

Sommers et al. (2017) validated the use of a handheld meter, STAT-Site M β-HB Photometer, to measure the concentrations of a β-hydroxybutyrate (BUTY) in free-living Grasshopper Sparrows (*Ammodramus savannarum*) and studied its effectiveness to help reduce some of the challenges associated with the analysis of plasma metabolites. In contrast to TRIG, BUTY is produced when lipids are being metabolized in the peripheral adipose tissue to meet energetic needs. Elevated concentrations of BUTY are indicative of individuals in a state of fasting with a decreasing body mass (Jenni-Eiermann & Jenni 1994; Smith et al. 2007). The handheld meter, STAT-Site M β-HB Photometer, used by Sommers et al. (2017) required only a small plasma sample, approximately 10µL, to accurately measure BUTY.

The objective of this research was to validate the use of a similar handheld meter to measure TRIG concentrations in male woodcock sampled in the field by comparing the results of

the meter to the laboratory analysis. The meter, CardioChek PA analyzer (part number 1708; PTS Diagnostics, Indianapolis, IN) was developed for human use in a clinical setting. The handheld meter is slightly larger in size and similar in function to a glucometer (Figure 2.1.).

Methods

Study Area and Capture Methods

Male woodcock were captured at ten study sites located within two state-managed areas (Ackley State Wildlife Area and Northern Highland American Legion State Forest) in Langlade (45° 8' N 89° 24' W) and Oneida counties (45° 47' N 89° 32' W), Wisconsin. Study sites were identified by selecting clearings within early-successional forests where male woodcocks were observed to be present. The presence was determined by observing at least one male woodcock performing its evening crepuscular courtship display. The following evening, technicians and I returned to capture the male woodcock and collect blood samples.

Male woodcock were captured from 20 April – 4 June 2018, and 18 April – 30 May 2019 using mist nets and audio lures. Mist nets were set up within clearings of early-successional forests. An mp3 player with speakers was placed near the mist nets while playing the calls of a male woodcock, which induced a territorial response from nearby male woodcock resulting in their capture in the net (McAuley & Longcore 1993).

Morphometric measurement of American woodcock

Once entangled in the mist nets, woodcocks were quickly removed upon capture to reduce stress and chance of injury. Each woodcock was fitted with a federal bird band, sexed, aged, and measured for physiological characteristics. Woodcocks were sexed using morphometric measurements (e.g. exposed culmen and tarsus length) and plumage characteristics (e.g. outer three primaries width) and aged using plumage characteristics (Sepik

1994; Pyle 2008). Following the collection of measurements and banding, female woodcocks were promptly released as only male woodcocks were used for this research.

Blood and plasma sample collection and handling

I collected blood samples from male woodcock to validate the use of the CardioChek PA analyzer for measuring the plasma metabolite TRIG. Blood samples were collected following the Guidelines to the Use of Wild Birds in Research (Fair et al. 2010). Blood samples were collected from the brachial vein using a 25-gauge needle and syringe. I collected a maximum of 600 µL blood sample from each male woodcock. Samples were then placed into two 300 µL heparinized blood centrifuge tubes to prevent blood coagulation (Owen 2011; Sheldon et al. 2008). After blood sample collection, the vein collection site on each male woodcock was treated with a clotting substance called Kwik-Stop® and cotton ball to prevent further bleeding. Each woodcock was then placed into a breathable cloth bag for 5 minutes and his health condition was reevaluated before being released at the point of capture (Fair et al. 2010).

One of the 300 µL whole blood samples was used for the CardioChek PA analyzer to measure TRIG concentrations in the field. I used TRIG test strips (part number 1716; PTS Diagnostics, Indianapolis, IN) and followed manufacturer protocols for using the handheld meter (PTS Diagnostics 2020a; PTS Diagnostics 2020b). Both the handheld meter and test strips were sensitive to environmental conditions including temperature, humidity, and light levels. The handheld meter and test strips required an ambient temperature between 20ºC - 30ºC for proper function (NOAA 2020). Because field conditions were often colder than the required temperature, the meter and test strips were placed into an insulated lunch bag with an air-activated hand warmer and a thermometer to monitor and maintain temperature. The handheld meter required humidity between 20-80%, so to reduce the effects of fluctuating humidity, the

samples were analyzed using the handheld meter in a vehicle with climate control running. The CardioChek PA analyzer is sensitive to direct light due to its dependence on light reflectance to measure enzymatic chemical reactions (PTS Diagnostics 2020a). To reduce the effects of light, all headlamps and vehicle lights directly adjacent to the handheld meter were turned off.

