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A Physiological Assessment of Wetland Habitats for Spring-migrating Ducks in the Agricultural Landscapes of the Southern Prairie Pothole Region

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A PHYSIOLOGICAL ASSESSMENT OF WETLAND HABITATS FOR SPRING-MIGRATING DUCKS IN THE AGRICULTURAL LANDSCAPES OF THE SOUTHERN PRAIRIE POTHOLE REGION

BY

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A PHYSIOLOGICAL ASSESSMENT OF WETLAND HABITATS FOR SPRING-MIGRATING DUCKS IN THE AGRICULTURAL LANDSCAPES OF THE SOUTHERN PRAIRIE POTHOLE REGION

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy in Wildlife and Fisheries Sciences degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidates are necessarily the conclusions of the major department.

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A PHYSIOLOGICAL ASSESSMENT OF WETLAND HABITATS FOR SPRING-MIGRATING DUCKS IN THE AGRICULTURAL LANDSCAPES OF THE SOUTHERN PRAIRIE POTHOLE REGION

ADAM K. JANKE

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The conversion of grassland and wetland ecosystems in the Prairie Pothole Region (PPR) has been a pervasive challenge for conservationists dating back to the early 1900s. The legacy of ever-increasing agricultural intensity in the southern portions of the PPR, including eastern South Dakota, has left many wetland ecosystems in a matrix of intensive agricultural production. With little surrounding nesting cover, these wetlands are thought to have limited potential for waterfowl reproduction but may still play an important role facilitating migration of waterfowl en route to northern breeding areas during spring. My research sought to understand the contributions of wetlands in intensively-farmed landscapes for spring-migrating ducks. I measured a number of biotic attributes of wetlands including the density of aquatic invertebrates and submersed macrophytes and abundance of spring-migrating ducks. I also measured concentrations of lipid metabolites circulating in plasma of female lesser scaup (*Aythya affinis*) and blue-winged teal (*Anas discors*) to understand refueling performance of migrants using wetlands with variable biotic and abiotic characteristics. Duck abundance, refueling performance, and prey abundance were generally similar across the upland cultivation gradient, if not slightly greater in more intensely-farmed landscapes. These results suggested wetlands in intensively-farmed landscapes in eastern South Dakota currently confer similar benefits to migrating waterfowl as those in less intensively-farmed landscapes. An analysis on wetland covariates and migrant refueling performance revealed density of fathead minnows (*Pimephales promelas*) in wetlands was negatively associated with refueling performance. Further analyses suggested this finding was likely the result of trophic effects of fathead minnows on invertebrate and plant communities in the wetlands. Taken together, my results suggested wetlands in agricultural landscapes in eastern South Dakota can provide novel refueling habitats for migrating ducks when factors such as artificial connectivity or water permanency that facilitate fathead minnow colonization and persistence are controlled. Further, they raise questions about whether wetlands in intensively-farmed landscapes are indeed resilient to adjacent land use or simply compensate for degradation through increased productivity characteristic of landscapes with intensive crop production. Answering this latter question is key for understanding agricultural impacts and setting wetland restoration priorities in the region.

CHAPTER 1: ON CULTIVATION, CONSERVATION, AND MIGRATION IN NORTH AMERICA'S DUCK FACTORY: AN INTRODUCTION

The challenges posed by agriculture for waterfowl conservation in North America's Duck Factory, the Prairie Pothole Region (PPR), have defined the discourse of conservationists for decades. Speaking about the state of the prairie breeding grounds at the first North American Wildlife and Natural Resources conference in 1936[a], John Huntington vice president of More Game Birds in America, the precursor to Ducks Unlimited, said,

> *"Settlement and utilization of the land (principally for agriculture) have brought about changes in this part of the producing plant which have been truly disastrous to the ducks. In the wake of the plow, approximately 80% of all duck breeding places have dried up. Of those that remain many have become so unattractive to the ducks that they are no longer used by them"*

In that same lecture, Huntington concluded, despite conservation and restoration efforts planned and ongoing, "… it seems doubtful whether maximum production of all the breeding grounds in the United States will ever again supply a sizable percentage of the continental duck crop." At the same time, those convened at the meeting and elsewhere were laying the ground work for North American wildlife conservation efforts to proceed throughout the 20th and early 21st century by developing innovative funding mechanisms like the Federal Aid in Wildlife Restoration Act of 1937 and the Migratory Bird Hunting Stamp Act of 1934 and organizing political and private financial support for wildlife conservation in the prairies through for example the incorporation of Ducks

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[a] U. S. Government Printing Office (1936) *Proceedings of the North American Wildlife Conference*, 518–523

Unlimited in the U.S. and Canada in 1937. So started a comprehensive approach for funding and delivering waterfowl conservation in North America, with a specific eye to the duck factory, that continues to present and has produced volumes of scientific literature, expended millions of dollars, conserved thousands of acres of grassland and wetland habitats, and yielded record waterfowl population size estimates. Despite the success of waterfowl conservation, the same fundamental challenges remain today, evidenced by recent conclusions of Hagy and colleagues[b] at the close of the 6th North American Duck symposium in 2013 where they said,

> *"…unless there is an immediate and significant change in a) wetland protection measures and b) agricultural policies that provide a disincentive to wetland drainage and conversion, the recent "good old days" of abundant wetlands for waterfowl are likely coming to a close"*

Whether the "good old days" lamented as perhaps bygone by Hagy and colleagues were the same as those lamented by Huntington, and his contemporaries like Frederick Lincoln, Logan Bennett, Jay "Ding" Darling, and other conservation icons at that 1936 conference we will perhaps never know. What is clear however, is that landscape modifications associated with agriculture are the premier challenge for waterfowl conservation, a fact as true today as it was in the heart of the dust-bowl era in 1936. Add to the mix the emerging, complex challenges of a changing climate and a few billion additional human mouths to feed, and the task for ensuring sustainable waterfowl and other wildlife populations in North America's Bread Basket during the 21st century seems evermore daunting. If, however, these challenges are going to be met it will be

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[b] Hagy et al. (2014) *Wildfowl* Special Issue 4, 343–367

done through sound policy and public engagement, founded on scientific understanding of the interactions between agricultural land uses, wetlands, and waterfowl and the cultural and economic benefits they confer. It is with this backdrop that my dissertation research was conducted, and it is my sincere hope that the insights gained here and the additional questions explored in response to this work may make a small, incremental step towards the decades-long effort to conserve the ecological contributions of North America's Duck Factory for future generations.

STUDY DESIGN

The primary question I set out to address in my dissertation was; what is the current condition of prairie wetlands in agricultural landscapes with respect to their potential contributions to spring-migrating ducks? Volumes have been written on the factors influencing wetland use and spatial distribution of breeding ducks in the PPR. Perhaps less appreciated and certainly less studied are the contributions prairie wetlands make to ducks moving through the southern PPR, en route to northern breeding ranges in the northern contiguous U.S., Canadian prairies, Boreal Forest, and Alaska. Millions of ducks annually use the PPR as the 'gateway' to the rest of North America's prime breeding habitats, but to date the role of the region for migrants, particularly wetland-foraging migrants, has received little consideration. Because well-over 95% of the southern PPR is outside the direct-management control of state or federal wildlife conservation agencies, addressing this question meant I needed to conduct a majority of my research on working farms and ranches where thousands of wetlands remain and are exposed to a wide-variety of disturbances and management strategies.

I conducted my research in the PPR of eastern South Dakota, an area decidedly important for breeding waterfowl in the U.S., but also a region with a relatively longer legacy of intensive agricultural production than northern portions of the PPR. The climate and geography of the region have precluded the near-complete conversion to agriculture as seen in the other southern portions of the PPR in southwestern Minnesota[c] and Iowa[d]. Therefore, eastern South Dakota seemed ideally suited to answer my research question because migrant ducks move through the region en masse annually and, in this landscape, I was able to find a diversity of wetlands distributed across a gradient of crop production intensity.

All counties east of the Missouri River in South Dakota comprise the PPR in the state with a total area of nearly 92,000 km^2. I constrained my study to area to the glaciated portions of the region that had at least 4 ha/km^2 of wetlands mapped by the National Wetlands Inventory in 1985[e] to exclude areas with low wetland densities. The final study area was 46,770 km^2 and comprised the core of the PPR in the state. The U.S. Fish and Wildlife Service or South Dakota Department of Game, Fish and Parks owned and managed only 2.4% of the land area, leaving the rest for private or tribal ownership and management. I stratified the area into 3 categories of upland cultivation intensities by calculating the proportion of non-developed uplands that were cultivated based on available satellite-based land cover maps[f]. The resulting classification divided the study area into high (18.3%), medium (47.6%) and low (34.1%) crop-intensity strata, from which I drew a random sample of 30 study areas to visit and sample wetlands and ducks.

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[c] Oslund et al. (2010) *Journal of Fish and Wildlife Management* 1, 131-135
[d] Miller et al. (2009) *Wetlands* 29, 1146-1152
[e] Johnson & Higgins. (1997) *Wetland resources of eastern South Dakota*. South Dakota State University
[f] Fry et al. (2011) *Photogrammetric Engineering and Remote Sensing* 77, 858-864

These study areas had a comparable ownership pattern as the rest of the study area: 97% of the 1,500 km^2 of land included in my study was privately owned, with most of it used for some kind of agricultural production, either pastoral or cultivation.

With the cooperation of over 300 farmers, ranchers, and landowners, I sampled 305 wetlands during three spring migration periods in 2013, 2014, and 2015. The wetlands ranged in size and character — ranging from a 500 m^2 seasonal basin to a 733 ha fishing lake — but were all potentially usable by spring-migrating ducks. Most wetlands were between 1 and 15 ha and had semipermanent hydroperiods. Only 2% of these wetlands were on lands completely owned by a state or federal wildlife agency, 11% had split public-private ownership, and the remaining 87% were owned entirely by private land owners. I set out to measure the potential contributions of these wetlands to migrating ducks, focusing specifically on their utility for migrants and the abundance of aquatic invertebrate prey.

Measuring habitat quality for a breeding duck is arguably simple: count the number of eggs laid and estimate their prospects of successfully hatching or fledging and compare those metrics across the range of habitat conditions of interest. Measuring habitat quality for migrants however, is more complex because migrants spend short periods at individual stopover locations and then depart for far-away breeding habitats, stopping in additional landscapes along the way. In a landscape like the PPR, where potential stopover locations number in the 100s of thousands, measuring the contribution of an individual location seems almost infeasible. However, we know migrants need energy-dense fats to fuel their flights[g] and, for female ducks, the need for those fats

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[g] Jenni & Jenni-Eiermann. (1998) *J. Avian Biol.* 29, 521-528

extends beyond the migratory period and factors into their ability to successfully lay clutches of eggs once they arrive on the breeding grounds[h]. Therefore, indexing lipid metabolism at an individual stopover location should be a suitable metric to measure the quality of a migratory stopover. The only direct-method to measure fat accumulation efficiency would be to capture the same individual on multiple occasions on the same stopover location to measure changes in fat content or an index thereof, like body mass — a daunting task for any study, and almost certainly impossible to pull off at any meaningful scale for migrating ducks in the PPR. Fortunately, measuring concentrations of metabolites circulating in plasma has been identified as an alternative to successive captures to index the trajectory of lipid reserves in a migrant from a single capture event[i]. Metabolites are like the fingerprints of metabolism, leaving a trail of evidence behind on the recent nature of metabolism by an individual, rising and falling during transitions among metabolic states. By measuring concentrations of key lipid metabolites circulating in plasma of migrating ducks, we can approximate the recent trajectory of lipid metabolism in birds collected among contrasting habitats — perhaps the ideal metric for measuring the quality of a habitat for a migrating bird. I used this approach with lipid metabolites in migrating ducks collected in my study areas to combine with more traditional habitat-quality metrics, like migrant abundance and food abundance, to comprehensively evaluate the contributions of wetlands in agricultural landscapes in the southern PPR.

My research focused on two duck species — blue-winged teal (*Anas discors*) and lesser scaup (*Aythya affinis*). Key similarities and differences between these two species

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[h] Ankney et al. (1991) *Condor* 93, 1029-1032
[i] Jenni-Eiermann & Jenni. (1998) *Biol Cons Fauna* 102, 312-319

made them ideally-suited to address my research questions. Both species are wetland-dependent during migration, meaning they spend all their time courting, resting, or foraging during migration on wetlands, in contrast to many waterfowl species that find substantial quantities of foods in the form waste grain in agricultural fields. Therefore, I could link the physiology of these two species to the quality of the wetlands in the landscape in which I encountered them. The second desirable attribute these two species shared was that they were ubiquitous and therefore could be reliably found across the range of land uses and wetland types that were the focus of my research. Finally, both species had diets comprising mainly invertebrates during migration and transition to breeding in the PPR, and therefore were thought to likely respond physiologically to factors influencing the abundance of their primary prey in wetlands. The two species diverged in their use of specific wetland types for foraging, with blue-winged teal using shallow-water foraging habitats in small wetlands or along the margins of larger wetlands and lesser scaup using large-open water foraging habitats. Therefore, collectively, the two species could conceivably use the entire range of possible foraging habitats for wetland-foraging ducks available during migration in the PPR. Collectively, these shared and divergent attributes made these two species ideal for addressing my research questions and for drawing broader inferences about the condition of wetlands for ducks in the southern PPR.

DISSERTATION CONTENT AND DIRECTION

In addressing my central research question, I have prepared 7 chapters in this dissertation. This introductory chapter and the final conclusions chapter were both written in first person and with a decidedly non-technical tone. I surmised that the reader would find sufficient technical writing in the research chapters of my dissertation, leaving the introductory material and conclusion chapters to communicate first the intent of the dissertation and then the implications thereof in a simpler, more accessible manner. It is my hope that biologists and conservationists interested in my results can find the most relevant information in the conclusions chapter, without concern for the important but dense technical details contained in the research chapters. I wrote my research chapters (2-6) using plural pronouns because they, like most scientific endeavors, were collaborative efforts and will ultimately be published with coauthors. Despite use of plural pronouns, I assume all responsibility for the content and conclusions in the chapters in their present form. The methods and procedures used throughout this dissertation were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (12-013A) and authorized by the U.S. Fish and Wildlife Service (Scientific Collecting Permit MB068976) and South Dakota Department of Game, Fish and Parks (Scientific Collector's Permits Number 25 and 7).

In my first two research chapters, I explored questions about the utility and possible limitations of using plasma metabolite concentrations in migration ecology research: first examining the consequences of sample quality on potential inferences drawn in such studies (Chapter 2) and then examining the utility of compositing two lipid metabolites into one index of refueling performance (Chapter 3). Building on insights gained in the first two chapters, I then explored the question central to my dissertation in Chapter 4 — what is the current contribution of wetlands in intensively farmed landscapes for spring-migrating ducks? — by combining all aspects of the data collected for my research. Building on understanding gleaned in Chapter 4, I then set out to examine wetland-specific factors influencing refueling performance of blue-winged teal and lesser scaup collected on wetlands in which I had collected substantial additional landscape and wetland-specific information. Finally, in Chapter 6, I took advantage of interannual variability in phenology of the four springs I sampled to evaluate how diets and physiological condition of blue-winged teal and lesser scaup varied among years.

It is my hope that the findings of this dissertation will find applications in the challenging, but perpetually important, work of conservation in the Duck Factory and perhaps more broadly.

CHAPTER 2: CONSIDERATIONS FOR SAMPLE QUALITY IN AVIAN PLASMA METABOLITE STUDIES

ABSTRACT

Field studies are increasingly employing concentrations of key metabolites circulating in plasma of wild birds to infer variation in physiological performance. An implicit assumption is that plasma samples collected in field environments yield unbiased measurements of concentrations of metabolites circulating in plasma. We explored the extent and apparent consequences of hemolysis on measurements of four common metabolites commonly used in field studies. We found extensive hemolysis, indicated by plasma hemoglobin concentrations, in our samples taken postmortem from ducks. The degree of hemolysis in a sample affected metabolite concentration estimates by artificially increasing absorbance or interfering with reactions. We also found investigator-associated variation in degree of hemolysis, suggesting inter-investigator variation in sampling methods or handling techniques could lead to variation in sample quality, which could subsequently be mistaken for spatial variability in metabolite concentrations. Research in hospital emergency departments has shown high-pressure environments are a catalyst for poor sample quality and manifest in poor patient outcomes. We suggest challenging environments characteristic of avian field studies have the same consequences for sample quality, and could lead to poor scientific inferences if the consequences of sample quality are not given full consideration.

INTRODUCTION

The utility and application of plasma metabolite concentrations for indexing daily lipid and protein dynamics in avian research has been the subject of considerable empirical research in field and laboratory settings (e.g., Jenni-Eiermann and Jenni 1994;1998, Guglielmo et al. 2005, Anteau and Afton 2008, Smith and McWilliams 2009). This proliferation of research was feasible because of the ease of blood sampling from capture birds and relatively inexpensive, high-throughput capabilities of modern metabolite assays conducted on microplate spectrophotometers. Accordingly, researchers from a wide variety of ecological or conservation disciplines are increasingly collecting plasma samples to address applied and theoretical questions. A liability of widespread adoption of metabolite concentrations in field research is that investigators with varying experiences with phlebotomy may be unfamiliar with potential pitfalls of improper blood sampling and handling. Several intricate steps are involved in procuring quality blood samples and there is great potential for sample degradation (Owen 2011); moreover, sampling under field conditions adds to the risk of sample degradation. Accordingly, there is a clear need to evaluate sources of sample degradation to ensure inferences drawn from metabolite concentrations are unbiased.

We recently noted the tendency for substantial variation in plasma coloration (e.g., Figure 1) in field studies on waterfowl across a range of conditions and investigators. Although plasma has natural color variation associated with circulation of pigments (Tella et al. 1998), some variation we observed seemed to fall outside values expected for carotenoids and therefore indicative of hemolysis, which has potentially negative consequences for inferences in avian metabolite studies (Owen 2011). Hemolysis is the rupturing of red-blood cell membranes that can occur during sample extraction or processing. When hemolysis occurs, it releases hemoglobin and other intracellular substances into surrounding plasma. Hemolysis can impact a wide variety of clinical metrics measured in blood through several mechanisms, including direct interference with reagents or reactions, dilution of plasma, or release of additional molecules of the substance being measured into plasma (Andreasen et al. 1996, Brady and O'Leary 1998, Cohen et al. 2007, Lippi et al. 2008). The potential for biased inferences based on hemolyzed samples in clinical applications has received considerable study and led to the formation of strict blood sampling and handling protocols to attempt to minimize such biases in the biomedical fields (*confer* Lippi et al. 2008). Here, we present the results of an evaluation of the extent of hemolysis that can occur in a field study, explore the apparent consequences of hemolysis on observed metabolite concentrations, and evaluate the nature of hemolysis impacts with a spike-recovery experiment with hemolyzed samples. We use these results and a review of the biomedical literature on hemolysis to discuss considerations for improving plasma sample quality in avian field research.

METHODS

We collected plasma samples from spring-migrating female lesser scaup (*Aythya affinis*) and blue-winged teal (*Anas discors*) associated with ongoing research in eastern South Dakota. We used shotguns to collect birds *in situ* so as to not bias metabolite concentrations with bait-capture methods (e.g., Dieter et al. 2009). Immediately after collection, we drew a ca. 1 mL blood sample via cardiac puncture with a 3 mL heparinized syringe and 16-21 gauge 38 mm needles. We removed the needle and transferred blood to a heparinized 1.5 mL microcentrifuge tube in the field and stored it in a cooler with ice packs. Field protocols specified that blood samples be handled carefully to avoid hemolysis and stored in a way that avoided freezing prior to centrifuging. We centrifuged whole blood within 4 hours of collection in the field with microcentrifuges that ran on power inverters in vehicles. After centrifuging for 5-10 minutes at 4,000 - 6,000 rpm, we transferred plasma to a non-heparinized microcentrifuge tube and stored it on ice packs until freezing at -20 C the evening of field sampling. We transferred plasma to a -80 C freezer within 1 month of collection until processing.

We measured concentrations of 4 metabolites (triglycerides, glycerol, uric acid, and β-hydroxybutyrate) and the concentration of plasma hemoglobin (Hb) in all samples. The four metabolites were each commonly used in avian research for indexing lipid metabolism (triglycerides, glycerol, and β-hydroxybutyrate) or protein breakdown (uric acid; e.g., Jenni-Eiermann and Jenni 1994, Guglielmo et al. 2005, Smith and McWilliams 2010). We measured all metabolite concentrations in duplicate on 96-well microplates and developed unique standard curves for each plate with duplicate wells of serially-diluted standards. We diluted all samples 3-fold with 0.9% NaCl solution. We followed protocols adjusted for small sample volumes and using commercially available reagents and standards following common protocols used in avian research (Guglielmo et al. 2005, Appendix 2). We reran all duplicates with coefficient of variation >15%.

We measured free glycerol (GLYC) with the Free Glycerol Reagent (Sigma Catalog F6428), reading absorbance at 540 nm and subtracting a background absorbance at 750 nm. We added Triglyceride Reagent (Sigma Catalog T2449) and reread absorbance (540 nm) and background absorbance (750 nm) to measure total triglycerides (TRIG), which includes the fraction of free glycerol measured in the first step. We used a serial dilution of the Glycerol Standard Solution (Sigma G7793) for plate standards. We subtracted free GLYC from total TRIG to calculate true TRIG. Package documentation for TRIG and GLYC reagents indicated highly hemolyzed or icteric samples should be avoided but did not provide information on the nature of hemolysis impacts on concentration estimates. Background readings we used were intended to correct for interference from background color variation, trace solids, or absorbance by microplate wells.

We measured concentration of β-hydroxybutyrate (BUTY) with a kinetic assay and the D-3 Hydroxybutyric Acid Assay Kit (R-Biopharm 10907979035) and β-Hydroxybutyrate Linearity Standards (Stanbio 2450). The assay read absorbance with no background reading at 492 nm every minute for 40 minutes and estimated BUTY concentration from change in absorbance over the first 30 minutes against change in absorbance in the last 10 minutes. The assay documentation did not explicitly note impacts of hemolysis on performance but noted interfering substances were possible and spiking trials could be used to evaluate robustness of the assay against such substances.

We measured concentration of uric acid (URIC) with endpoint assay (Teco Diagnostics U580) and a 2.97 mmol/L standard solution mixed from uric acid powder (Sigma G7126). We did not use the kit standard because it was too low for values commonly seen in birds (Seaman et al. 2005). We measured absorbance at 520 nm and background absorbance at 700 nm. The kit documentation did not report any potential interference from hemolysis in samples, but Cohen et al. (2007) previously reported hemolysis can interfere with URIC acid concentration estimates in avian plasma.

We were interested in quantitatively estimating the degree of hemolysis in our plasma samples, rather than subjectively scoring color variation as has been reported in some previous work (e.g., Cohen et al. 2007). Hemolysis releases contents of red blood cells, including hemoglobin (Hb), into plasma. Although plasma has low baseline concentrations of Hb (0.03 g/dL in humans; Lippi et al. 2008), we surmised that color variation indicative of hemolysis could be quantitatively expressed based on concentration of Hb. We measured Hb in plasma with an endpoint assay with the Hemoglobin Colorimetric Assay Kit from Cayman Chemical Company (700540). We predicted increases in plasma Hb concentrations would reflect increased hemolysis in samples and capture color variation noted in our samples, which was qualitatively supported by inspection of samples and Hb concentrations in our study (e.g., Figure 1).

We tested for differences in mean concentration of Hb in plasma samples between the 2 species in our study with a t-test. We tested for differences in mean Hb concentration among 5 investigators that did a majority of the collections in our study (≥30 samples each, range: 31 to 292 samples per investigator) with an Analysis of Variance (ANOVA). All investigators used the same type of equipment (e.g., needles, syringes, centrifuges, heparin) and collection methods (shooting with shotguns) and were trained in the same manner, so we should expect no inter-investigator variation. We surmised any variation in Hb concentrations among investigators may indicate sensitivity of Hb concentrations, and thus hemolysis, to handling methods that varied slightly among investigators. We tested for investigator differences with an Analysis of Covariance (ANCOVA) that included a covariate for species and used a Tukey Honest Significant Difference test for post-hoc multiple comparisons. Hb concentrations were right-skewed, so we natural log-transformed concentrations in all analyses. We plotted the relationship between each individual metabolite and log-transformed Hb concentrations to evaluate systematic biases in metabolite concentrations estimates associated with Hb. We tested for linear relationships between metabolites concentrations and log-transformed Hb with a simple linear model and interpreted slope coefficients to quantify the strength of the relationship between Hb and metabolite concentration estimates. To evaluate potential exclusion rules for Hb concentrations in plasma, we plotted the Pearson's correlation coefficient between log-transformed Hb and metabolite concentrations across a range of cutoff values from the 5th to the 95th percentile of log-transformed Hb concentrations observed in our study. We truncated the data set at each sequential cutoff value and plotted the correlations and the proportion of the sample excluded.

To evaluate the impacts of hemolysis on estimates of metabolite concentrations we conducted a spiking-recovery experiment with a random sample of 50 plasma samples distributed across a range of Hb concentrations. We sorted samples from birds collected in 2015 ($n = 104$) into 6 equally-spaced Hb concentration bins and randomly selected equal portions (or all samples in the bin in cases with low numbers) to reach a total sample size of 50. We loaded each sample into 4 wells on each plate, following the same assay procedures described above for all 4 metabolites (BUTY, GLYC, total TRIG, and URIC). We spiked 2 wells for each sample with 5 μL of dH_20 and 2 wells for each sample with 5 μL of a mid-concentration standard from the serial dilution for each assay. We used the 0.705 mmol/L standard for the TRIG and GLYC assays, 1 mmol/L standard for the BUTY assays, and 0.7425 mmol/L for the URIC assays. We re-ran all four wells for a sample in cases where spiked or non-spiked duplicates had CV >20% and diluted and reran samples when concentrations were outside the standard curve.

Our analyses of the spiking experiment sought to evaluate whether Hb concentrations interfered with the recovery of the spiked concentration in each individual sample. Therefore, we included all 4 wells from each sample in a generalized linear mixed effects model that included a random intercept term for the sample and tested for a significant interaction between log-transformed Hb concentration and a dummy variable indicating whether the sample was spiked. Using each individual well as the sample unit and an associated random intercept term allowed us to functionally test whether variation introduced by Hb was greater than intra-assay variation in sample concentration estimates. The additive spike term accounted for the predicted increase in metabolite concentration for each spiked sample and a significant interaction with Hb would indicate concentrations changed differently in response to the spiking treatment at different Hb concentrations. No support for the interaction term would therefore suggest Hb in the sample did not interfere with the recovery of the spiked concentration. We tested the significance of the spike term with ANOVA on a model without the interaction term with all other main and random effects. We conducted the analysis for each metabolite except true-TRIG, because it is a calculation from assays of total TRIG and GLYC, rather than directly measured.

RESULTS

We included 318 female blue-winged teal and 231 female lesser scaup plasma samples in our study. Hb concentrations for both species were right-skewed (Figure 2). Mean Hb concentration was 0.556 g/dL (SD = 0.732, range = 0.009 – 8.096 g/dL) for blue-winged teal and 0.731 g/dL (SD = 0.683, range = 0.069 – 4.594 g/dL) for lesser scaup. Lesser scaup had higher mean log-transformed Hb concentrations than blue-winged teal ($t = -4.973$, $P < 0.001$). There was variation in mean log-transformed Hb concentrations among the 5 primary investigators in the study ($F_{4,525} = 11.65$, $P < 0.001$; Figure 3). The multiple comparisons test revealed Investigator 2 had lower ($P \leq 0.003$) mean Hb concentrations than all but 1 other investigator ($P = 0.212$; Figure 3). Log-transformed Hb concentration was positively associated with all the metabolites but had varying magnitudes of effects (Figure 4). Listed in increasing order of slope estimates (*b* ± SE) were: BUTY (0.156 ± 0.030, $P < 0.001$), URIC (0.169 ± 0.032, $P < 0.001$), true TRIG (0.282 ± 0.044, $P < 0.001$), GLYC (0.744 ± 0.049, $P < 0.001$), and total TRIG (1.026 ± 0.057, $P < 0.001$). The correlation and cut off value analysis revealed similarly variable correlations among the metabolites and generally increasing correlations with higher Hb cut off values (Figure 5). Log-transformed Hb did not have a significant impact on the recovery of known concentrations of GLYC ($P = 0.418$), total TRIG ($P = 0.528$), or BUTY ($P = 0.427$). There was however a slight impact of Hb on recovery of URIC spikes ($P = 0.048$) and the regression coefficient on the spike-by-Hb interaction indicated increased Hb concentrations reduced recovery of the 0.743 mmol/L spike ($b = -0.024$,SE = 0.012, $t = -1.97$).

DISCUSSION

Our results provide evidence for potential biases in plasma metabolite studies resulting from unaccounted variation in hemolysis and have implications for design and analysis of plasma metabolite studies. In general, most samples had low Hb concentrations, as expected for plasma. However, there was a long-right tail on the distribution of Hb concentrations for both species, indicating substantial hemolysis had occurred in the process of collecting and preserving some samples. Our analyses of metabolite concentrations and our spike-recovery experiment suggested that, for some assays (mainly total triglycerides and glycerol), Hb can bias concentration estimates high and, for others (uric acid), Hb can interfere with detection of the metabolite in the sample, potentially biasing estimates low. Further, our analyses suggested variation in blood sampling or handling skill by individuals following the same protocol can produce variation in sample quality. Such unaccounted for variation in sampling methods and biases in metabolite concentration estimates has potential for negative impacts on inferences drawn in metabolite studies and should therefore be given full consideration in study design, training protocols, and data analyses.

Many factors during sampling and processing could have given rise to the hemolysis we measured during our study. First, we are unaware of any studies evaluating the consequences of taking blood samples postmortem. Although blood samples in our study were taken within seconds or minutes of mortality, blood was not circulating at the time of sampling, which may directly or indirectly lead to hemolyzed samples. Anecdotal accounts from personal experience of the authors sampling live and dead birds supports the notion that darker-red samples are more common from postmortem samples, though lighter shades of red in plasma samples seem ubiquitous (D. Swanson, University of South Dakota personal communication; personal observations). The occasional difficulty in extracting blood postmortem could result from the lack of circulation or exsanguination following shooting and may lead to overly aggressive blood sampling methods (e.g., too much negative pressure on the syringe plunger) that ruptures red blood cells. Evaluating potential biases associated with sampling blood postmortem may be merited in light of the increasing number of studies collecting plasma from dead birds (e.g., Tidwell 2010, Anderson and Lovvorn 2011, Anteau and Afton 2011, Casady 2013). Investigator differences in Hb concentrations we found however seem to suggest post-collection (i.e., after-shooting) methodological variation may account for a majority of hemolysis observed in our study. The investigator that had the lowest mean Hb concentrations in our study was also the most familiar with factors influencing plasma quality, suggesting thorough training and protocols and emphasizing patience could potentially guard against factors leading to hemolysis. Such factors include, the size of the needles used during blood draw; the presence of positive or negative pressure on the syringe during blood draw; the anticoagulant used (EDTA can lead to hemolysis); the stability of blood storage temperatures after collection and before centrifugation; the balance, speed, and duration of centrifugation; and incidental transfer of red-blood cells below the supernatant to the plasma vial.

Our results suggest exclusion of highly-hemolyzed samples is necessary to gain appropriate insights for all 4 metabolites we measured. The presence of Hb in the sample appeared to directly interfere with detection of uric acid based on the results of the spiking experiment, which is consistent with previous work on avian plasma (Cohen et al. 2007). BUTY, TRIG, and GLYC all tended to elevate in the response to increased Hb, either because additional molecules were released from red-blood cells after hemolysis or because interfering substances associated with hemolysis (i.e., Hb) absorbed at the same wavelengths as the reactions in the assay. This latter conclusion is consistent with the tendency for Hb to absorb most intensively between 540 and 580 nm (see figure 2 in Sonntag 1986), the range in which GLYC and TRIG (540 nm) assays read absorbance and well outside background absorbance readings (700 nm). There was a comparatively weak correlation between Hb and BUTY concentrations, which was likely because the kinetic assay, where concentration is estimated from changes in absorbance through time rather than at a single point as in endpoint assays, corrects for increased baseline absorbance in the presence of Hb (Guder 1986). Similarly, true TRIG was less correlated with Hb than GLYC and total TRIG, which may have resulted from control over baseline Hb absorbance in the GLYC determination step.

The biomedical community classifies any sample with >0.03 g/dL plasma Hb as hemolyzed (Lippi et al. 2008). In our study, only 2 samples from each species fell below this cut off, which probably suggests baseline plasma Hb concentrations in birds are higher than in humans, though we were unable to find baseline concentration estimates in the literature. The cut off analysis (Figure 5) does not seem to offer much promise in identifying a cut off value under which there is little correlation between Hb and total TRIG, true TRIG, or GLYC concentrations unless an appreciable proportion (i.e., >80%) of the data are excluded. Inspection of the plots suggests that log-transformed Hb concentrations > 0 (1 g/dL Hb) are consistently problematic for both species, so this value may serve as a suitable minimum cutoff. This criteria would exclude 11.6% of blue-winged teal and 22.1% of lesser scaup samples in our study. Discriminating highly-hemolyzed samples without the additional analytical step of quantitative Hb measurements used here may be feasible with the intensity of red coloration in the samples (Figure 1). However, this technique may need further evaluation because of the latent color variation in plasma and demonstrated inconsistencies among observers in ranking coloration of plasma (Tella et al. 1998). Further work with that directly simulates hemolysis in otherwise clean samples (e.g., Andreasen et al. 1996) would be an improvement over the correlational and spike-recovery methods we used and may provide a more concise approximation of an ideal cutoff criteria.

The high-stakes, high-pressure environment of emergency departments has long been recognized in the biomedical community as a substantial liability for the accuracy and reliability of laboratory diagnostics and ultimately patient outcomes (Lippi et al. 2011). If the conditions of the emergency department are enough to lead to poor sample quality among highly-trained professionals in biomedical disciplines, it seems the challenging conditions of field research and likely low familiarity with phlebotomy among field ecologists, may be a catalyst for poor sample quality. Therefore, we encourage investigators to give full consideration to the factors likely to impact sample quality and to consider exclusion criteria for highly hemolyzed samples. Careful blood handling techniques should include the following considerations (Lippi et al. 2008, Owen 2011): use the largest-diameter needle feasible (<21 ga. for postmortem samples); take the smallest blood volumes necessary for experiments and suitable replication; expel whole blood from syringes after removing needles; ensure blood samples are cooled and remain at a constant temperature before centrifuging; centrifuge samples as soon as feasible; ensure centrifuges are balanced and running at appropriate speeds and for appropriate durations; and ensure only plasma from the supernatant is transferred to the final vial. Although our study and review has focused on plasma metabolites, hemolysis can plague a diversity of other commonly measured substances in plasma (e.g., creatine kinase; Sonntag 1986, Lippi et al. 2008) and should therefore receive consideration across a broad portfolio of research methods employing avian blood samples.

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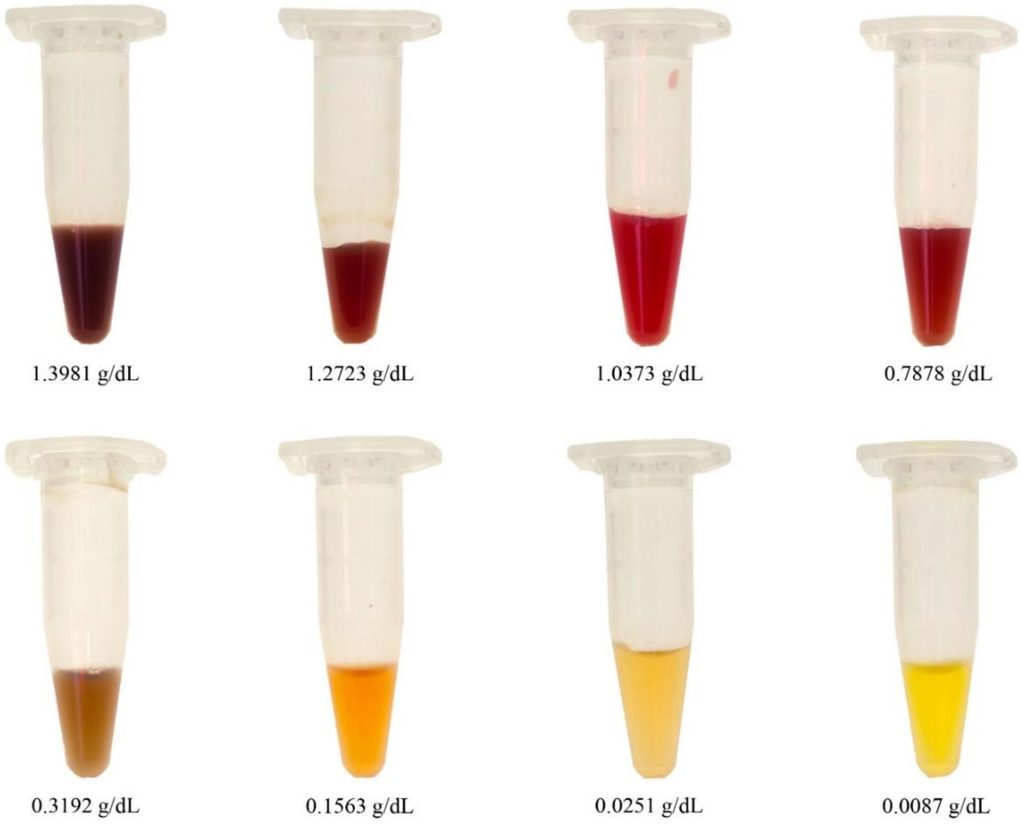
Figure 1. Observed color variation and associated hemoglobin concentrations of plasma samples taken postmortem from spring-migrating lesser scaup and blue-winged teal in eastern South Dakota.



1.3981 g/dL
1.2723 g/dL
1.0373 g/dL
0.7878 g/dL
0.3192 g/dL
0.1563 g/dL
0.0251 g/dL
0.0087 g/dL

Figure 2. Histogram of observed hemoglobin (Hb) concentrations in plasma samples taken postmortem from spring-migrating lesser scaup and blue-winged teal in eastern South Dakota.

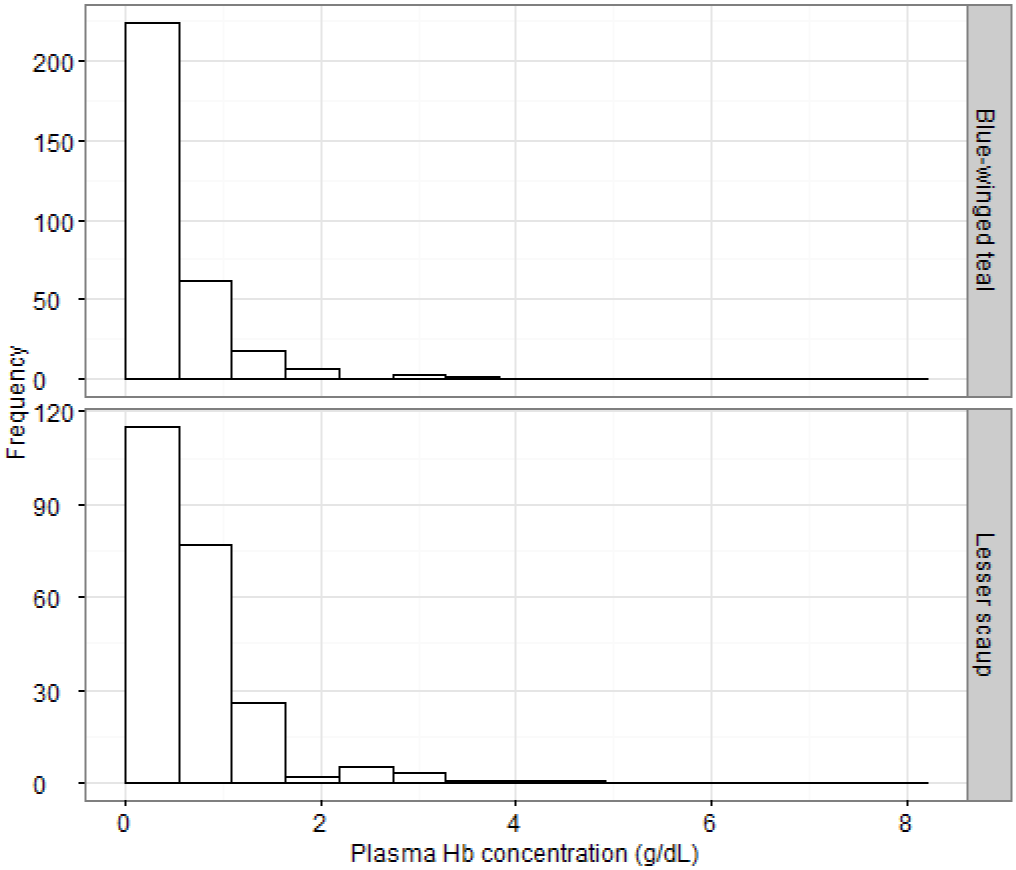


Figure 3. Least-squared means (±95% CI) of log-transformed hemoglobin (Hb) concentrations in plasma samples from spring-migrating lesser scaup and blue-winged teal collected postmortem by 5 different investigators in eastern South Dakota. Least-squared means control for species differences. Letters below each observation represent differences in a post hoc multiple comparisons test among each individual investigator pair.