The CardioChek PA analyzer measured samples using test strips that were inserted into the handheld meter. Approximately 15µL of whole blood was applied to the test strip and a TRIG value was reported after a few minutes. To quantify the accuracy of the handheld meter, each sample was analyzed 10 times, each with a new test strip. Following the completion of the 10 samples, I calculated coefficients of variance (CV) to measure the precision of the handheld meter.

The second of the two 300 µL heparinized blood centrifuge tubes was centrifuged to separate plasma from the rest of the whole blood (4,800 x g for 10 minutes on an HWLAB min centrifuge). After centrifugation, the plasma was pipetted from the centrifuge tube and placed into a 1mL cryotube. Cryotubes were labeled and placed into coolers before being transported to the location where samples could be stored in a -20ºC freezer until the end of the field season (< 7 weeks; Williams et al. 2007; Smith et al. 2007; Owen 2011).

At the end of the field season, plasma samples were packed in coolers with dry ice and then shipped overnight to Dr. Scott McWilliams' Bird Lab at the University of Rhode Island. In the lab, colorimetric assay kits (Triglyceride Reagent part number T2449, Sigma-Aldrich Inc., St. Louis, MO) were used to analyze the concentration of TRIG in each of the plasma samples. Using the Kolmogorov-Smirnov normality test, I verified that the results from the CardioChek PA handheld meter and the laboratory analysis met the assumptions for linear regression. I used linear regression in R statistical environment, using the *stats* package to compare TRIG

concentrations from the handheld meter to the TRIG concentrations analyzed in the lab (R Core Team 2019).

Results

The concentrations of TRIG analyzed in the lab were weakly correlated with the handheld meter, CardioCheck PA analyzer, ($F_{1,33}$ = 9.638, P = 0.004, r^2 = 0.23; 95% C.I. = 0.074– 0.355; slope = 0.21x; Figure 2.2). TRIG results from the lab had a higher mean of 1.51 mmol/L (Range = 0.482-2.784, SD = 0.540) relative to the handheld meter (mean=0.821 mmol/L, Range = 0.570-1.461, SD = 0.243; t (34) = 8.533, P < 0.001). The confidence interval (CI) for the line of best fit did not include the line with a slope of 1 (95% CI = 0.074– 0.355), indicating a difference in the actual handheld meter results and expected results (Figure 2.2). Even though the handheld meter did not provide the expected results for accuracy, the meter was found to be consistent and repeatable. The high precision of the results from the handheld meter was indicated with a CV <15% (mean CV = 7.62%; Figure 2.3).

Discussion

Results indicated that the handheld meter, CardioChek PA analyzer, was inaccurate when compared to the results completed in the laboratory analysis. Even with the high precision and repeatability of the handheld meter, results from the meter were weakly correlated with results completed in the laboratory analysis. Sommers et al (2017) validated the use of a similar handheld meter, STAT-Site M b-HB Photometer, for measuring concentrations of BUTY, which was found to be highly precise (CV = 5.4%) and highly correlated with concentration results from the laboratory ($F_{1,19}$ =764, P<0.001, r^2 =0.98; slope = 1.07, 95% C.I. = 0.985– 1.147). Had the handheld meter I tested been able to accurately measure TRIG concentrations, it would have reduced or eliminated many of the challenges associated with the collection and analysis of

plasma samples in the field. Unfortunately, this handheld meter did not demonstrate accuracy in measuring TRIG concentrations under the conditions experienced in a field setting.

The handheld meter had several limitations that may have influenced its ability to accurately measure TRIG concentrations under field conditions. The CardioChek PA analyzer was originally developed for use in the controlled environment of a clinical setting. In a clinical setting, the environmental and light levels are controlled and stable, reducing or eliminating the impact on the function of the handheld meter. The use of the handheld meter occurred in the late evenings during the spring months (March - June). Weather and field conditions during the fieldwork were not optimal since both the handheld meter and test strips were sensitive to temperature, light, and humidity (NOAA 2020).