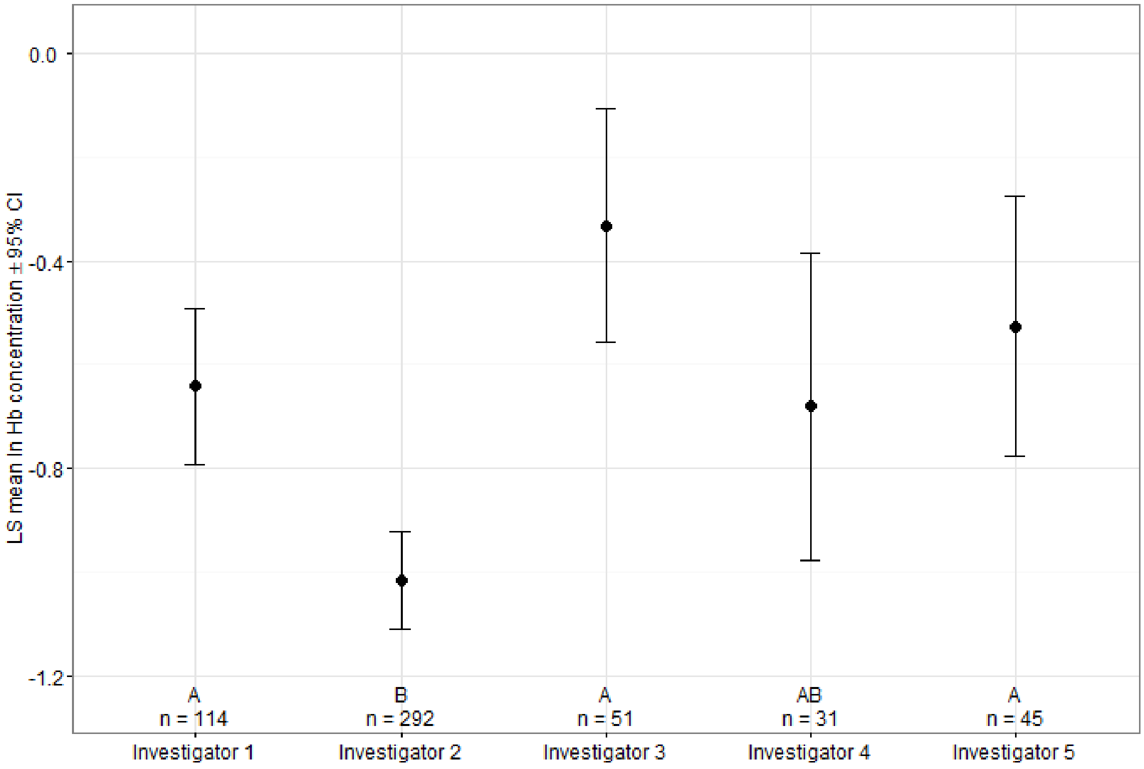


Figure 4. Scatter plots of log-transformed hemoglobin (Hb) concentrations and metabolite concentrations measured in plasma of spring-migrating lesser scaup and blue-winged teal collected in eastern South Dakota.

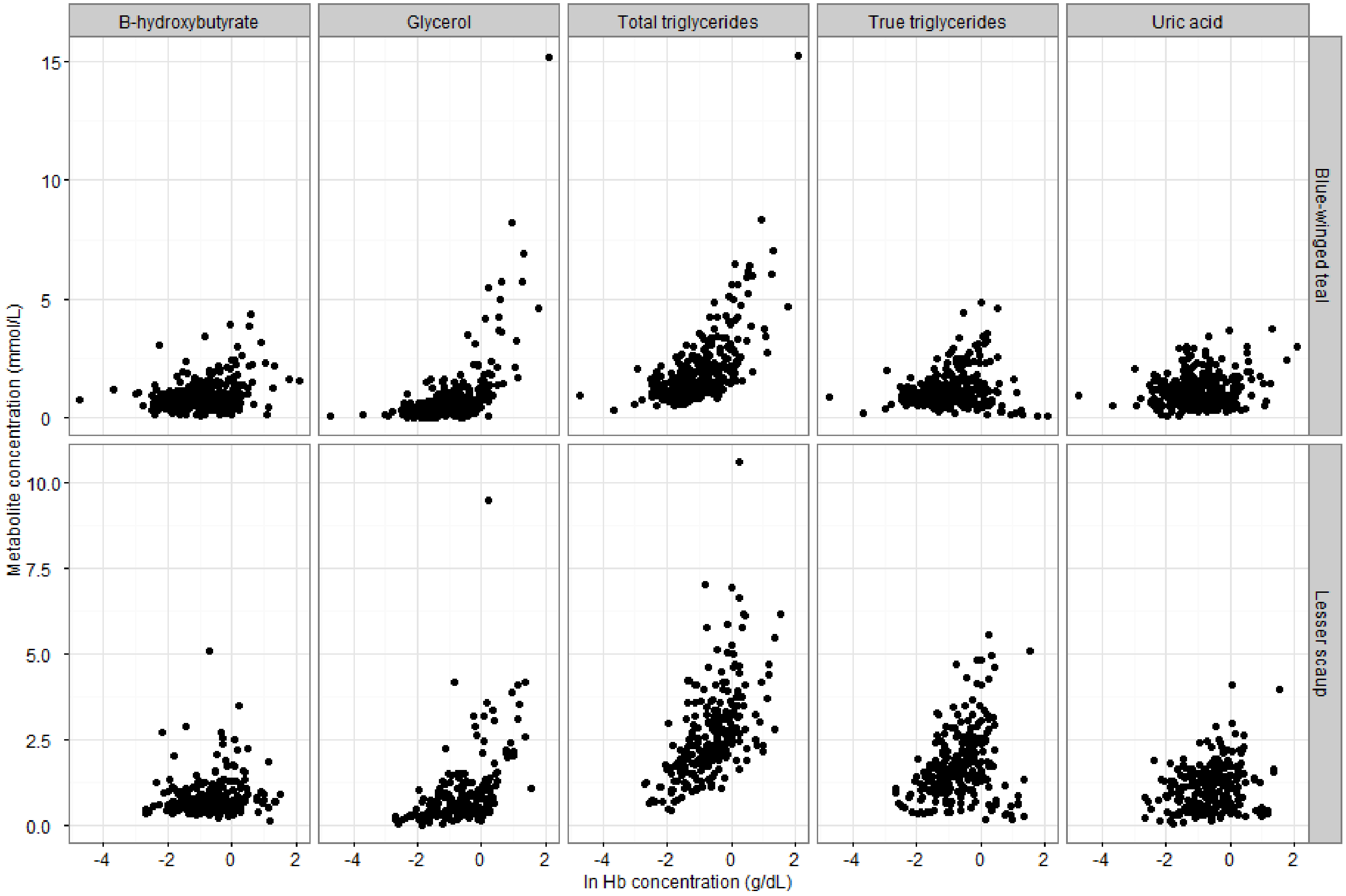
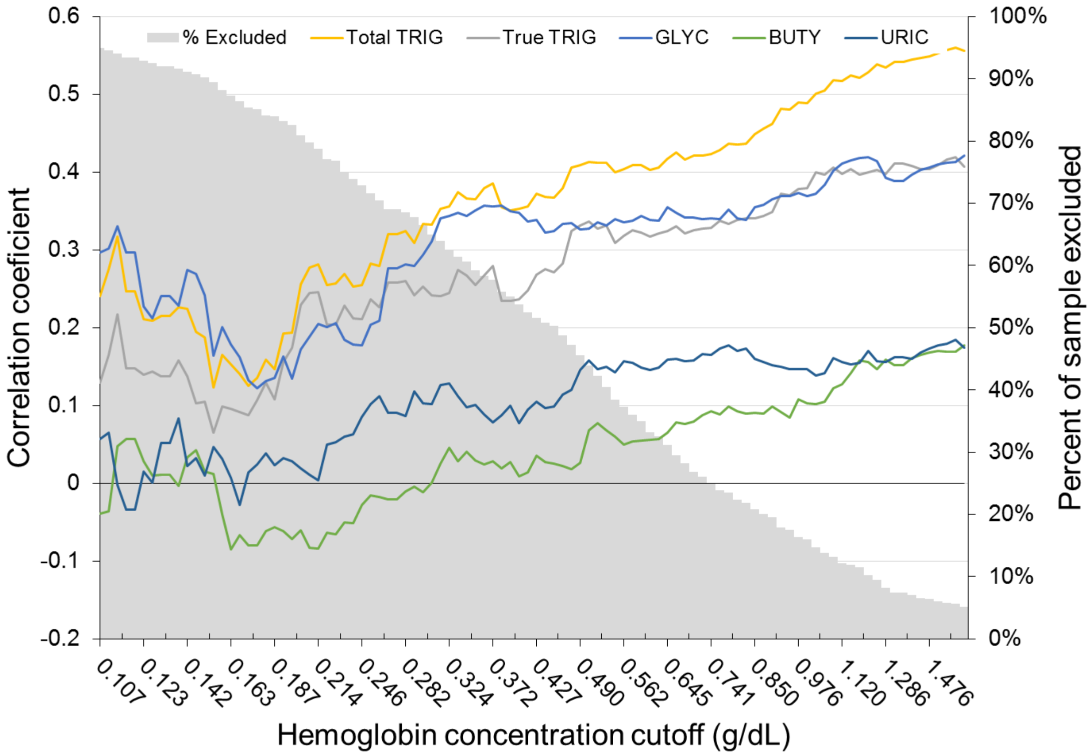


Figure 5. Pearson's correlation coefficient between log-transformed plasma hemoglobin and metabolite concentrations for samples across a range of exclusion criteria along the x-axis. The secondary y-axis corresponds to the background fill and indicates the proportion of samples excluded at each hemoglobin concentration cutoff value. Samples are pooled observations from lesser scaup and blue-winged teal plasma collected postmortem from spring-migrating females in eastern South Dakota.



CHAPTER 3: TOWARDS A GENERALIZABLE MEANS OF INTERPRETING AVIAN LIPID METABOLITE PROFILES

ABSTRACT

Concentrations of lipid metabolites circulating in plasma of birds can reflect recent lipid dynamics, which is important in the regulation of migration and subsequent breeding success across taxa and ecosystems. Two lipid metabolites with greatest potential applications – triglycerides (TRIG) and β-hydroxybutyrate (BUTY) – each individually index either lipid deposition (TRIG) or lipolysis (BUTY) and are generally used individually to interpret patterns of lipid metabolism. Here, we evaluated the potential utility of compositing both of these metabolites into a single index of refueling performance, taking advantage of the contrasting responses and partial redundancy of each metabolite. We developed a composite index from both metabolites based on previous independent research and called the resulting standardized score the refueling index. We evaluated correspondence between this refueling index and more traditional means of expressing variation in metabolite concentrations with metabolite concentrations from 17 species, compared the refueling index with 18 previously published predictive models of mass change, and evaluated the utility of the refueling index for predicting mass change in an independent field study. Our review of the literature and evaluation with independent data revealed the potential utility of the refueling index for compositing information contained in lipid metabolites and may facilitate stronger inferences into the lipid dynamics of birds across gradients of interest.

INTRODUCTION

The last 20 years have seen a surge of studies reporting concentrations of key lipid metabolites circulating in plasma as indicators of metabolic state and thus realized habitat quality for birds. Such studies are common in migration research because of the demonstrated importance of lipid metabolism for migration (Jenni and Jenni-Eiermann 1998) and cross-seasonal life-history constraints imposed by poor refueling performance among migrating birds (e.g., Drent et al. 2006, Newton 2006, Drent et al. 2007). Plasma metabolite profiles offer high potential for avian research (Jenni-Eiermann and Jenni 1998) and have been used to address a breadth of questions ranging from basic ecological adaptations of birds (e.g., Jenni-Eiermann et al. 2010, Seewagen et al. 2013) to impacts of anthropogenic modifications on migrant physiology (e.g., Cerasale and Guglielmo 2010, Anteau et al. 2011, Seewagen et al. 2011, Liu and Swanson 2014). Concomitant with the proliferation of plasma metabolite field studies has been the growth of a body of literature evaluating the potential utility, limitations, and applications of various lipid metabolites in characterizing lipid and mass dynamics in birds.

Plasma metabolite research has focused primarily on 2 key lipid metabolites – triglycerides (TRIG) and β-Hydroxybutyrate (BUTY) – to characterize lipid dynamics (Jenni-Eiermann and Jenni 1994, Cerasale and Guglielmo 2006a). Triglycerides are elevated in response to increased dietary intake or *de novo* lipogenesis and therefore concentrations circulating in plasma are indicative of an anabolic state. β-hydroxybutyrate is a ketone body that accumulates during lipid catabolism associated with fasting or exercising, and therefore is indicative of a catabolic state (Ramenofsky 1990). These two metabolites index contrasting metabolic states, and are therefore used independently or in combination to indicate the short-term trajectory of lipid metabolism in an individual or mean lipid dynamics of a sample.

Although the utility of each metabolite to indicate recent mass change is well-established (e.g., Jenni-Eiermann and Jenni 1994, Williams et al. 1999, Jenni and Schwilch 2001, Cerasale and Guglielmo 2006a, Zajac et al. 2006), an approach to combine information contained in both metabolites into a generalizable composite index of relative mass change has received little attention. Rather, most investigators report results for each metabolite separately and some studies have reported inconsistent signals in TRIG and BUTY concentrations in the same samples (e.g., Seaman et al. 2006, Lyons et al. 2008). Given their stated utility for indexing contrasting metabolic states, such contrasting results imply unexplained variation may reside in the underlying relationship between TRIG and BUTY that may help to approximate individual or study-site-level refueling performance (Schaub and Jenni 2001). Here, we developed a method to composite both metabolites into a single index of refueling performance and then evaluated its utility across a number of studies and species. Our review of the literature and preliminary evaluation of the utility of this combined index provides an initial attempt towards generalizing results of metabolite concentrations into a single index that captures information contained in both metabolites simultaneously. Such an approach offers potential utility for interpreting lipid metabolite profiles in studies of migrants or other times in the annual cycle where primarily lipid-based mass changes are common and of interest (e.g., winter fattening, egg production).

METHODS

Refueling index development

We were interested in evaluating correspondence between previously published approaches to composite information contained in each lipid metabolite. Previous work has combined information about TRIG and BUTY using 2 techniques; multiple regression models (Jenni-Eiermann and Jenni 1994, Anteau and Afton 2008) and Principal Components Analysis (PCA) of concentrations (Schaub and Jenni 2001). A PCA is calculated within each individual study based on the correlation between each individual metabolite, whereas multiple regression procedures fit models to observed mass change data and metabolite concentrations. We found 3 studies that reported using multiple regression models to predict mass changes with concentrations of BUTY and TRIG (Jenni-Eiermann and Jenni 1994, Cerasale and Guglielmo 2006a, Anteau and Afton 2008) and only 2 of these reported equations. We evaluated correspondence between model predictions from these two models and then evaluated the relative utility of multiple regression and PCA later with independent observation from metabolite concentrations from previous studies. The first predictive equation for mass change and TRIG and BUTY was published by Jenni-Eiermann and Jenni (1994) for mass change over ~6 hours in the morning for garden warblers (*Sylvia borin*);

$$\Delta mass = 0.259 + 0.120 \times TRIG - 0.350 \times \ln(BUTY + 0.5)$$

The Jenni-Eiermann and Jenni (1994) model had an r^2 value of 0.608. The second published model with both metabolites we found was from Anteau and Afton (2008) for daily mass change of lesser scaup (*Aythya affinis*);

$$\Delta mass = -54.49 + 11.82 \times TRIG - 28.65 \times \ln(BUTY)$$

The Anteau and Afton (2008) model had an r^2 value of 0.750. Direct comparisons of these two models was constrained by the different sizes of the birds (Garden Warbler = 18.2 g and Lesser Scaup 850 g; Dunning 1992) and the different time-scales over which mass change was calculated. To facilitate direct comparisons, we generated predictions from each model with 1000 random values of TRIG and BUTY concentrations generated from a mean distribution from Guglielmo et al. (2005) and z-standardized the predictions from each equation. These standardized predictions therefore weighted each metabolite proportionally to the original scale, but were on the same standardized scale of relative mass change. Thus, correspondence between these two standardized predictions would indicate high relative correspondence between the two independent predictive models. We plotted predictions for each model and reported their correlation. We then took the median standardized prediction from the 2 models and fit a new regression equation to predict median standardized scores based on the original TRIG and transformed BUTY (+0.5) concentrations. We used the natural-log plus 0.5 transformation to maintain consistency with previous studies that used the transformation to improve model fit. The resulting regression equation predicted average standardized scores from the two original equations and became the focus of the rest of our inferences here. We called the resulting prediction the refueling index because it is a relative score indicating the recent nature of mass change based on information contained in both TRIG and BUTY concentrations.

Refueling index evaluation

Comparison with other metrics—To understand the generalizability of the refueling index across a range of taxonomic groups, seasons, and study areas we used measurements of TRIG and BUTY from ongoing or completed studies (Thomas and

Swanson 2013, Liu and Swanson 2014, this dissertation) on migrating birds captured in the Upper Midwest USA. All metabolite concentrations were measured following standard protocols on a microplate spectrophotometer with commercially available reagents and standards (Guglielmo et al. 2005, Liu and Swanson 2014, Appendix 2). We included 18 data sets from 17 unique species ranging in study-specific body masses from 6.3 g (ruby-crowned kinglet *Regulus calendula*) to 716 g (lesser scaup). Our sample included a diversity of foraging guilds and habitats as well as seasonal variation between fall migrants and spring migrants, and therefore represented a wide variety of applications of metabolite concentrations. We calculated the refueling index for each individual and then evaluated similarities between the refueling index and individual metabolite concentrations and the first axis from a PCA on the correlation matrix of TRIG and BUTY. We reported the coefficient of determination (r^2) on a regression between each metric and the refueling index to quantify similarities across the range of observed metabolite concentrations in each data set.

Comparison with other models—We reviewed the literature to find and report linear regression models that predicted change in body mass with concentrations of single metabolites (TRIG or BUTY) to evaluate correspondence between our refueling index and other predicted mass change estimates. In cases where only slope estimates were provided, we estimated intercepts from figures by digitally measuring axes. We reported coefficients of determination when available or calculated them from reported test statistics. We took a random sample of 20 individuals from each data set described above for a test data set of 360 independent TRIG and BUTY concentration observations. We calculated the Pearson’s correlation coefficient between model predictions and the refueling index for each individual in this test data set. We followed procedures for transformations described in each study (e.g., ln, $\log_{10}$) and mean-centered concentrations within each data set to mimic the behavior of residuals when used. Limiting our analysis to 20 individuals from each study ensured any species-specific variation in BUTY and TRIG correlations were not overrepresented in the evaluation of mean correlation coefficients across taxa and studies. Evaluating the correlation coefficient of model predictions and the refueling index allowed us to evaluate the correspondence between the refueling index and relative predictions from independent studies of mass change and metabolite concentrations.

Comparison with independent data—We evaluated the potential utility and limitations of the refueling index for indexing changes in body mass of individuals on short (i.e., daily) time scales with independent mass change and metabolite concentrations from a field study that replicated techniques used by Anteau and Afton (2008). We measured 24-hour mass change and metabolite concentrations in plasma of hatch-year, flighted blue-winged teal (*Anas discors*) prior to onset of fall migration in north-central South Dakota during August 2014. We focused on flighted hatch-year birds to ensure individuals were not actively growing or undergoing remigial molt, which we assumed would not be representative of conditions experienced in other periods of the annual cycle (i.e., migration). We determined age following standard protocols (Carney 1992) and weighed and drew blood from individuals with fully-grown 9th primary feathers. We captured individuals in swim-in style traps baited with corn left out overnight and checked once between 0800 and 1200. We weighed and uniquely marked birds on the first capture occasion and drew blood and took repeat mass measurements on birds recaptured on subsequent days. We weighed birds to the nearest gram with a 500 g Pesola spring-scale in the field and palpated crops to ensure there was not substantial day-to-day variation in undigested food in the crop that may bias mass measurements. We prioritized recaptured individuals to minimize time between removal from the trap and sampling blood to avoid associated biases (<25 minutes; Guglielmo et al. 2002). There was a dry roosting platform and abundant food available for birds in the traps prior to removal each morning, so traps should not have induced fasting directly or stressed captured birds (until arrival of investigators).

We measured BUTY and TRIG concentrations with commercially available reagents and standards (Appendix 2). Preliminary analyses indicated regression analyses *sensu* Anteau and Afton (2008) were not predictive of raw mass change on successive days. Further exploratory analyses revealed larger individuals had lower daily mass changes than smaller individuals. Therefore, we evaluated whether metabolite concentrations or combinations thereof improved model fit on a regression of recapture mass that included an offset term for initial mass (previous day mass). We evaluated the contributions of four expressions of metabolite concentrations; concentration of TRIG and BUTY individually, the first principal component from a PCA on the correlation matrix of TRIG and BUTY (Schaub and Jenni 2001), and our refueling index. We interpreted the utility of each metric based on improvements in model fit based on regression coefficients and associated test statistics.

RESULTS

The standardized model predictions from Jenni-Eiermann and Jenni (1994) and Anteau and Afton (2008) were highly correlated (Pearson's $r = 0.999$; Figure 1). The resulting regression model to predict the median standardized score from each model, or the refueling index, was:

$$Refueling\ index = -0.162 + 2.700 \times TRIG - 8.207 \times \ln(BUTY + 0.5)$$

There was a moderately strong relationship between raw TRIG concentrations and the refueling index among the 18 data sets we included (mean r^2 = 0.335, range = 0.008-0.670; Table 1). The relationship with BUTY tended to be stronger (mean r^2 = 0.795, range 0.474 – 0.958; Table 1). The relationship with the PCA was generally strong as well (mean r^2 = 0.745; Table 1) but 3 data sets had low r^2 values (<0.2; Table 1). Inspection of the PCA results for those 3 data sets indicated TRIG and BUTY both loaded in the same direction on the first axis, whereas in all other models the terms loaded in opposite directions and had stronger correlations (r^2 mean = 0.883, range = 0.674 – 0.983).

We found 7 studies reporting a total of 18 individual models that predicted mass change over time scales ranging from hours to 7 days (Table 2). All studies reported a significant negative correlation between mass change and BUTY concentrations and one of these studies reported non-linear effects (Table 2). Predictive equations within these studies had a mean r^2 of 0.532 (Table 2). Five of the studies reported significant positive association between TRIG concentrations and mass change, while two reported insignificant associations between mass change and TRIG concentrations (Table 2). The mean r^2 value of studies reporting a significant effect of TRIG was 0.489, which dropped to 0.380 when 0 was included in for 2 models without TRIG effects (Table 2). Correlations between predictions from these models and refueling index from our diverse taxa were all positive and generally strong (mean r = 0.706, range = 0.482-0.897; Table

2). Models with BUTY tended to be more closely correlated with the refueling index (mean $r = 0.844$) than models with TRIG (mean $r = 0.529$).

We recorded 1-day mass change and collected plasma samples from 49 individuals in the recapture study. Concentration of TRIG at recapture ($b \pm SE = 5.49 \pm 2.40$, $t = 2.40$, $P = 0.026$), the PCA (3.21 ± 1.59, $t = 2.02$, $P = 0.049$), and the refueling index (1.63 ± 0.72, $t = 2.25$, $P = 0.029$) all marginally improved prediction of recapture mass from initial mass, but BUTY concentration alone did not (-11.81 ± 14.02, $t = -0.842$, $P = 0.404$).

DISCUSSION

Our literature review demonstrates the utility of TRIG and BUTY to index changes in mass of birds from single capture events, which has found wide application in field studies. However, generalizing concentration estimates of TRIG and BUTY simultaneously has received comparatively little attention, and our initial assessment here suggests this is a meaningful avenue to consider in future research. Although limited in replication in the literature, our evaluation of two independent composite indices of TRIG and BUTY revealed remarkably strong correspondence between standardized model predictions, which then had generally high correlations with predictions from other single-metabolite models across a range of study species and experimental diets. This result, coupled with results from other field and laboratory studies reviewed here, suggests generalizing the two concentrations into a single composite index of refueling performance for comparison within a study could improve understanding of the significance of metabolites and their implications for gauging refueling performance beyond inferences gained from interpreting concentrations individually.

Our review of the literature revealed substantial inter-study variation in the strength of relationships between mass change and metabolite concentrations. Some studies reported no correlation between mass change and TRIG (Seaman et al. 2005, Dietz et al. 2009) while others showed strong effects (Table 2). Similarly, within the same study, Cerasale and Guglielmo (2006a) reported a fourfold difference in slopes of TRIG and mass change among three similarly-sized passerines. Concentrations of BUTY were more consistently negatively correlated with mass change, but the strength of the relationships were variable, and in one study non-linear with functionally no correlation to mass change during mass loss (Cerasale and Guglielmo 2006a). These results, coupled with documented effects of dietary-associated variation in metabolite concentrations (Smith et al. 2007, Smith and McWilliams 2009), suggest that a universal approach to converting metabolite concentrations to mass change is unlikely. However, among studies we reviewed there were general patterns which suggested compositing lipid metabolites into a single index such as ours would aid in interpreting the likely relative trajectory of lipid metabolism of individuals with comparable diets.

Among the mass-change equations in our literature review, BUTY was more consistently (all studies) and significantly (higher r^2 values) related to mass change than TRIG. This was also the case in both of the multiple regression equations included in the refueling index, where BUTY had steeper and more significant slopes (Jenni-Eiermann and Jenni 1994, Anteau and Afton 2008). This finding contrasts with conventional recommendation to focus inferences on TRIG for indexing mass change among free-living birds (e.g., Jenni-Eiermann and Jenni 1994, Schaub and Jenni 2001, Cerasale and Guglielmo 2006b). Using TRIG alone has been advocated because it is directly involved with lipid deposition (i.e., mass gain) following dietary intake and hepatic lipogenesis (Ramenofsky 1990), whereas BUTY elevates in response to fat catabolism (i.e., mass loss) but also during transition among metabolic states (Jenni-Eiermann and Jenni 1991). Thus, TRIG is a leading indicator of transition from feeding to fasting, whereas BUTY is a lagged indicator of recent starvation or catabolism (Seaman et al. 2006). However, the consistent support for BUTY in models predicting mass change in the literature perhaps suggests its utility in field studies has been understated, or at least suggests it stands to contribute to understanding of physiological condition of birds in addition to TRIG concentrations.

Considering TRIG and BUTY in the same model seems to improve predictions of mass change consistently. Jenni-Eiermann and Jenni (1994) reported individual and combined coefficients of determination, which increased from 0.436 for TRIG alone, to 0.511 for BUTY alone, to 0.608 with both combined. Anteau and Afton (2008) only reported test statistics (t-values) for individual terms, but converting them to r^2 values (which ignores the intercept) revealed a similar increasing pattern from 0.227 for TRIG alone, to 0.492 for BUTY alone, to 0.750 for the model combining both terms and the intercept. Cerasale and Guglielmo (2006a) passingly reported that including BUTY and TRIG in the same model raised r^2 from 0.350 from individual models to 0.390 in a combined model for yellow-rumped warblers (*Setophaga coronata*). Further, some studies have found utility in compositing both metrics with the PCA approach to draw inferences from both metabolites simultaneously (Schaub and Jenni 2001). However, our analysis of concentrations from many different species and studies indicated PCA may be volatile, and in some cases misleading, when axes fail to load in the biologically meaningful opposite directions. Such failures may be the product of the either small sample sizes, inconsistencies in metabolite concentration estimates (e.g., Chapter 2), or from rapid transition to a feeding state following a fast, where both metabolites would remain high. The refueling index we have proposed would not fall victim to this data-specific constraint, because it explicitly expresses the directional effects of each metabolite on refueling performance, but otherwise generally relates closely to inferences drawn from a properly loaded PCA.

Our evaluation with independent mass change served as an initial case study to evaluate the relative utility of the refueling index against other approaches. Our field study was constrained by a number of factors, including using spring scales for mass measurements with potentially low precision and working with non-migrating hatch-year birds with access to supplemental food. Despite these potential short-comings, the evaluation showed the potential robustness of the refueling index to capture individual variation in daily mass dynamics, even when only one metabolite (TRIG) was predictive of mass change on its own. This result, coupled with the literature review above and the correspondence between individual metabolite concentrations and model predictions across many species suggests the index is consistent with inferences gained from individual metabolites. Further, the results suggest in some cases the refueling index may reveal additional variation included in the partial redundancy of both metabolite concentrations that would be missed when only examining one concentration.

The refueling index is advantageous because it conforms to the general patterns highlighted in our literature review, which showed relatively strong predictive value for BUTY concentrations and slightly reduced but still important predictive value for TRIG concentrations for mass change in a variety of species. Similarly, it could be applied without constraints of underlying correlation structure or sample size that constrain the utility of PCA. It is important to note that this model would not overcome issues with interpreting plasma metabolite profiles with interfering factors such as sampling variation (Guglielmo et al. 2002, Mandin and Vézina 2012), sample quality (Chapter 2), or dietary variation (Smith et al. 2007, Smith and McWilliams 2009). However, if investigators control for these factors through regression or study design, an improved ability to draw inferences from both metabolites together seems likely. Further research comparing the refueling index against individual metabolite concentrations for predicting mass change or for distinguishing between study sites with independent mass change observations would be useful to validate the refueling index and understand its generalizability. Similarly, important questions remain about how dietary variation may influence interpretation of lipid metabolites. If dietary shifts in metabolite concentrations are only additive (i.e., increase or decrease concentrations proportionally) the refueling index would still lead to robust differentiation of relative refueling rates. However, if dietary variation changed the ratio of TRIG to BUTY, the refueling index may over or underestimate differences in refueling performance among individuals in a study. Nevertheless, we argue the refueling index is likely to provide a better approximation of mass change trends than individual metabolites alone, and could therefore find wide application in studies of the relative trajectory of lipid metabolism of wild birds.

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Figure 1. Correspondence between z-standardized model predictions of relative mass change based on concentration of random Triglycerides and β-hydroxybutyrate concentrations from models reported by Anteau and Afton (2008) and Jenni-Eiermann and Jenni (1994). The black line is a 1-to-1 line indicating the location of perfect correspondence between z-standardized predictions in the 2 models.

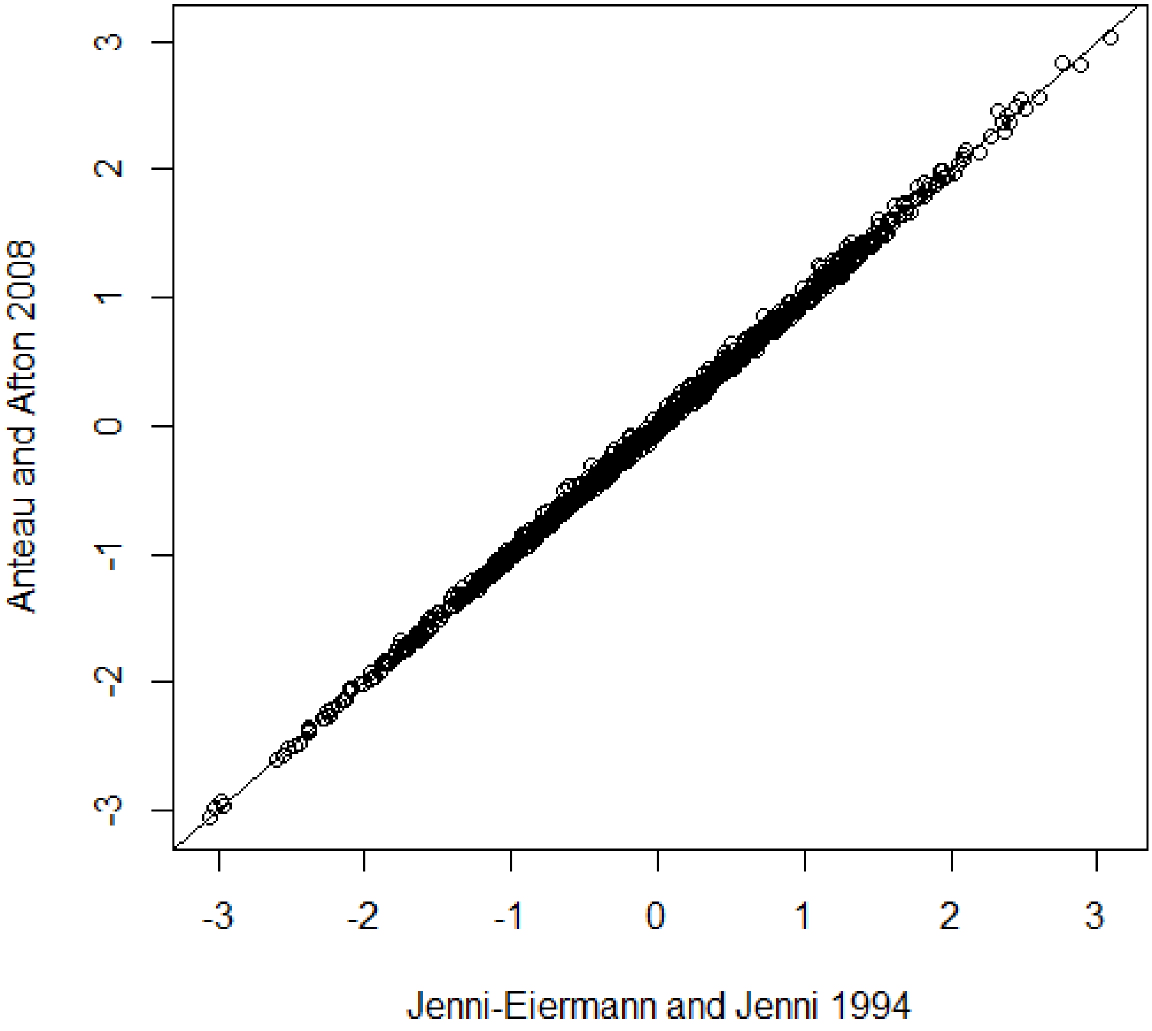

Table 1. Coefficient of determination (r^2) of regression models between the refueling index described in the text and individual triglycerides (TRIG) concentrations, β-hydroxybutyrate (BUTY) concentrations, and the first axis of a Principal Component Analysis (PCA) of the correlation matrix between TRIG and BUTY for a diversity observations from previous studies of migrating birds in the Upper Midwest U.S.

		Refueling Index		TRIG			BUTY			PCA
Study[a]	*n*	Mean	SD	Mean	SD	r^2	Mean	SD	r^2	r^2
Thomas and Swanson 2013										
Least sandpiper	67	-0.778	7.26	1.927	1.08	0.337	2.113	1.80	0.843	0.886
Semipalmated sandpiper	52	2.992	6.39	1.690	0.83	0.348	1.030	1.36	0.885	0.883
Liu and Swanson 2014, Fall										
Lincoln's sparrow	40	-2.297	5.36	0.830	0.80	0.237	1.519	1.33	0.836	0.849
Myrtle warbler	42	-3.226	4.91	0.714	0.54	0.011	1.725	1.73	0.911	0.175
Nashville warbler	41	-2.146	5.39	0.699	0.43	0.265	1.474	1.67	0.958	0.794
Orange-crowned warbler	66	-5.118	4.92	0.636	0.61	0.256	2.085	1.43	0.892	0.847
Ruby-crowned kinglet	23	-0.501	4.63	0.634	0.56	0.175	0.978	0.94	0.893	0.827

Red-eyed vireo	22	0.753	2.91	0.583	0.30	0.008	0.651	0.45	0.921	0.674
Warbling vireo	100	-0.496	3.68	0.751	0.52	0.177	0.957	0.70	0.852	0.817
Liu and Swanson 2014, Spring										
Common yellowthroat	31	2.203	4.58	1.377	0.70	0.521	0.793	0.63	0.854	0.943
Least flycatcher	40	5.902	2.80	1.316	0.65	0.426	0.265	0.26	0.592	-0.026
Swainson's thrush	47	4.921	4.05	1.689	1.08	0.605	0.489	0.37	0.474	0.973
Tennessee warbler	22	1.419	3.72	1.070	0.62	0.570	0.734	0.41	0.827	0.970
Traill's flycatcher	36	3.990	2.97	0.949	0.51	0.264	0.368	0.34	0.777	0.010
Warbling viero	28	-1.479	4.61	0.938	0.82	0.346	1.273	0.88	0.766	0.930
Yellow warbler	51	2.027	5.74	1.410	1.04	0.434	0.925	0.94	0.770	0.920
Janke spring 2012										
Blue-winged teal	48	1.212	3.29	1.505	0.74	0.379	0.966	0.57	0.626	0.957
Lesser scaup	43	3.273	4.61	2.094	1.08	0.670	0.892	0.55	0.632	0.983

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[a] least sandpiper, *Calidris minutilla*; semipalmated sandpiper, *C. pusilla*; Lincoln's sparrow, *Melospiza lincolnii*; Myrtle warbler, *Dendroica coronata*; Nashville warbler, *Oreothlypis ruficapiilla*; orange-crowned warbler, *Oreothlypis celata*; ruby-crowned kinglet, *Regulus calendula*; red-eyed vireo, *Vireo olivaceus*; warbling vireo, *Vireo gilvus*; common yellowthroat, *Geothlypis trichas*; least flycatcher, *Empidonax minimus*; Swainson's thrush, *Catharus ustulatus*; Tennessee warbler, *Oreothlypis peregrina*; Traill's flycatcher, *Empidonax alnorum*; Yellow warbler, *Setophaga petechial*; blue-winged teal, *Anas discors*; lesser scaup, *Aythya affinis*

Table 2. Predictive equations from published studies evaluating relationships between individual mass change and concentrations of triglycerides (TRIG) or β-hydroxybutyrate (BUTY) and associated correlation coefficient (*r*) with the composited refueling index described in the text.