The manufacturer-recommended temperature for the handheld meter is between 10-40 °C, while the recommended temperature for the test strips is 20-30 °C (PTS Diagnostics 2020a; PTS Diagnostics 2020b). Temperatures during the field season regularly dropped well below freezing temperatures (NOAA 2020). To reduce the impact of temperature, both the handheld meter and test strips were placed inside of an insulated lunch bag a minimum of one hour before use with an air-activated hand warmer and a thermometer to maintain operational temperature. Even with this preventive measure, the air temperature still may have impacted the accuracy of the handheld meter. Analyzing ten samples took approximately 20 minutes (~2 minutes per test strip) which may have exposed the meter to a temperature below the recommended range. Future research should take into consideration the effects of temperature during the time of use. If the CardioChek PA analyzer is being used within an enclosed area, allow the area to reach the recommended temperature before and during use.

Humidity was more difficult to control in the field. The manufacturer's recommendation was to maintain the handheld meter between 20-80% humidity (PTS Diagnostics 2020a). As with temperature, humidity fluctuated greatly through the evening during these spring months (NOAA 2020). To reduce the impact of humidity, the meter was only used in the research vehicle while the vehicle's climate control was turned on. Adding a meter for measuring humidity may help in reducing the effects of humidity on the CardioChek PA analyzer. Before using the CardioChek PA analyzer, researchers could ensure that the humidity is within the recommended range and, if not within that range, attempt to correct the humidity in the area of use.

Finally, the impact of light on the meter was likely problematic. The CardioChek PA analyzer uses light reflectance to measure enzymatic chemical reactions. The dependency of light for measuring concentrations of TRIG made the handheld meter sensitive to direct light. The manufacturer recommended that the meter not be used in direct light, such as sunlight or under a lamp (PTS Diagnostics 2020a; PTS Diagnostics 2020b). To reduce the effects of light on the handheld meter, all headlamps and internal vehicle light directly adjacent to the meter were turned off. A vehicle's headlights farthest from the meter were needed to allow us to see what we were doing, which still may have impacted the function of the meter.

All concerns about environmental and field conditions that may affect the accuracy of the handheld meter were communicated with the manufacturer before use in the field. Best methods for using the handheld meter in the field were created and put into practice using the manufacturer's responses to our concerns as well as the manufacturer's user guides. The CardioChek PA analyzer is designed with internal sensors that display warning signs whenever the temperature reaches a high or low threshold and/or if the light was too bright (PTS

Diagnostics 2020a). A “low temperature” warning was the only warning sign that was ever displayed during our use of the handheld meter. When this occurred, the meter was placed back into the insulated lunch bag and given time to reach operating temperature.

Future research using the CardioChek PA analyzer needs to take into consideration the effects of environmental conditions such as temperature, humidity, and light on its ability to accurately measure TRIG concentrations. One recommendation for future use of this meter would be to validate its use in a controlled setting similar to clinical use. Testing of the meter in a laboratory setting would require a captive population of birds but would allow for the testing of the handheld meter’s accuracy and precision. Also, a laboratory setting would allow us to test the accuracy and precision of the meter for various periods between blood collection and the analysis of samples. If the meter was found to be accurate and precise for an hour or more following blood collection, then it may be possible to collect blood samples in the field and transport these samples to a location for analysis where environmental conditions are controlled.

Using a handheld meter in a field setting has many advantages because it gives researchers the ability to quickly assess body condition while reducing the challenges associated with handling plasma samples. This research shows that there are limitations to using the handheld meter that must be taken into consideration. This precision could lead to a false sense of accuracy and cause researchers to believe an individual’s body condition is better or worse than reality. The poor accuracy of results from this handheld meter used under field conditions to analyze TRIG must be addressed before future use in wildlife field research.

Figure 2.1: The CardioChek PA analyzer is a small handheld meter that is similar in size and ease of use as a glucometer. Paired with the triglyceride test strips, also pictured, allows it to be easily transported and used in a field setting.

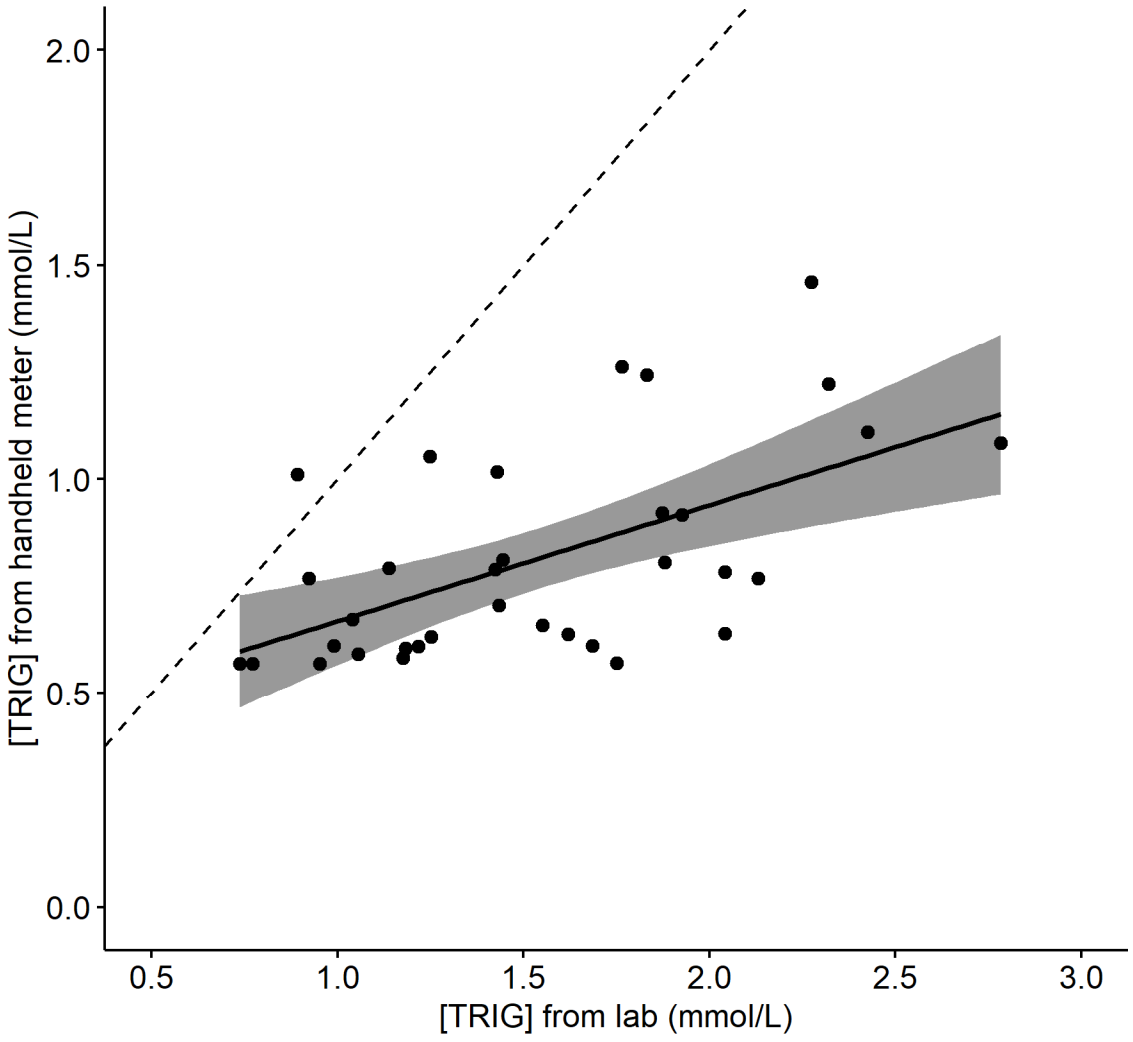

Figure 2.2: Triglyceride (TRIG) results from the CardioChek PA analyzer were weakly correlated with results completed in the laboratory using colorimetric assays. The line of best fit from a linear regression is shown by the solid line ($F_{1,33} = 9.638$, $P = 0.004$, $r^2 = 0.23$; 95% C.I. = 0.074– 0.355; slope = 0.21x). The 95% confidence interval for the slope of the line (gray shaded area) of best fit does not include the line with a slope of 1 (dotted line) indicating a difference in handheld meter results and expected results**.**