Study and species[a]	*n*	Body mass (g)[b]	Response units	Equation	Equation r^2	*r* with Refueling Index
Jenni-Eiermann and Jenni 1994						
Garden warbler	80	18.2	g/morning	-0.082 + 0.204 x TRIG	0.436	0.586
Garden warbler	80	18.2	g/morning	0.669 - 0.495 x ln(BUTY+0.5)	0.511	0.897
Williams et al. 1999						
Western sandpiper	20	27.8	g/day	-0.05 + 7.61 x residual ln(TRIG)	0.368[c]	0.572
Western sandpiper	19	27.8	g/day	-0.30 - 1.74 x residual ln(BUTY)	0.588[c]	0.893
Jenni and Schwilch 2001						
Reed warbler	76	12.3	g/hour	0.146 + 0.116 x ln(TRIG) - 0.40272[d]	0.470	0.512
Reed warbler	77	12.3	g/hour	0.185-0.133 x ln(BUTY+0.5) - 0.0444[d]	0.490	0.897

Seaman et al. 2005[e]						
Western sandpiper - low fat diet	16	27.8	g/day	-0.1 - 0.3 x residual ln(BUTY)	0.829[c]	0.744
Western sandpiper - high fat diet	28	27.8	g/day	0.25 - 0.06 x residual ln(BUTY)	0.133[c]	0.744
Cerasale and Guglielmo 2006a						
Cedar waxwing	36	31.6	g/hour	0.14 + 1.79 x residual log10(TRIG+1)	0.720	0.482
Cedar waxwing	36	31.6	g/hour	*Non-linear regression with* BUTY	0.710	---
White-crowned sparrow	32	24.4	g/hour	0.1 + 0.92 x residual log10(TRIG+1)	0.630	0.482
White-crowned sparrow	32	24.4	g/hour	*Non-linear regression with* BUTY	0.710	---
Yellow-rumped warbler	39	11.8	g/hour	0.06 + 0.4 x residual log10(TRIG+1)	0.350	0.482
Yellow-rumped warbler	39	11.8	g/hour	0.06 - 0.43 x residual log10(BUTY+1)	0.350	0.757
Cerasale and Guglielmo 2006b						
White-crowned sparrow	45	24.4	g/hour	(log10(TRIG+1) - 0.44) x 0.43^{-1}	0.449[c]	0.587
White-crowned sparrow - high phospholipid diet	14	24.4	g/hour	(log10(BUTY+1) - 0.46) x -0.88^{-1}	0.661[c]	0.892

White-crowned sparrow - low phospholipid diet	13	24.4	g/hour	(log10(BUTY+1) - 0.43) x -0.32^{-1}	0.425[c]	0.892
Dietz et al. 2009[e]						
Red knot	202	148	g/day	-1.121 - 2.285 x ln(BUTY)	0.440	0.880

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[a] garden warbler, *Sylvia borin*; western sandpiper, *Calidris mauri*; reed warbler, *Acrocephalus scirpaceus*; cedar waxwing, *Bombycilla cedrorum*; white-crowned sparrow, *Zonotrichia leucophrys*; yellow-rumped warbler, *Setophaga coronata*

[b] Body mass estimates from Dunning (1992)

[c] r^2 values calculated from F statistics reported in citation

[d] Additional additive term is from time covariate held at constant of 6 hours as in citation

[e] Authors reported testing TRIG model and found no significant relationship

CHAPTER 4: EVALUATING THE FUNCTIONAL ROLE OF WETLANDS IN AN INTENSIVELY FARMED LANDSCAPE FOR SPRING-MIGRATING DUCKS

ABSTRACT

Understanding stressors imposed by agricultural land uses is a challenge for wildlife conservation across a breadth of ecosystems. The challenge is pervasive in temperate grassland ecosystems, where climate and rich organic soils have facilitated widespread conversion to intensive agricultural production. In the Prairie Pothole Region of central North America, an expansive agricultural footprint has grown since settlement in the late 19th century and intensified with improvements in technology and government supports. The unique coupled grassland-wetland ecosystem in the region has been altered extensively, but many wetland ecosystems remain in a matrix of intensively-farmed uplands. Here, we comprehensively evaluated contributions of those wetlands for spring-migrating ducks by studying two wetland-obligate foragers — lesser scaup (*Aythya affinis*) and blue-winged teal (*Anas discors*) — as they attempted to accumulate or maintain lipid reserves en route to their northern breeding ranges during spring migration. We measured three metrics important to these species during migration in wetlands distributed across a range of upland cultivation intensities expressed at fine and coarse scales. We found no systematic negative responses in invertebrate prey abundance, abundance of migrants, or lipid metabolism of migrant females across the upland cultivation gradient. Further, some metrics, namely abundance and physiology of blue-winged teal and some key invertebrate prey densities were higher in more intensively farmed landscapes. Collectively, these results suggested wetlands in modern intensively farmed landscapes make important contributions to spring-migrating ducks in the region,

despite the likely negative impacts of upland cultivation surrounding them. Further, our results raise important questions about the factors underlying the apparent resiliency of wetlands in intensively farmed landscapes in the region to upland cultivation that have implications for restoration and conservation in these landscapes.

INTRODUCTION

Grassland and wetland ecosystems and the organisms that depend on them are threatened globally by conversion to agriculture and other land uses. These pressures are heightened in temperate regions, where increasing global demands for food and biofuels are exerting unprecedented pressures for conversion of native ecosystems (Fargione et al. 2008, Clay et al. 2014). In many locations, these pressures are outpacing efforts to conserve biodiversity and ecosystem integrity, leading to widespread declines in populations and species extinctions (Tilman et al. 1994, Hoekstra et al. 2005). These pressures have been pervasive in the Prairie Pothole Region (PPR) of southern Canada and northern U.S., where closely-coupled wetland-grassland ecosystems have been extensively converted to agriculture, threatening migratory birds and many other organisms that depend on these ecosystems (Samson and Knopf 1994, Askins et al. 2007, Doherty et al. 2013).

The full extent of the agricultural footprint in the PPR is difficult to estimate because of pastoral land uses and the paucity of information on historical wetland function or extent. Doherty et al. (2013) reported 54% of the US portion of the PPR was converted to cropland by 2006, and recent studies have reported substantial additional grassland conversion since that time (Wright and Wimberly 2013, Johnston 2014, Reitsma et al. 2015) driven by increased commodity prices and government programs

(Claassen et al. 2011, Mehaffey et al. 2011, Rashford et al. 2011). Widespread wetland drainage and alteration in the region coincided with upland conversion, reducing the total extent of wetlands (Dahl 1990, Miller et al. 2009, Oslund et al. 2010) and changing the character of remaining wetlands (Genet and Olsen 2008, Miller et al. 2011, McCauley et al. 2015). Despite intense anthropogenic modifications to the PPR, the region remains a globally important ecosystem for migratory birds and annually hosts over half of the breeding populations of many of North America's waterfowl (Anatidae; Batt et al. 1989). This distinction has earned the region its colloquial designation as 'North America's Duck Factory' and made it a focal point for conservation and restoration of wetlands in proximity to grassland nesting habitats important for upland-nesting waterfowl (NAWMP 2012, Reynolds et al. 2006, Doherty et al. 2015).

In addition to the widely recognized importance of the PPR for breeding waterfowl, the region lies between key wintering areas, in the southern U.S. and central America, and breeding areas, in the northern contiguous U.S., Canada, and Alaska (Baldassarre 2014), and is used extensively by waterfowl during migration (e.g., Haukos et al. 2006, Gray 2010, Krementz et al. 2011, Finger 2013). Conditions experienced by migrants during spring can impact individual reproductive success and population productivity (Arzel et al. 2006, Drent et al. 2006, Newton 2006, Devries et al. 2008). Many waterfowl studies have demonstrated carry-over effects from conditions experienced on wintering areas to population reproductive success on breeding areas (e.g., Kaminski and Gluesing 1987, Raveling and Heitmeyer 1989, Drent et al. 2007, Guillemain et al. 2008), leading to an increased appreciation of the importance of non-breeding habitats for completion of the annual cycle (Sedinger and Alisauskas 2014, Alisauskas and DeVink 2015). Accordingly, the value of wetlands in the PPR for continental waterfowl production extends beyond breeding to include an important role in facilitating spring migration.

Despite this important annual function of PPR wetlands, little is known about the condition of wetlands used by spring-migrating waterfowl in the region or the impacts of agricultural intensification on these wetlands. Spring-migrating waterfowl need to accumulate or at least maintain nutrients necessary for reproduction and to fuel migration (Ankney et al. 1991, Jenni and Jenni-Eiermann 1998). All species use wetlands in some capacity, with some foraging mostly in uplands and gaining supplemental nutrition or roosting on wetlands (LaGrange 1985, Lagrange and Dinsmore 1989, Abraham et al. 2005, Pearse et al. 2011, Stafford et al. 2014), whereas others depend entirely on wetlands for invertebrate or plant diets, roosting, and courtship. Myriad factors associated with agricultural intensification, including altered hydrology (Euliss and Mushet 1996, van der Kamp et al. 1999, van der Kamp et al. 2003, Voldseth et al. 2007, McCauley et al. 2015), increased sedimentation (Gleason and Euliss 1998, Gleason et al. 2003), altered disturbance pathways (Kantrud et al. 1989), and introduction of agricultural chemicals (Donald et al. 1999, Sura et al. 2012, Main et al. 2014), have been predicted to degrade wetlands in intensively farmed regions of the PPR. These degradation pathways could thus constrain migrants and ultimately lead to delayed reproduction and reduced population productivity (Anteau and Afton 2004, Drent et al. 2007, Anteau and Afton 2011).

We sought to understand the condition of wetlands for spring-migrating waterfowl across a gradient of upland crop cultivation intensity in the southern PPR in eastern South Dakota. We focused our analyses on two sentinel species — lesser scaup (*Aythya affinis*) and blue-winged teal (*Anas discors*) — that were ubiquitous in the region, relied exclusively on wetland-based foods during migration, and used a diversity of wetland types. These differences in our study species allowed us to comprehensively evaluate the status of many wetland types, from shallow water foraging habitats of blue-winged teal, to large, deep-water foraging habitats of lesser scaup, that comprised the whole range of potential wetland foraging conditions used by waterfowl during migration in the region. We used a spatially-explicit stratified sampling strategy to sample a diversity of wetland types distributed across a gradient of upland cultivation intensity. We evaluated three key response variables across this land use gradient to evaluate the potential contributions of wetlands in different landscape contexts for spring migrating waterfowl. We first examined variability in the availability of the primary invertebrate prey of both species in likely foraging locations in wetlands across the land use gradient. Next we evaluated the numerical responses of both species to variation in upland cultivation to evaluate whether they were selecting for or avoiding wetlands with variable upland cultivation intensities. Finally, we measured the tendency for females of each species to accumulate or catabolize lipid reserves on wetlands distributed across the upland cultivation gradient based on concentrations of key lipid metabolites circulating in plasma (Jenni-Eiermann and Jenni 1994, Guglielmo et al. 2005). Measuring the physiological responses of migrating birds in response to the upland cultivation gradient yielded a more objective measure of habitat quality for migrants than coarser indices of food availability or numerical abundance alone (Van Horne 1983, Jones 2001). Collectively, these three response variables represented a comprehensive assessment of the contributions of wetlands to migrating ducks in the PPR and provide implications for conservation and restoration of wetlands in this intensely modified landscape.

METHODS

Study area selection

We evaluated the condition of a diversity of wetland types along the southern edge of the PPR. The PPR is characterized by millions of small depressional wetlands created by a rapid retreat of the Wisconsin Glacier ca. 10,000 years BP. The PPR was historically a range of tall grass prairie in the eastern and southern extents and mid- to short-grass prairie dominating with a decreasing precipitation regime to the west. We focused our analyses in the southeastern corner of the PPR in eastern South Dakota, where temperatures were comparatively mild and precipitation relatively greater than in the rest of the region (Millett et al. 2009). The eastern portion of the state was 90.5% privately owned (Doherty et al. 2013), with only 1.8% of the land owned and managed by state or wildlife federal conservation agencies. Most of the area was converted from grasslands to annual crop production (49.0%) or was managed grasslands used for grazing or hay production for livestock (40% grassland, pasture, hay; Han et al. 2012). Despite widespread crop production in the region, a number of social and ecological factors have combined to facilitate continued persistence of landscapes with relatively low crop production intensity throughout eastern South Dakota (Figure 1). Further, the region still hosts exceptionally high densities of prairie wetlands distributed across a gradient of land uses (Johnson and Higgins 1997). This gradient of land uses and associated wetland ecosystems was the focus of our study.

To facilitate sampling across this cropping intensity gradient in our study area, we developed a spatially-explicit sampling frame from which to draw fixed-area study sites with variation in upland land use. We used a 4-km radius (50 km^2 area) moving window over the 2006 National Land Cover Dataset (NLCD; Fry et al. 2011) to characterize the intensity of upland cultivation for each 30 x 30 m raster cell inside the study area. This moving-window approach allowed for a gradient-wise assessment of land use across the entire study area, rather than constraining sample site selection to fixed geopolitical boundaries. Further, the moving window size (4 km radius) represented a biologically meaningful spatial scale for our analyses because 4 km was the approximate mean minimum daily movement rate of radio-marked lesser scaup monitored during spring migration in the PPR (A.D. Afton, Louisiana State University, unpublished data). We defined upland cultivation intensity for each pixel as the ratio of pixels classified as cultivated crops to the sum of pixels defined as upland cells, which included all categories except developed lands (i.e., houses, roads, or developments), open water (i.e., large wetlands, lakes or rivers) or barren land. Therefore, the resulting classification strategy represented the proportion of lands that could have been cropped that were, based on classifications in the NLCD. The resulting raster had values ranging from 0 to 98%.

We further constrained potential candidate pixels in the study area by calculating the area of seasonal and semipermanent wetlands mapped by the National Wetlands Inventory (NWI; Wilen and Bates 1995) within the 4 km radius surrounding each cell. The NWI was based on ca. 1985 wetland imagery, and was therefore not a perfect approximation of wetland abundance for each pixel, but did provide a coarse approximation of the abundance and type of wetlands across the study region. We added 2 wetland-based constraints to our sampling frame. First, we excluded all areas that had <200 ha of seasonal or semipermanent wetlands within the 4 km radius. Excluded cells generally fell in large lakes, urban areas, or areas outside the extent of the prairie pothole landscape along the southern and eastern margins of the study area. We then stratified the remaining cells into those containing ≥200 ha of semipermanent wetlands (hereafter high wetland density) and those containing <200 ha of semipermanent wetlands (hereafter low wetland density). This constraint ensured we sampled across a range of wetland densities and crop production intensities within the study area, and that we maintained representation of wetlands suitable for spring migrating lesser scaup (i.e., large semipermanent wetlands; Anteau and Afton 2009, Kahara and Chipps 2012). The area included in the sampling frame was 46,770 km^2, approximately 51% of the area east of the Missouri River in South Dakota.

We classified the upland cultivation intensity layer into 3 strata — low (0-33.3% of uplands cropped), medium (33.3-66.6% of uplands cropped), and high (>66.6% of uplands cropped) — and then merged it with the 2 wetland density strata to yield 6 unique strata. We used a Generalized Random Tessellation Stratified (GRTS) sample to select study site centroids within each stratum. GRTS samples combine elements of simple random and systematic sampling designs to generate a spatially balanced stratified sample (Stevens and Olsen 2004). GRTS ensured sites in each stratum were distributed across the study area appropriately, rather than spatially clustered in regions with higher densities of candidate cells. For example, more candidate cells were in the low upland cultivation intensity stratum in the northwest corner of the study area than in the southern portion of the state, where annual crop production was more intensive (Figure 1). This spatial pattern in candidate sites could potentially confound with variables that follow a similar latitudinal gradient (e.g., migration physiology or chronology, geologic history of landscapes) and thereby constrain inferences from a simple random sample that would have followed a similar clustered distribution. The spatially balanced stratified sample ensured sites were distributed evenly across latent gradients that could have confounded with the upland cultivation intensity gradient that was the focus of our study. We selected 6 study area locations from the initial sampling frame and included 18 additional sites in an oversample to use in cases where drought prohibited sampling or we could not gain sufficient access to privately-owned lands. We sampled 2 sites per stratum (12 sites) during 2013 and 2014 and 1 site per stratum (6 sites) during 2015 for a total of 10 study areas in each upland cultivation intensity strata and a total of 30 study areas over the 3 years of the study. Sites from previous years were not revisited in subsequent years to allow for greater spatial replication in the study over the 3 years.

Wetland selection

We manually digitized all seasonal, semipermanent, and permanent wetlands on each study area following classification procedures described in Johnson and Higgins (1997) and Cowardin et al. (1979) to serve as a sampling frame for wetland surveys. We mapped wetlands based primarily on their extent during a relatively wet year (2010) and assigned hydrological classes based on apparent permanency of the basin between the wet year (2010) and a drier year (2012). We used National Aerial Imagery Program (NAIP) images for wetland classification, which were high resolution (1 m) true-color images acquired during later summer or early fall. We stratified wetlands into 2 categories (seasonal wetlands and semipermanent or permanent wetlands) and then randomly selected wetlands with a simple random sample weighted by the area of the basin within the study area boundary to randomly sort the wetlands for sampling prioritization. The area-based weighting strategy ensured numerically abundant small wetlands were not overrepresented in our sample. We sought permission to sample the first *n* wetlands in each category, where *n* was 10 times the proportion of the total area of each wetland category within the study site, or 2 if the proportion was <20%. We sequentially pursued permission from land owners to sample wetlands until 10 wetlands had been identified (hereafter survey basins). If we found a wetland to be dry or vegetation-choked, and therefore unusable for our study species, at the start of the season, we replaced it with the next wetland in the sample. In the rare occasion where nearly all wetlands in study areas were dry during the study year, we sampled all available wetlands in the study area. If there were < 8 wetlands with open water on the study area or if we were denied permission for >50% of the wetlands, we sampled on the next suitable study area in the stratum.

Duck surveys

We conducted two duck abundance surveys on each basin timed to coincide first with peak migration of earlier-migrating lesser scaup and then to coincide with the later peak of migrating blue-winged teal. We gauged migration progression each spring to capture early and late migration peaks and conducted survey rounds across all study areas within 11 days, progressing among study areas from south to north. The timing between successive surveys varied with the chronology of migration, averaging 10 days between surveys in the late spring of 2013 and 18 days between surveys in the warm spring of

2015. All surveys were conducted when we estimated at least 70% of the surface water of all basins in the study area were ice-free, to ensure all basins were available for foraging. Most basins were completely ice-free during surveys, particularly by the second survey round, but in cold years (i.e., 2013), peak migration of lesser scaup coincided with late ice thaws. We conducted surveys from shore or small boats following established methods for waterfowl counts on prairie wetlands (Cowardin et al. 1995, Reynolds et al. 2006). We started surveys within a study area at approximately sunrise on days without heavy precipitation that impaired visibility and with winds <30 kph. Whenever possible, we surveyed basins from vantages with high-powered spotting scopes and binoculars to not flush birds to unsurveyed basins. On basins with emergent vegetation or low-visibility, we walked in and around the basin to ensure we could see all open water (Cowardin et al. 1995). To minimize potential for double-counting individuals on near-by basins, we attempted to survey large, roosting wetlands first and minimized flushing, then surveyed smaller wetlands where birds were less likely to relocate after being flushed later in the survey day. All basins on a study area were surveyed the same day, generally before 12:00 hrs. No collections, sampling, or other study-related disturbances were conducted on the study areas 5 days prior to a survey to avoid biasing counts with disturbances.

Wetland sampling

We characterized aquatic invertebrate prey abundance for lesser scaup and blue-winged teal in likely foraging locations in each randomly selected wetland. Both species consume large quantities of invertebrates during migration (Chapter 6, Anteau and Afton 2008a, Hitchcock 2009, Tidwell et al. 2013) and on breeding grounds in spring (Swanson et al. 1974, Afton and Hier 1991); thus, we were interested in evaluating if key invertebrate prey abundances varied across the upland cultivation intensity gradient. We established 2-5 (depending on basin area) sampling transects in each wetland by extending random compass bearings from the center of the wetland. We sampled prey abundance for blue-winged teal along the margins or in shallow water at 2 locations on each transect in all wetlands. In wetlands without an open water zone or with interspersed vegetation before the transition to the open water zone, the first sampling location occurred at the first open water patch >1 m from shore and the second location was half-way from the first location to the center of the basin, or 5 m from the edge of the emergent vegetation in the open water zone. In wetlands with a defined open water zone and no emergent vegetation or a thick ring of emergent vegetation, the sampling locations were 1 and 5 m from shore or the edge of dense emergent vegetation. At each of these sampling locations, we took a 0.5 m long horizontal sweep-net sample in the top 20 cm of the water column with a D-framed sweep net. In depths <20 cm, we sampled the entire water column. We presumed 20 cm was the maximum foraging depth of blue-winged teal (Guillemain et al. 2007) and therefore invertebrates captured in the sample represented potential invertebrate prey abundance. We characterized lesser scaup invertebrate prey abundance in all semipermanent or permanent wetlands that had an open water zone >0.5 ha and ≥0.5 m deep. We used a 0.5 m long horizontal D-framed sweep-net sample along the surface of the benthos to characterize invertebrate prey availably for lesser scaup at 2 locations along each transect (<3 m deep). The first sampling location was 10 m from the edge of the wetland or emergent vegetation ring and the second location was 50 m beyond the first location. We rinsed samples in a 750 μm wash bucket in the field, uniquely labeled samples, and preserved them in 70% ethanol dyed with Rose Bengal in Whirl-Pak bags (Nasco Company, USA).

We recorded electrical conductivity with a multiparameter meter (HI 8731N, Hanna Instruments, USA) along each sampling transect to characterize the chemical characteristics of the wetlands that could influence invertebrate communities (Euliss et al. 2004). We also sampled fish communities in all wetlands. We deployed 2-5 Gee-style minnow traps overnight to characterize small-bodied fish densities and species composition in all wetlands with sufficient water depths to submerse the entrance to the traps. We set 1 experimental gill net in wetlands >0.5 m deep with an open water zone to characterize large-bodied fish species composition and relative abundance. All fish sampling on a site was done within the same 2 days on the latter-end of the sampling season.

Duck collections

We attempted to collect spring-migrating female lesser scaup and blue-winged teal on the randomly-selected wetlands within all study areas. In some cases (7% of blue-winged teal and 13% of lesser scaup collected), we opportunistically collected ducks within the study area boundaries but off the randomly selected wetlands because of logistical constraints or low abundance on the randomly selected wetlands. We were not able to collect any female lesser scaup on 6 of the study areas because of low abundance or complete absence during migration. All collections were done using shotguns with non-toxic shot (Envrion-metal, Inc., Sweet Home, OR) from shore or small boats. We attempted to focus collections on actively foraging individuals when possible and noted whether the individual was observed foraging prior to collection. We did not commence collections until ≥6 hours past sunrise to ensure individuals had an opportunity to forage within the landscape in which they were collected and to allow metabolite concentrations in plasma samples to asymptote following potential overnight fasts or migratory flights (Mandin and Vézina 2012). Immediately after collection we extracted a small (ca. 1 mL) blood sample via cardiac puncture with a heparinized syringe and 16-21 ga. needle and transferred it to 1.5 mL heparinized microcentrifuge tubes. We stored blood samples in a cooler until centrifuging in the field within 4 hours at 4,000-6,000 rpm for 5-10 minutes. We transferred plasma to a new 1.5 mL microcentrifuge tube and froze the sample at -20 C the evening of collection. Within 1 month of collection, we transferred samples to -80 C until processing.

Laboratory methods

We composited samples from benthic and surface locations in each wetland in the laboratory and rinsed them in a 500 µm sieve. We searched samples under 10X magnification dissecting scopes to enumerate and preserve key forage taxa. We focused our analyses and sampling on 3 taxa that are important prey items for spring-migrating lesser scaup and blue-winged teal (Chapter 6, Appendix 1, Anteau and Afton 2008a, Hitchcock 2009, Tidwell et al. 2013): Mollusca, which included all Gastropoda and Sphaeriidae; Amphipoda and; Chironomidae. We identified all Gastropoda to families and Amphipoda to genus (*Hyalella* spp. or *Gammarus* spp.). We calculated biomass (mg dry weight) of each taxa to convert counts to biomass to better approximate forage availability. We calculated mean individual dry mass of each Amphipoda genus and Mollusca family with a random sample of individuals from wetlands across all 3 years of the study dried to a constant mass at 60 C. Chironomidae larvae were ubiquitous in our samples and demonstrated substantial inter-wetland variation in size, likely because of the high taxonomic diversity of the family and potential variation in instar stages among wetlands. To account for this inter-wetland variability in biomass, we weighed a randomly-selected sample of 30 individuals from each wetland with ≥30 individuals. We then used the mean individual dry weight from these wetlands to convert counts from wetlands with <30 individuals to biomass.

We conducted necropsies on all birds to confirm individuals had not commenced rapid follicle growth (RFG) based on the diameter of the 3 largest ovarian follicles. We did not find any evidence for RFG in lesser scaup but did incidentally collect blue-winged teal that had ovaries indicative of RFG (mean size of 3 largest follicles > 5mm; Janke et al. 2015), which we subsequently removed from analyses. We measured concentrations of key lipid metabolites circulating in plasma to index refueling performance of the migrating females we collected. We used commercially available assays and standards to measure concentration of 2 key lipid metabolites in plasma samples — β-Hydroxybutyrate (BUTY) and triglycerides (TRIG: Appendix 2). We measured BUTY with a kinetic assay with the D-3 Hydroxybutyric Acid Assay Kit (R-Biopharm 10907979035) and β-Hydroxybutyrate Linearity Standards (Stanbio 2450). BUTY is a ketone body that elevates during lipid catabolism and remains elevated during transitions from fasting to feeding, and therefore serves as an index of mass loss or lipid catabolism (Ramenofsky 1990). We measured TRIG with two sequential endpoint assays by first measuring total free glycerol with the Free Glycerol Reagent (Sigma Catalog F6428) and then measuring total TRIG with Triglyceride Reagent (Sigma Catalog T2449). We calculated true TRIG as total TRIG minus free glycerol and used that in all analyses. TRIG indicates the opposite metabolic state as BUTY because it elevates in response to lipid accumulation associated with dietary intake and hepatic lipogenesis and is therefore indicative of improved foraging conditions. Together, these two metabolites indicate the relative trajectory of lipid metabolism over short (i.e., hours to days) time scales, which facilitates inferences specifically to the landscape where we collected the individual (Jenni-Eiermann and Jenni 1994, Williams et al. 1999, Anteau and Afton 2008b). More static indicators of physiological condition, such as size-corrected body mass or lipid reserves, respond more slowly to variation in foraging conditions, and therefore would not have been suitable for our analyses. Metabolites functionally provide a real-time assessment of the foraging conditions experienced by migrants within a small spatial scale (Guglielmo et al. 2005).

Geospatial analyses

Water surface area and availability is highly dynamic in prairie wetlands (Kahara et al. 2009, Niemuth et al. 2010), so we anticipated static wetland availability maps used for wetland sampling would not represent availability of wetlands in each study area during migration. Therefore, we acquired high-resolution (≤1.5 m) true-color (2013, 2014) or color-infrared (2015) aerial imagery from fixed-winged aircraft during May of the year of sampling (Niemuth et al. 2010). We mosaicked and geographically referenced imagery to use as a base map to identify and classify all available surface water within 2 km of the boundary of each study wetland or collection location. We classified surface water into 3 categories distinguishable in the imagery and relevant for our study species: 1) non-wetland surface water, which included all lotic water bodies (i.e., rivers, streams, open drainage ditches) and artificial surface water (e.g., stock dams, waste-water treatment facilities); 2) interspersed wetlands, which included all wetland surface water with interspersed persistent emergent vegetation or patch sizes <0.5 ha and; 3) open wetlands, which included all open water patches in wetlands or lakes >0.5 ha.

We manually digitized upland land use in each study year based on study year photographs, recent NAIP imagery, and LANDSAT-based remotely sensed land cover surfaces (i.e., NLCD or CROPSCAPE; Han et al. 2012, Homer et al. 2015). We defined uplands as all areas outside the extent of wetlands in a wet year image used for the sampling frame described previously. By focusing on upland habitats outside of wetland basins, we were able to draw similar comparisons of cropping extent among study areas with variable wet-areas during the study year. This approach would underestimate total upland area in sites where wetland area declined because of drier conditions during the study years, but was preferable because it provided a static indicator of upland cultivation intensity on each site, independent of climatic conditions during the study year. We focused upland classification on distinguishing between perennial herbaceous vegetation and cultivated crops, excluding land uses associated with developments (e.g., houses, buildings, roads), trees, or non-wetland surface water. Perennial herbaceous vegetation primarily comprised grazed grasslands but also included hay fields (including alfalfa), idle herbaceous vegetation associated with conservation practices (e.g., Conservation Reserve Program) or publicly owned lands, and idle herbaceous vegetation on wetland margins. We included alfalfa in the grassland category for logistical reasons (i.e., it was difficult to distinguish between hayed fields and alfalfa in study year photographs) and because it represents a perennial vegetation type that is likely hydrologically and structurally more comparable to grasslands than annually disturbed cultivated crops

(Voldseth et al. 2007). Land classified as crops included all fields that were cultivated in the previous growing season, including winter wheat. To characterize land-use at spatial scales beyond those captured in our study year aerial imagery, we used the 2011 National Land Cover Database (Homer et al. 2015), classified into upland and crop categories described with our study area selection procedures. We maintained consistency with the classifications in the manual digitizing by classifying upland cells as all pixels classified as shrub/scrub, herbaceous, hay/pasture, and cultivated crops, excluding developed lands, water, and tree categories and expressing upland cultivation as the ratio of cropped pixels to upland pixels.

Statistical analyses

We were interested in understanding how three key response variables we identified as most consequential for spring migrating ducks varied across the upland cultivation gradient. We used a space-for-time design to address this question, substituting substantial spatial replication for temporal replication or randomization to address our question. This space-for-time approach introduces the potential for variation in response variables that is unrelated to the land use gradient. Therefore, we employed a 2-stage analytical approach to control for latent variability in response variables among wetlands and then tested for an influence of the upland cultivation covariates across ‘average’ (i.e., controlled-for) wetland conditions. Introducing this control on the response ensured we did not find variation across the upland cultivation gradient that was related to underlying spatially autocorrelated factors, such as water permanency or fish presence that often increases in intensively farmed landscapes (e.g., Anteau et al. 2011, Wiltermuth 2014, McCauley et al. 2015) but is not a direct result of upland cultivation itself.

We used generalized linear mixed models with each individual response term and associated covariates (see below) with the *lmer* function in the *lme4* package in Program R (Bates et al. 2015). Mixed models were advantageous for our analyses because they allowed us to control for dependency in observations made in the same year and on the same study areas with random effects while retaining statistical power for main effects of covariates and the upland cultivation terms. In the first stage of each analysis, we identified the best baseline combination of covariates that we predicted *a priori* to influence comparisons of the response variable across space. We describe each individual covariate suite below for each individual response variable. We had no *a priori* predictions or interest in specific combinations of covariates, but rather were interested in finding the most parsimonious combination of covariates that controled for variability among wetlands. Therefore, we tested all possible combinations of each covariate and used the model with the lowest Akaike's Information Criteria (AIC) value as the baseline model. We then added the upland cultivation main effect term to the baseline model and evaluated the impact of the term with a parametric bootstrap with the *PBmodcomp* function in the *pbkrtest* package in R (Halekoh and Hojsgaard 2014). The parametric bootstrap procedure described by Halekoh and Hojsgaard (2014) iterated 1000 Likelihood Ratio Tests with simulated data from the baseline model and calculated a P-value from the observed test statistic in the fully constrained model with the land use term. The bootstrapping procedure is superior to a Likelihood Ratio Test on the two models alone because it explicitly accounts for variation explained by the random effect terms. We interpreted the direction and strength of the upland cultivation term when the bootstrapping procedure indicated significant support ($\alpha = 0.05$) based on the regression coefficient estimate and 95% confidence interval. We reported marginal and conditional r^2 for baseline models following Nakagawa and Schielzeth (2013) to indicate the strength of the main effects (marginal r^2) and random and main effects (conditional r^2). In cases of significant support for the land use term indicated by the parametric bootstrapping procedure, we reported improvements in conditional and marginal r^2 of the fully constrained model. We z-transformed all continuous covariates to improve model convergence and to allow for direct interpretation of the upland cultivation term (Schielzeth 2010).

We expressed upland cultivation intensity at 3 spatial scales for wetland-specific analyses (invertebrate prey abundance and duck abundance) and 2 scales for collection locations in the physiology analysis. The first scale we considered on wetlands was a 50 m buffer around the maximum wet-area extent of the wetland. This fine-scale buffer size allowed us to evaluate associations between immediately adjacent land use and our response variables without regard for larger-scale land use surrounding the wetland. We expressed upland cultivation at this scale as the proportion of the area surrounding the wetland basin that was classified as cropland in the manual digitizing. Because our inferences in the physiology analysis was on individual collection locations, rather than specific wetlands, we did not include this scale in that analysis. The second scale we considered was a 2-km radius buffer around the margins of wetlands and collection locations to characterize the local landscape in which the wetland or bird occurred. We expressed upland cultivation at this scale as the proportional area of manually-digitized cropland to upland area (perennial vegetation + cropland) within the buffer. The final scale we evaluated was a 4 km radius buffer around collection locations and wetlands. Four-kilometers is the maximum extent of mean daily movement rates reported for non-breeding ducks in recent satellite-telemetry studies (Beatty et al. 2014, Beatty et al. 2015; A. D. Afton, Louisiana State University unpublished data) and therefore served as an approximation of the relevant landscape available to a migrant during a single stopover event. Our manually digitized land cover layer only extended 2 km from basin margins, so we used NLCD cropland and upland categories at this scale to quantify the proportions of uplands that were cropped. An exploratory analysis comparing our manually digitized land cover and NLCD upland cultivation within 4 km circular buffers (50 km^2) found high correspondence ($r = 0.93$) between the two estimates, suggesting NLCD provided suitable resolution in this 4 km buffered area.

Invertebrate prey abundance – We conducted three separate analyses for invertebrate prey abundance, one each for the 3 taxa we identified as most important prey types for our study species. We converted sample biomass to densities (mg/m^2) by correcting for the mean width of the D-framed net that was submerged at the sampling location and the horizontal length of the sample (0.5 m). We natural-log transformed densities (+1) to approximate a normal distribution and improve model fit. We evaluated 8 individual covariates for inclusion in the baseline model to control for latent variability in factors potentially influencing invertebrate abundance. We included a categorical variable for basin hydroperiod (seasonal, semipermanent, or permanent) to control for potential variation in invertebrate communities among basins with variable water permanency (Kantrud et al. 1989). We also included a continuous covariate for basin depth and basin perimeter-to-area ratio, to control for variation in water permanency (indexed by depth) or the shape of the wetland and vegetation interspersion (Weller and Spatcher 1965, Fairbairn and Dinsmore 2001). We included a covariate for the square-root of conductivity of the wetland, which indexes connectivity with ground water and has substantial impacts on biotic communities of wetlands (Euliss et al. 2004, van der Kamp and Hayashi 2009). Similarly, we included three candidate covariates to account for variable climatic conditions leading up to the sampling event to account for variable water inputs or drought conditions (Euliss et al. 2004). We used the Standardized Precipitation and Evapotranspiration Index (SPEI) to provide a spatially explicit approximation of climatic conditions in the time leading up to sampling (Vicente-Serrano et al. 2013). We calculated SPEI values over 6, 12, and 72 month time horizons with temperature and precipitation observations from the Parameter-elevation Regressions on Independent Slopes Model (PRISM Climate Group, Oregon State University) for each wetland basin (Post van der Burg et al. 2016). We included 2 covariates to account for the influence of fish on aquatic invertebrate communities and densities in prairie wetlands (e.g., Zimmer et al. 2000, Anteau et al. 2011, Hanson et al. 2012, Maurer et al. 2014). The first fish covariate was a binomial term to indicate the presence or absence of fish in the wetlands detected with either minnow traps or the gill nets. The second fish covariate was a quadratic term for fathead minnow (*Pimephales promelas*) catch per unit effort (CPUE; mean captures per day, corrected for trapping duration and number of traps), which have been previously shown to influence prey abundance for lesser scaup in the study region (Anteau et al. 2011) and have trophic impacts on wetland biotic communities (Zimmer et al. 2002).

Duck abundance – We used total counts of lesser scaup and blue-winged teal on each basin across the 2 survey periods as the response variable in abundance analyses. Lesser scaup were most abundant during the first survey and blue-winged teal were most abundant on the second survey. However, combining counts across both periods allows for annual variation in the attenuation of migration and should have more appropriately quantified the cumulative use of wetlands during migration. We natural log-transformed total counts (+1) to improve model fit. We constrained the lesser scaup analysis to basins >0.5 m deep and with an open water zone >0.5 ha to focus inferences on potentially suitable habitats (Anteau and Afton 2009, Kahara and Chipps 2012). We evaluated three individual covariates for inclusion in baseline models to account for basin size and shape that we predicted could influence duck abundance. The covariates were basin depth, $\log_{10}$ transformed basin-to-perimeter ratio of the survey wetland, and $\log_{10}$ transformed basin-to-perimeter ratio of all wetlands within a 2 km radius buffer of the wetland margin. The latter covariate accounted for variation in potential foraging habitat surrounding the wetland that could influence the distribution and abundance of migrating ducks (Fairbairn and Dinsmore 2001, Webb et al. 2010).

Duck physiology – We composited TRIG and BUTY into a single index (hereafter refueling index) following Chapter 3. The refueling index is a relative account of the trajectory of lipid accumulation or catabolism predicted from the relationship between TRIG and BUTY. Lower scores indicated reduced refueling performance, and by extension may be suggestive of reduced refueling conditions in wetlands. Before compositing the metabolite concentrations, we conducted an exploratory analysis to ensure concentrations did not vary systematically with time from sunrise or time until sunset, a relative date of collection within the migration season, year, pair status or foraging status of the individual upon collection (Guglielmo et al. 2002, Mandin and Vézina 2012). We did not find any systematic variation in these factors so we used raw metabolite concentrations to calculate the index. We excluded highly-hemolyzed samples with plasma hemoglobin concentrations >1 g/dL to avoid associated biases (Chapter 2). We evaluated a covariate for the basin-to-perimeter ratio of the wetland on which the bird was collected (square-root transformed) and the basin-to-perimeter ratio ($\log_{10}$ transformed) of available wetlands within 2 km radius buffer of the collection location to control for wetland and landscape-scale variation in potential foraging habitats that may influence refueling performance (Chapter 5).

RESULTS

Wetland sampling

We sampled 305 wetlands on 30 unique study areas over 3 years. Most wetlands had semipermanent hydroperiods (65%), 30% had seasonal hydroperiods, and 5% were large permanent water bodies (i.e., lakes) generally managed for recreational fisheries. Only 3.6% of wetlands ($n = 11$) were cultivated in the previous growing season as indicated by the absence of persistent emergent vegetation or presence of crop residue in the basin. All of the basins that were cultivated in the previous growing season were classified as having seasonal hydroperiods. Most wetlands were entirely privately owned (86%), 3% were entirely on lands managed by the U.S. Fish and Wildlife Service or South Dakota Department of Game, Fish, and Parks, and 11% were mixed public-private ownership. The upland cultivation terms for the 3 spatial scales considered around wetlands generally ranged from 0 to 100% (Figure 2). Upland cultivation was right-skewed at the 50 m scale because many wetlands had small perennial vegetation buffers immediately adjacent to them even when in otherwise intensively farmed landscapes (Figure 2).

Invertebrate prey abundance

We included 304 wetlands in the analysis on invertebrate prey abundance in surface water foraging habitats of blue-winged teal. The baseline model for Mollusca densities included the binomial term for fish presence or absence, $\log_{10}$ transformed basin perimeter-to-area ratio, and both SPEI terms and explained 63% (conditional r^2) of the variation in Mollusca densities (Table 1). The upland cultivation terms did not have significant additional support over the baseline model at any spatial scale ($P \geq 0.277$; Table 1). The baseline model for Amphipoda densities included terms for the hydrologic regime and maximum depth of the wetland, and the quadratic fathead minnow CPUE term. The baseline model explained 28% of the variation in Amphipoda densities (Table 1). There were no associations between Amphipoda densities and upland cultivation at any of the spatial scales ($P \geq 0.053$; Table 1). The baseline model for Chironomidae densities included the hydrologic regime categorical covariate and the 6-month SPEI term and explained 42% of the variation (Table 1). Chironomidae densities did not vary with upland cultivation at the 50 m or 2 km scales, but were significantly greater in wetlands with higher upland cultivation intensity in the 4 km buffer ($P = 0.032$; $b \pm$ SE, 0.164 ± 0.069; Table 1).

We included 128 wetlands in the analysis of invertebrate prey abundance along the benthos in open-water foraging habitats for lesser scaup. The baseline model for Mollusca densities included a term for $\log_{10}$ transformed basin perimeter-to-area ratio and explained 37% of the variation in Mollusca densities (Table 1). There was no support for the upland cultivation terms influencing Mollusca densities ($P \geq 0.276$; Table 1). The baseline model for Amphipoda densities included the 6 month SPEI term and explained 13% of the variation (Table 1). There was evidence for higher Amphipoda densities in wetlands with more upland cultivation within the 50 m band surrounding the wetland ($P = 0.013$; 0.346 ± 0.141; Table 1) but no associations at the other 2 scales ($P \geq 0.064$; Table 1). The baseline model for Chironomidae densities included the categorical term for hydroperiod and the 6-month SPEI term. The model explained 36% of the variability in Chironomidae densities (Table 1). There was no association between Chironomidae densities and upland cultivation at any of the scales ($P \geq 0.083$; Table 1).

Duck abundance

We included 304 wetlands in the abundance analysis for blue-winged teal. The best baseline model included a term for $\log_{10}$ transformed basin perimeter-to-area ratio and explained 33% of the variation in abundance (Table 2). There was a positive association between blue-winged teal abundance and upland cultivation at the 50 m scale ($P = 0.041$; 0.174 ± 0.077; Table 2) and the 2 km scale ($P = 0.013$; 0.224 ± 0.092; Table 2). Abundance and upland cultivation were not significantly related in the 4 km scale ($P =0.051$). We included 128 semipermanent or permanent wetlands with an open water zone and maximum depth ≥0.5 m in the lesser scaup abundance analysis. The baseline model included a term for $\log_{10}$ transformed basin perimeter-to-area ratio and explained 52% of the variation in abundance (Table 2). There was no support for inclusion of any of the upland cultivation terms ($P \geq 0.926$; Table 2).

Duck physiology

We included 233 female blue-winged teal collected on 125 unique wetland basins in the physiology analysis. The baseline model included a term for the perimeter-to-area ratio of the basin on which the individual was collected and explained 27% of the variability in the refueling index (Table 3). There were no significant associations between the refueling index and upland cultivation at the 2 km scale ($P = 0.061$; Table 3) but there was a positive association with upland cultivation in the 4 km scale ($P = 0.010$; 0.739 ± 0.271; Table 3). We included 137 female lesser scaup collected on 62 unique wetland basins in the lesser scaup physiology analysis. The baseline model included a term for the perimeter-to-area ratio of the basin on which the individual was collected and explained 15% of the variability in refueling index (Table 3). There was no evidence for any association between the refueling index and upland cultivation in either of the 2 spatial scales ($P \geq 0.426$; Table 3).