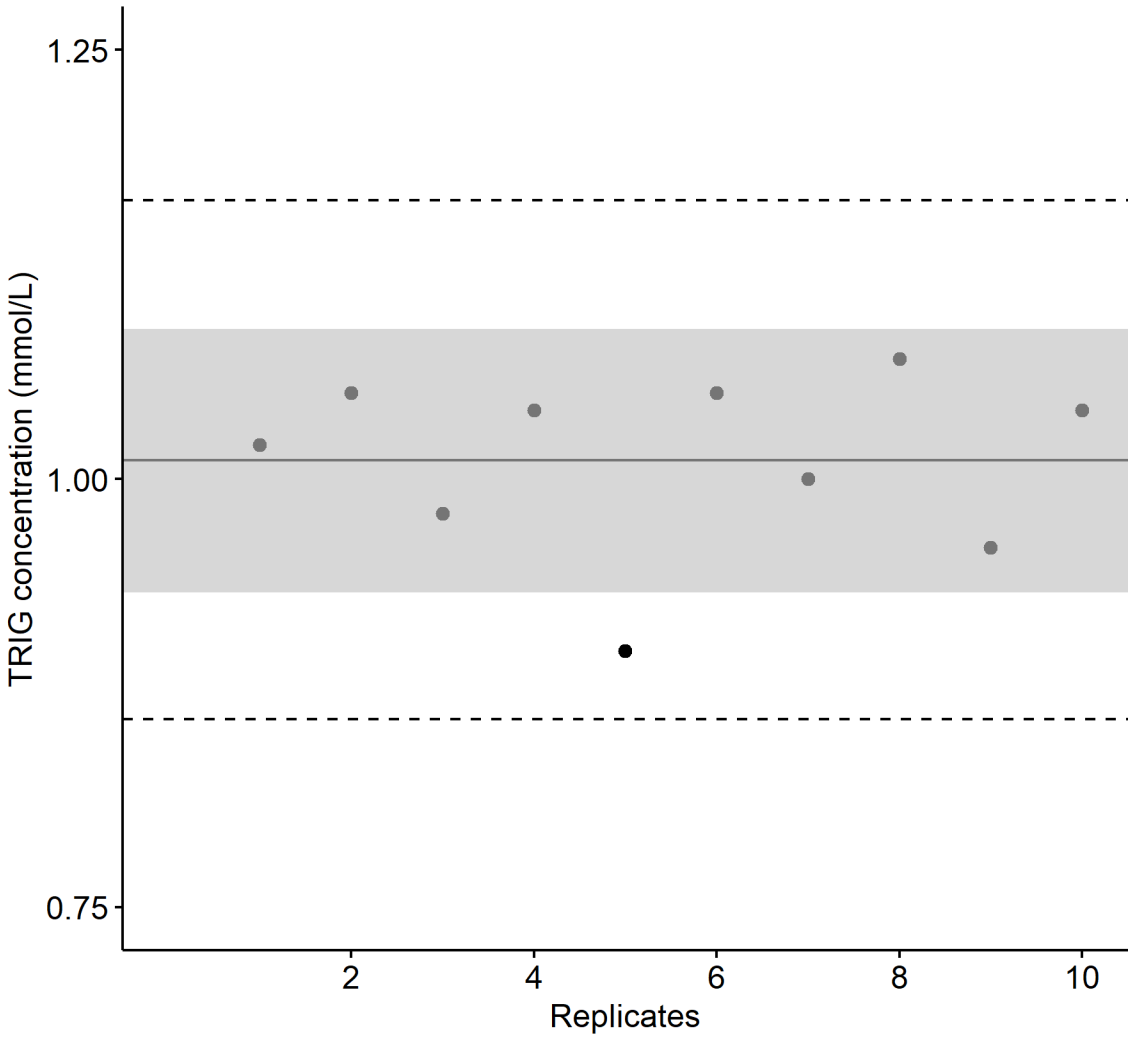

Figure 2.3: Example results from a single sample show the precision and repeatability of the handheld meter, CardioChek PA analyzer. Overall results from the handheld meter were precise with a coefficient of variation of 7.62% (gray box = mean ± 7.62%). Acceptable CV for replicates completed in a laboratory setting was a CV <15% (dashed lines = ± 15%).

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