DISCUSSION

Our study was the first intensive attempt to understand contributions of wetlands in intensively-farmed landscapes to wetland-foraging waterfowl during spring migration in the PPR, an otherwise intensively studied region of global significance for waterfowl production. Results of our comprehensive assessment suggested upland cultivation around wetlands in the region was not associated with any detectable decreases in forage availability, duck abundance, or physiological performance and, in some cases, was associated with increases in the key metrics we monitored over those in less intensively-farmed landscapes. Across all metrics we monitored on a wide-spectrum of wetland types used during migration, we observed few significant associations with upland cultivation and among those metrics in which we found significant variation, the direction of the effects were always positive. These results imply that ‘average’ wetlands in modern intensively-farmed landscapes in eastern South Dakota currently confer at least comparable or perhaps better migration habitats for wetland-foraging ducks en route to northern breeding ranges during spring. This finding has implications for understanding the potential for agricultural landscapes to play a relevant role in migrations of wetland-foraging waterfowl and raises questions about the underlying mechanisms giving rise to observed similarities of wetlands across the wide range of upland land uses we examined.

The apparently equivocal association between upland cultivation and our metrics seem to contrast with the implicit assumption that factors associated with upland cultivation such as increased sedimentation (Martin and Hartman 1987, Gleason and Euliss 1998), nutrient enrichment (Neely and Baker 1989), hydrologic alterations (van der Kamp et al. 2003, Voldseth et al. 2007), or pesticide inputs (Grue et al. 1986, Main et al. 2014) would manifest in measurable impairments. Extensive sampling in a diversity of lotic ecosystems has clearly linked agricultural land use practices to biotic impairments in those systems (e.g., Allan et al. 1997, Genito et al. 2002, Allan 2004) and some work even in the PPR has shown negative consequences of crop production on microfauna in ephemeral wetlands (Euliss and Mushet 1999) or in controlled experiments (e.g., Dieter et al. 1996). However, other than the well-established negative association between breeding waterfowl densities and upland cultivation (e.g., Austin et al. 2001), studies have consistently shown equivocal relationships between intensive agricultural land use and biotic communities in wetlands in the PPR (Guntenspergen et al. 2002, Tangen et al. 2003, Hanson et al. 2012). Further, the tendency for apparent resiliency of wetland-invertebrates to anthropogenic disturbances extends beyond prairie wetlands and is characteristic of aquatic invertebrate communities in a diversity of wetland ecosystems globally (Batzer 2013).

Considering the underlying mechanisms that gave rise to similar wetland function across the upland cultivation gradient is useful for understanding the true consequences of upland cultivation on prairie wetlands. First, it is important to recall the question we asked in this research, which was what are the current contributions of wetlands in intensively farmed landscapes, relative to those observed in modern landscapes that are not intensively farmed? This question does not however address a more salient question, which is if we were to restore grasslands to all the cultivated uplands in this system, what response would we expect to see in wetlands we sampled? Understanding the mechanisms giving rise to the similar condition of wetlands across the upland cultivation gradient is necessary to answer the latter question and has substantial implications for understanding these wetlands and evaluating potential management and conservation strategies in this landscape. Here we explore two potential hypotheses to explain the similarities we observed and discuss their respective implications.

The first hypothesis to explain the relatively similar wetland conditions across the upland cultivation gradient is that wetlands in this system were inherently resilient to the presumed pathways for degradation in response to upland cultivation. The dynamic nature of wetland ecosystems has conceivably favored evolution of life-history traits among wetland-dependent organisms that are tolerant of a wide range of ecological perturbations (Batzer 2013). Therefore, although agricultural land use practices change nutrient balances, sedimentation patterns, or introduce pesticides, these disturbances may simply mimic historical processes with which wetland invertebrates and waterfowl evolved, leading to no detectable negative impacts of upland cultivation. Further, the positive responses in some invertebrate taxa we observed that were reflected in increased abundance, and to a lesser extent improved physiology, of blue-winged teal could result from enrichment of wetlands conferred by upland cultivation. Increased nutrient inputs associated with crop production have been shown to impact invertebrate communities in prairie wetlands (Hann and Goldsborough 1997), which could benefit blue-winged teal. Similarly, conversion of upland catchments from perennial vegetation to cropland alters water-level dynamics in prairie wetlands (Euliss and Mushet 1996, van der Kamp et al. 1999), which could facilitate increased sediment oxidation and improve potential productivity of wetlands in intensively farmed landscapes. Finally, climate-driven variation in water levels of prairie wetlands (e.g., Niemuth et al. 2010) facilitates frequent disturbances by land owners seeking to farm or hay margins of dry wetlands. These disturbances, namely burning, disking, or mowing, have been shown to positively impact invertebrate production in managed wetland ecosystems throughout North America (e.g., Murkin et al. 1982, de Szalay and Resh 1997, Gray et al. 1999, Davis and Bidwell 2008), and arguably could have comparable, albeit unintended, positive impacts on wetlands in agricultural landscapes (Davis and Bidwell 2008).

The second hypothesis to explain observed similarities in wetland quality for migrating ducks across the upland cultivation gradient is that wetlands in intensively farmed landscapes have been degraded due to negative agricultural impacts discussed above (e.g., sedimentation, nutrient enrichment, pesticide inputs), but that degradation has only lowered the productivity of the wetlands to the level seen in modern unfarmed wetlands. This pattern could result because upland crop production is not a randomly applied ‘treatment’; rather, constraints in soil productivity, climatic conditions, and economic factors interact to drive conversion of grasslands to crop production (Stephens et al. 2008, Rashford et al. 2011) and land-retirement programs prioritize parcels with less crop production potential (Claassen et al. 2008). In South Dakota, lands with the fewest constraints to crop production (e.g., steep slopes or poor drainage) are farmed most intensively and those with the most constraints are generally not cropped (Reitsma et al. 2015). Arguably, the same factors driving the distribution of upland cultivation could drive productivity potential of wetlands. If this were true, we might find improved wetland quality metrics in landscapes where there is currently more upland cultivation, if the negative constraints attributed to upland cultivation were to be removed. Therefore, the similarities we observed across the upland cultivation gradient may actually be a signal of degradation that occurred in these landscapes. If this hypothesis is true, it suggests there is potential to achieve improved wetland ecosystem function in modern, intensively farmed landscapes if the most salient impacts of upland cultivation were removed.

It is important to note that the statistical control we used in our analyses (i.e., wetland size, depth, recent climate, and fish communities) could have masked impacts of factors that reduce wetland function in intensively farmed landscapes but are not directly associated with upland cultivation. First, we only sampled extant wetlands in these landscapes because drained or filled wetlands were by definition unavailable to ducks. A number of studies in the PPR and anecdotal accounts on our study areas revealed that drained wetlands are more common in intensively farmed landscapes (Miller et al. 2009, Oslund et al. 2010). Furthermore, consolidation of wetlands through surface or subsurface drainage is a common practice in intensively farmed landscapes (Turner et al. 1987, Bartzen et al. 2010) and leads to reduced availability of small, seasonal wetlands and increased prevalence of large wetlands with more permanent hydroperiods (Miller et al. 2011, Anteau 2012, Wiltermuth 2014, McCauley et al. 2015, Vanderhoof and Alexander 2015). The attendant increase in water permanency and artificially inflated connectivity, which is naturally low among prairie wetlands (Leibowitz and Vining 2003), can lead to increased colonization and persistence of fish, which have cascading and well-documented impacts on wetland function for waterfowl (Chapter 5; Bouffard and Hanson 1997, Zimmer et al. 2002, Hanson et al. 2005, Anteau et al. 2011, Maurer et al. 2014). Studies in prairie wetlands have demonstrated within-wetland factors, such as fish or submersed macrophyte communities, have more detectable impacts on invertebrate communities than watershed factors, such as upland cultivation (Tangen et al. 2003, Anteau et al. 2011, Hanson et al. 2012). We have shown that those within-wetland factors manifest in physiological impacts for spring migrating waterfowl (Chapter 5). Therefore, although we show that wetlands in a matrix of extensive upland cultivation have no detectable differences for spring-migrating waterfowl, subsurface and surface drainage practices associated with intensive upland cultivation can alter the ecology of remaining wetland ecosystems in intensively farmed landscapes (Genet and Olsen 2008, Blann et al. 2009, Miller et al. 2011, Wiltermuth 2014, McCauley et al. 2015). Accordingly, our study aids in framing the appropriate focus for restoration or conservation of wetlands in agricultural landscapes. Our results suggest looking beyond upland cultivation to factors that systematically alter the hydrology, and by extension biotic communities, of prairie wetlands should be the principal focus for restoration or management in agricultural landscapes.

Regardless of the underlying mechanisms giving rise to the observed consistency in wetland function across the upland cultivation gradient, our results illustrate potential for remaining prairie wetlands in modern agricultural landscapes to confer refueling benefits for spring-migrating waterfowl en route to northern breeding ranges. Wetlands in intensively farmed prairie landscapes have historically not received attention in conservation efforts because of demonstrated degradation of more ephemeral wetlands (Euliss and Mushet 1999) and a focus on landscapes with high perennial cover for upland nesting birds (Reynolds et al. 2006). Our results suggest wetlands in intensively farmed landscapes, which comprise a large area of the southern PPR, perhaps confer novel habitats for spring-migrating waterfowl. Further, because of the importance of aquatic invertebrates for breeding waterfowl (Swanson et al. 1974, Swanson et al. 1985, Cox et al. 1998), our results suggest wetlands in agricultural landscapes may provide comparable potential for breeding ducks if constraints on upland nesting cover or nest success in those landscapes are understood and addressed (Higgins 1977, Cowardin et al. 1985, Stephens et al. 2005). Additional research in the region could seek to understand the full potential of wetlands in intensively farmed landscapes and understand the underlying mechanisms giving rise to comparable wetland function for migrant ducks across the wide range of upland cultivation intensities in our study. Understanding these mechanisms will improve ecological understanding of wetlands, but more importantly provide a better picture of the restoration and conservation potential of remaining wetlands in the intensively farmed regions of this globally important agricultural and wetland ecosystem.

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Table 1. Model summaries and parametric bootstrapping results comparing baseline models of key blue-winged teal and lesser scaup invertebrate prey densities with more constrained models including covariates for upland cultivation in 50m, 2 km, or 4 km buffers surrounding wetlands sampled in eastern South Dakota during April and May 2013 through 2015. P-values are based on a parametric bootstrap comparing the less constrained baseline-model with each individual land use term. Regression coefficients and associated standard error and t-statistics are shown for each individual land use term.

Species/ Response variable	Marginal r^2	Conditional r^2	P	b	SE	t
Blue-winged teal						
Mollusca density						
Baseline model	0.175	0.627	---	---	---	---
50 m buffer	0.175	0.626	0.942	-0.001	0.119	-0.008
2 km buffer	0.174	0.628	1.000	-0.022	0.168	-0.132
4 km buffer	0.179	0.626	0.277	0.208	0.186	1.119
Amphipoda density						
Baseline model	0.184	0.281	---	---	---	---
50 m buffer	0.190	0.295	0.053	0.097	0.048	2.003

2 km buffer	0.185	0.288	0.384	0.054	0.055	0.985
4 km buffer	0.186	0.284	0.396	0.045	0.057	0.797
Chironomidae density						
Baseline model	0.318	0.429	---	---	---	---
50 m buffer	0.321	0.429	0.165	0.078	0.058	1.346
2 km buffer	0.329	0.436	0.137	0.109	0.069	1.573
4 km buffer	0.337	0.425	**0.032**	**0.164**	**0.069**	**2.364**
Lesser scaup						
Mollusca density						
Baseline model	0.059	0.370	---	---	---	---
50 m buffer	0.071	0.395	0.276	-0.258	0.225	-1.148
2 km buffer	0.074	0.414	0.431	-0.259	0.309	-0.838
4 km buffer	0.060	0.380	0.959	0.014	0.325	0.042
Amphipoda density						
Baseline model	0.076	0.135	---	---	---	---

50 m buffer	0.115	0.134	**0.013**	**0.346**	**0.141**	**2.459**
2 km buffer	0.077	0.142	0.723	0.064	0.158	0.406
4 km buffer	0.075	0.141	1.000	-0.015	0.160	-0.095
Chironomidae density						
Baseline model	0.174	0.363	---	---	---	---
50 m buffer	0.174	0.370	0.793	-0.051	0.142	-0.363
2 km buffer	0.202	0.381	0.083	0.276	0.158	1.751
4 km buffer	0.185	0.372	0.313	0.173	0.162	1.070

Table 2. Model summaries and parametric bootstrapping results comparing baseline models of blue-winged teal and lesser scaup abundance with more constrained models including covariates for upland cultivation in 50 m, 2 km, or 4 km buffers surrounding wetlands surveyed in eastern South Dakota during April and May 2013 through 2015. P-values are based on a parametric bootstrap comparing the less constrained baseline-model with each individual land use term. Regression coefficients and associated standard error and t-statistics are shown for each individual land use term.

Species	Marginal r^2	Conditional r^2	*P*	*b*	SE	*t*
Blue-winged teal						
Baseline model	0.239	0.334	---	---	---	---
50 m buffer	0.253	0.338	**0.041**	**0.174**	**0.077**	**2.249**
2 km buffer	0.265	0.343	**0.013**	**0.224**	**0.092**	**2.428**
4 km buffer	0.260	0.349	0.051	0.205	0.100	2.059
Lesser scaup						
Baseline model	0.451	0.516	---	---	---	---
50 m buffer	0.449	0.516	0.966	-0.008	0.128	-0.059
2 km buffer	0.449	0.517	0.926	0.016	0.133	0.119

4 km buffer	0.449	0.518	1.000	0.004	0.133	0.027

Table 3. Model summaries and parametric bootstrapping results comparing baseline models of blue-winged teal and lesser scaup refueling performance with more constrained models including covariates for upland cultivation in 2 or 4 km circular buffers surrounding collection locations. Refueling performance was measured with concentrations of key lipid metabolites circulating in plasma of migrating females of each species in eastern South Dakota during April and May 2013 through 2015. P-values are based on a parametric bootstrap comparing the less constrained baseline-model with each individual land use term. Regression coefficients and associated standard error and t-statistics are shown for each individual land use term.

Species	Marginal r^2	Conditional r^2	*P*	*b*	SE	*t*
Blue-winged teal						
Baseline	0.025	0.274	---	---	---	---
2 km buffer	0.044	0.269	0.061	0.502	0.257	1.950
4 km buffer	0.066	0.282	**0.010**	**0.739**	**0.271**	**2.723**
Lesser scaup						
Baseline	0.053	0.153	---	---	---	---
2 km buffer	0.053	0.174	0.767	0.173	0.367	0.470
4 km buffer	0.061	0.201	0.426	0.401	0.387	1.035

Figure 1. Location of 30 randomly selected study areas sampled during spring migration (April – May) during 2013 to 2015 across a gradient of upland cultivation intensity in eastern South Dakota. The inset map shows the location of the Prairie Pothole Region (PPR; shaded region) and the location of eastern South Dakota in North America (black box). Areas outside the landscape gradient in eastern South Dakota were excluded from the sampling frame because they have too few wetlands or laid outside the traditional extent of the PPR in the state.

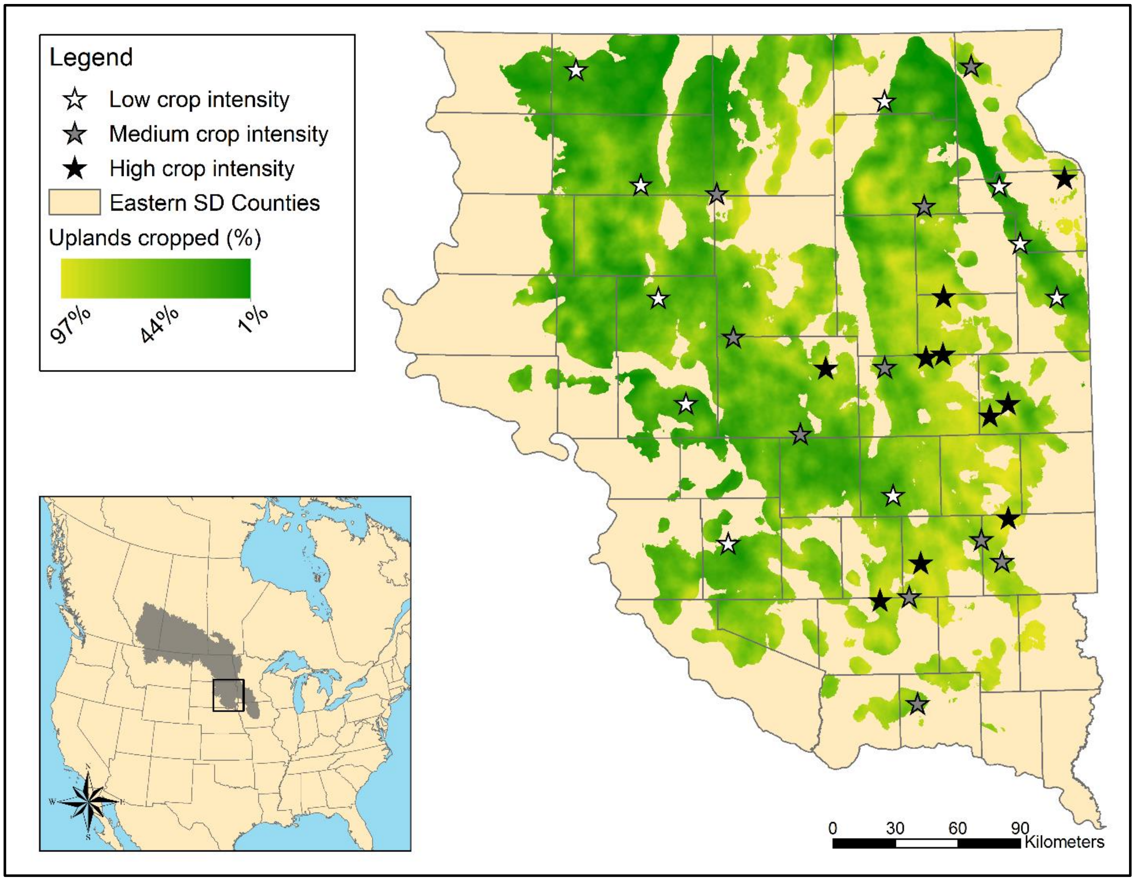
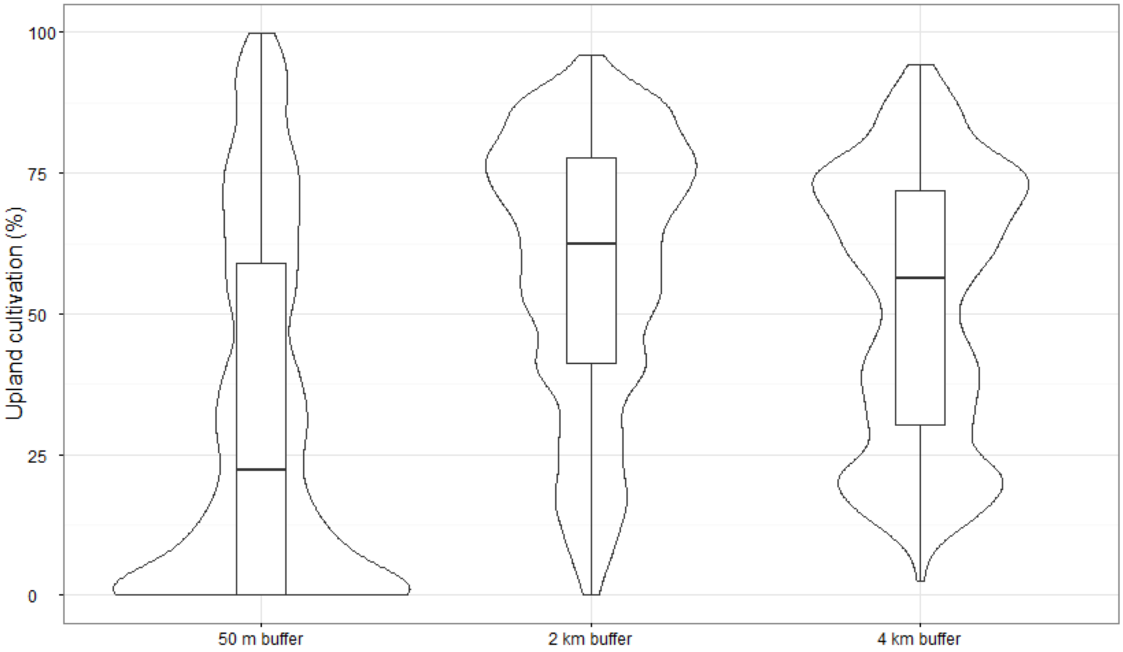


Figure 2. Box plot and violin plot showing the distribution of observations of upland cultivation metrics in 3 buffer sizes around 305 wetlands sampled during spring migration in eastern South Dakota during 2013 through 2015. The boxplots show range, inter-quartile range, and medians of observations in each buffer while the violin plots show the density of observations across the range of upland cultivation.



CHAPTER 5: IMPACTS OF WETLAND CONDITIONS ON REFUELING PERFORMANCE OF TWO SPRING-MIGRATING DUCKS

ABSTRACT

The capacity of a migrating bird to accumulate and maintain sufficient lipid reserves to fuel migration and facilitate subsequent reproduction is the ideal currency for gauging the contribution and quality of migration stopover habitats. We used concentrations of lipid metabolites circulating in plasma of spring-migrating female lesser scaup (*Aythya affinis*) and blue-winged teal (*Anas discors*) to evaluate the consequences of variation in biotic and abiotic attributes of stopover wetland habitats on lipid dynamics, or refueling performance, of migrants. We found little evidence for most wetland attributes to influence blue-winged teal refueling performance. Lesser scaup refueling performance was positively associated with density of Chironomidae in foraging locations, density of submersed aquatic vegetation in wetlands, relative density of conspecifics using the wetland during migration, and size and shape of surrounding wetlands. Refueling in both species was negatively correlated with high densities of fathead minnows (*Pimephales promelas*). Taken collectively, the biotic factors associated with improved refueling performance of lesser scaup are known from previous work to respond negatively to high densities of fathead minnows, suggesting changes in wetland trophic structure coincident with the introduction and proliferation of fathead minnows were the primary attribute affecting lipid dynamics of lesser scaup, and to a reduced extent blue-winged teal, during migration. Such impairments to lipid accumulation during migration could manifest in cross-seasonal and cross-ecosystem effects as breeding ducks recoup lipid deficits accrued during migration on the breeding grounds. Accordingly,

restoration and management actions aimed at reducing impacts of fathead minnows on stopover habitats used by spring-migrating ducks may have positive impacts on migration performance and ultimately population productivity of these species.

INTRODUCTION

North American migratory waterfowl (Anatidae) comprise a wide range of foraging, migrating, and breeding strategies and occur throughout the entire continent, from southern wintering areas in South America to northern breeding ranges on the Arctic coastal plain (Baldassarre 2014). Such ecological diversity, along with their demonstrated socioeconomic importance (e.g., Vrtiska et al. 2013), has prompted research on their ecology and management throughout the annual cycle, with a focus on reproduction and over-winter survival (Kaminski and Elmberg 2014). Reproductive ecology studies have demonstrated the importance of early arrival and clutch formation on the breeding grounds in determining individual reproductive success (e.g., Dzus and Clark 1998, Blums et al. 2005, Elmberg et al. 2005), which is facilitated by physiological condition during the transition to breeding (Prop et al. 2003, Devries et al. 2008). Further, waterfowl use nutrient reserves during early clutch formation nearly universally across a breath of body sizes and breeding latitudes (Ankney et al. 1991, Klaassen et al. 2006, Alisauskas and DeVink 2015, Janke et al. 2015). Therefore, some role of nutrient reserves among waterfowl during the transition from wintering to breeding is functionally universal, suggesting a comprehensive understanding of waterfowl ecology must extend beyond conditions experienced at the poles of their annual cycle and include the critical spring-migratory period (Arzel et al. 2006, Sedinger and Alisauskas 2014, Stafford et al. 2014).

The potential for conditions experienced off the breeding grounds to impact population productivity of waterfowl has arguably been best illustrated by the substantial population growth of midcontinent snow geese (*Chen caerulescens*; Alisauskas 2002). Despite liberalization of harvest to impose additional mortality on this relatively K-selected population, they have sustained record population sizes and reproduction in years with suitable climatic conditions (Alisauskas et al. 2011) because of functionally unlimited food supplementation during winter and spring migration (Jefferies et al. 2004, Abraham et al. 2005). Although these Arctic breeding, large-bodied birds are more reliant on nutrients they carry with them to the breeding grounds than smaller-bodied temperate breeding ducks, the insights gained about the potential for non-breeding conditions to lift constraints on population growth are compelling reasons for understanding the ecology of other waterfowl populations during spring. Indeed, research on the smaller-bodied mallard (*Anas platyrhynchos*) and Northern pintail (*Anas acuta*) has revealed apparent linkages between wintering areas and population productivity (Kaminski and Gluesing 1987, Raveling and Heitmeyer 1989), which are by extension mediated by conditions experienced during spring migration. Mallards and Northern pintails are buffered from many potential nutritional constraints during spring migration however because of their tendency to exploit waste-grain in crop fields in the same manner of snow geese (LaGrange 1985, Pearse et al. 2011). Few studies have examined the ecology of spring-migrating waterfowl that do not exploit waste-grain during migration.

Two exceptions exist for the paucity of information on migration ecology of wetland-foraging ducks: a comprehensive body of work on the ecology of Eurasian teal (*Anas crecca*) in Western Europe and recent work on lesser scaup (*Aythya affinis*) migration ecology in the upper Midwest. As noted for mallards and Northern pintail, Eurasian teal population productivity relates to conditions on wintering areas, suggesting an important role of spring migration in mediating annual productivity (Guillemain et al. 2008). Work throughout the primary migration route of Eurasian teal revealed foraging conditions encountered during spring migration were essential because they relied exclusively on dietary intake to fuel migration rather than using nutrient reserves (i.e., ‘income migrants’; Arzel et al. 2007), and that food availability was often low during migration (Arzel et al. 2009). In North America, concern over reduced population abundance of lesser scaup led to predictions about the role of spring migration habitats in reducing population recruitment capacity (Austin et al. 2000, Afton and Anderson 2001). Field studies examining this so-called Spring Condition Hypothesis documented declines in lipid reserves among lesser scaup during spring migration and upon arrival at breeding areas (Anteau and Afton 2004; 2009a) that provided credence to the role of spring habitat conditions in mediating population productivity and was subsequently supported with evidence for reduced population recruitment over the same time frame (Arnold et al. *In press*).

Clearly, conditions encountered during spring migration can have important impacts on population dynamics of wetland-foraging ducks, and therefore understanding factors associated with improved physiological outcomes for ducks using wetland habitats would improve management of these systems. In work further evaluating the ecology of spring-migrating lesser scaup, Anteau and Afton (2011) argued that concentrations of key lipid metabolites circulating in plasma of lesser scaup collected throughout the upper Midwest indicated they were actively catabolizing lipid reserves, perhaps in response to reductions in invertebrate prey densities due to wetland degradation (Anteau and Afton 2008a). Although this work provided a circumstantial link between observed reductions in lipid reserves at northern latitudes and wetland habitats encountered during spring migration, it does not explicitly tie the physiology of the ducks to specific wetland attributes associated with lipid catabolism. Such a direct link between wetland attributes and lipid metabolism of spring-migrating wetland-foraging ducks is arguably the ideal metric for characterizing wetland habitat quality during this important life-history phase (*sensu* Van Horne 1983, Jones 2001). Therefore, we sought to evaluate fine-scale relationships between migrant lipid metabolism and attributes of wetlands used by two wetland-foraging ducks in the southern Prairie Pothole Region (PPR) of the northern U.S. We focused our analyses on lesser scaup and blue-winged teal (*Anas discors*) because they forage exclusively in wetlands during migration, are ubiquitous, and use a diversity of wetland types. Additionally, comparison of the two species offers interesting contrasts with regard to body size variation (i.e., small-bodied blue-winged teal and larger lesser scaup), timing of migration (late-migrating blue-winged teal and early lesser scaup), and ultimate location of breeding habitats (comparatively lower latitudes among blue-winged teal). Our analysis should elucidate the role of wetland heterogeneity in determining the physiology of migrating females as they accumulate or maintain lipids for migration or the forthcoming breeding season.

METHODS

Study wetland selection

We worked in the Prairie Pothole Region of eastern South Dakota that comprised most counties east of the Missouri River. The region was characterized by thousands of isolated prairie wetlands in a matrix of intensive agricultural land used for crop production, grazing, or conservation (Johnson and Higgins 1997, Naugle et al. 2001). A majority of the region (ca. 96%) was privately owned, interspersed with small tracts of publicly owned and managed lands for waterfowl and other wildlife. We sampled 30 unique 50 km^2 study areas that comprised a total of 1500 km^2 that were 97% privately owned. Sites were selected with a Generalized Random Tessellation Stratified Sample to assure broad spatial coverage across eastern South Dakota and stratified across an agricultural land use gradient that was the focus of a concurrent study (Chapter 4). We sampled 12 study areas during April to May 2013 and 2014 and 6 study areas during April to May 2015.

We manually digitized wetlands on each study area based on aerial imagery from a wet year (2010 Farm Services Agency National Aerial Imagery Program [NAIP] images; 1 m resolution) to define a sampling frame to randomly select study wetlands. We distinguished between 2 wetland categories in this mapping procedure: wetlands with a seasonal hydroperiod and those with a semipermanent or permanent hydroperiod (Stewart and Kantrud 1971). We defined seasonal wetlands as those that were distinguishable with wetland vegetation in the 2010 NAIP photograph but then dry in the 2012 NAIP photograph from that dry year. Semipermanent wetlands had water or visible persistent emergent wetland vegetation in both years. We also informed our classification strategy with previous wetland classifications from the National Wetlands Inventory (Wilen and Bates 1995) based on the Cowardin et al. (1979) wetland classification methods and ca. 1985 imagery. We used an area-weighted random sample to select 10 wetlands on each study area to pursue permission from land owners to include in our study. We used an area-based weighting to avoid over-sampling small, numerically-abundant basins. We stratified the sample to include seasonal and semipermanent or permanent basins in equal proportion to their availability (area) on the study areas based on the digitized wetland maps. We omitted and replaced basins when we were denied permission, or in cases where the basin was entirely vegetation-choked or dry, until we had obtained permission to sample 10 wetlands or all available wetlands on the study site in cases of low wetland densities.

Field methods

We attempted to collect spring-migrating female lesser scaup and blue-winged teal on as many of the randomly-selected basins as possible within constraints imposed by use or abundance of each species on the basin, feasibility of collecting on the basin, and permits that allowed for ca. 10 individuals per study area per year. Therefore, our inferences are constrained first by the use of the wetland by the species and then by our ability to collect on the basin. The first constraint (use) is relevant for our inferences, because we were only interested in evaluating variation in wetlands used by each species during migration. The second constraint could introduce an unknown bias into the study because of underlying factors that may constrain our ability to collect on some wetlands (e.g., surrounding vegetation, size, depth). Comparison of the collection and survey data from all wetlands included in our study indicated we collected a migrating female lesser scaup on 52% of wetlands on which we detected them during migration and a migrating blue-winged teal on 45% of the wetlands on which we detected them during migration. Our explicit focus on randomly-selected study areas and then randomly selected wetlands within those relative small study areas is unique among most studies collecting migrating ducks and may minimize potential biases associated with our collection method.

We collected ducks from shore and small boats with shotguns and non-toxic shot (Envrion-metal, Inc., Sweet Home, OR). We noted the location of the collection on aerial photographs in the field and later geographically referenced them in ArcGIS. We constrained the time of collections to ≥6 hours after sunrise to improve the likelihood individuals had not recently arrived from nocturnal migration and had opportunity to forage in the landscape in which we collected them. We also attempted to focus collections on actively foraging individuals to further tie the individual and associated metabolite profiles to the basin on which it was collected. Immediately after collection, we drew a 0.5-1.5 mL sample of whole blood from the heart of the dead bird with a heparinized 3 mL syringe and 16-20 gauge 38 mm needles. We transferred whole-blood to a heparinized 1.5 mL microcentrifuge tube and stored it near ice packs to avoid freezing. Within 4 hours, we centrifuged whole-blood at 4,000 – 6,000 rpm for 5-10 minutes to separate plasma from red blood cells. We transferred plasma to a non-heparinized microcentrifuge tube and froze it at -20 C. Within a month of collection we transferred plasma to a -80 C freezer until further processing.

We conducted surveys of lesser scaup and blue-winged teal abundance on each basin twice during spring migration. The 2 survey occasions were timed to coincide with peak migration of lesser scaup early in the season and with peak blue-winged teal migration later (ca. 2 weeks) in the season. We used conventional survey methods used for waterfowl counts on prairie wetlands (e.g., Cowardin et al. 1995, Reynolds et al. 2006) to count all individuals on the basin and minimize the potential for double-counting individuals on multiple basins. We commenced surveys around sunrise on days with low winds (<30 kph) and high visibility. We first observed basins from high vantage points with high-powered spotting scopes and binoculars. We then walked through emergent vegetation when present to ensure we counted all individuals not visible from high vantage points. We did not collect ducks or otherwise disturb wetlands for 5 days preceding surveys to minimize investigator-related biases in counts.

We sampled aquatic invertebrates, fish, and vegetation on each wetland on one occasion during the spring migration period. We established 2-5 sampling transects on the basin radiating from the center with random compass bearings. We sampled invertebrate availability for blue-winged teal at 2 locations along transects by passing a D-framed sweep net through 0.5 m distance of surface water. At sampling locations >20 cm deep, the net only sampled the top 20 cm of the water column, which was the maximum height of the net and represented the likely maximum foraging depth for blue-winged teal and similarly-sized dabbling ducks (Guillemain et al. 2007). The first sampling location was 1 m from the edge of the water or the first open water area in which a blue-winged teal could land and forage along the transect. The second sampling location was half-the-distance from the middle of the basin to the first sampling location, or 5 m from the edge of the emergent vegetation zone in wetlands with an open water zone. We sampled invertebrate availability for lesser scaup at 2 locations along the transect in wetlands with an open water zone >0.5 ha (Anteau and Afton 2008a). We passed the sweep net along the benthos for 0.5 m length to sample the likely foraging location of diving lesser scaup in open water areas. Sampling locations were constrained to water depths <3 m. The first sampling location was 10 m beyond the edge of the emergent vegetation ring and the second location was 50 m beyond the first. We recorded the sampling depth at each sampling location to calculate the area of the net that was submerged and to characterize the depth profile of the basin. We rinsed samples through a 750 μm sieve in the field, separately composited blue-winged teal and lesser scaup invertebrate samples across all transects, and stored samples in 70% ethanol dyed with Rose Bengal in Whirl-pak bags.

We used a 36-tine lake rake at 3 locations perpendicular to transects at each individual sampling location to characterize the abundance of submersed aquatic vegetation (SAV) in the wetland. Although SAV had not started growing at the time of our sampling, we sampled residual SAV from the previous growing season, which related to aquatic invertebrate abundance and wetland quality for spring-migrating ducks in previous studies in the region (Anteau and Afton 2008a). SAV density was expressed as the proportion of tines obstructed by SAV across all sampling locations in the wetland. We sampled fish to document presence/absence, characterize communities and relative abundance among all wetlands in the study. We used 2-5 Gee-style minnow traps in all wetlands deep enough to submerse the traps (>20 cm) and used an experimental gill net in wetlands with an open water zone and ≥1 m deep. We recorded the time we set traps and the time we returned on the following day to account for total effort of each trap. We enumerated fish by species and released them back into the wetland.

Laboratory methods

We rinsed invertebrate samples in the lab in a 500 μm sieve and searched under 10X magnification dissecting scopes to enumerate and preserve all aquatic invertebrates. We generally identified key taxa to families, except for Amphipoda, which we identified to genus (*Hyalella* and *Gammarus*). The same investigator (AKJ) conducted quality control on identification of all organisms to ensure consistency across sites and years. We converted counts to biomass estimates based on the average dry weight (mg/individual) of a random subset of individuals in each taxa taken across samples from all 3 years of the study. We noted substantial inter-wetland variation in size of Chironomidae larvae and therefore measured basin-specific biomass of Chironomidae larvae in each sample with ≥30 individuals and used the mean mass for samples with fewer than 30 individuals.

We conducted necropsies on all birds to confirm they had not transitioned into reproductive state based on the size of the 3 largest ovarian follicles. We did not note evidence of rapid follicle growth in any lesser scaup but collected blue-winged teal with mean follicle size >5mm indicative of transition into breeding (Janke et al. 2015). Those individuals were removed from analyses. We measured concentrations of β-hydroxybutyrate (BUTY) and true triglycerides (TRIG) in plasma to serve as an indication of recent (ca. 1 day; Jenni-Eiermann and Jenni 1994, Anteau and Afton 2008c) lipid metabolism. β-hydroxybutyrate is a ketone body and elevates in plasma coincident with the breakdown of somatic lipids, and has therefore been reported to negatively correlate with recent changes in lipid mass (i.e., lipid catabolism; Jenni-Eiermann and Jenni 1994). Conversely, true-triglycerides measured in plasma indicates lipid transport from dietary break-down or *de novo* lipogenesis to somatic tissue and therefore correlates with short-term increases in lipid mass (i.e., lipid accumulation; Jenni-Eiermann and Jenni 1994). We used commercially available assays adjusted for small volumes to measure TRIG and BUTY by means of endpoint assays and kinetic assays, respectively, as described in Appendix 2. We ran all samples in duplicate and re-ran samples with high inter-assay variation in concentration estimates (CV > 20%). We also measured concentration of plasma hemoglobin with a colorimetric assay (Hemoglobin Colorimetric Assay Kit, Cayman Chemical Company, Ann Arbor, MI) to identify and exclude highly-hemolyzed samples (defined as plasma hemoglobin ≥1g/dL) that could impair measurements of metabolite concentrations (Chapter 2).

We obtained high-resolution (≤1.5 m) geographically referenced true color (2013, 2014) or color infrared (2015) aerial imagery of each of the study sites during May of the year the site was sampled to quantify the availability of surface water. Imagery was acquired from small, fixed-winged aircraft flying over the study sites and taking digital photographs that were later mosaicked and geographically referenced (Niemuth et al. 2010). We manually digitized all available surface water from the imagery in ArcGIS 10.3 (ESRI, Redlands, CA). We classified surface water into 3 categories: 1) non-wetland surface water, which included streams, drainage ditches, and excavated ponds (i.e., waste-water treatment facilities, stock dams); 2) interspersed wetlands, which included wetlands or portions of wetlands with interspersed emergent vegetation or patches <0.5 ha deemed usable by blue-winged teal but not by lesser scaup and; 3) open wetlands, which included open water patches >0.5 ha without emergent vegetation. We mapped surface water in these categories within a 2 km radius buffer of collection locations to quantify the area of potentially suitable wetland habitat around each individual. Previous work with radio-marked non-breeding ducks indicated that daily movement rates were generally <4 km/day (Beatty et al. 2014, Beatty et al. 2015; A. D. Afton, Louisiana State University unpublished data), so we assumed that a 4 km diameter area was representative of the landscape immediately available to ducks during spring migration stopovers.

Analysis methods

We conducted an exploratory analysis with diagnostic plots and linear regression on metabolite concentrations and variables including date, time-since sunrise, relative day within the migrating period in the year, latitude of collection, and whether the individual was observed foraging or paired upon collection to ensure there were no associated biases (Guglielmo et al. 2002, Mandin and Vézina 2012). We found no systematic variation in metabolite concentrations and any of these variables, so we proceeded with analyses on raw metabolite concentrations. We were interested in characterizing the refueling performance of spring-migrating lesser scaup and blue-winged teal with respect to a suite of wetland-related covariates. We used the refueling index proposed in Chapter 3 to composite information contained in each lipid metabolite into a single index of refueling performance on an individual. The refueling index was advantageous over individual metabolite concentrations because it accounts for unique information contained in BUTY, an indicator of lipid catabolism, and TRIG, a leading indicator of lipid accumulation, while also accounting for the inherit redundancy in information contained in the two contrasting metabolic indicators.

We evaluated the importance and impacts of wetland covariates describing biotic and abiotic factors associated with collection wetlands and surrounding wetland availability on variation observed in the refueling index for each species. The covariates we examined fell into 3 general categories, representing the biotic community (invertebrates, submersed macrophytes, fish, and ducks), structural attributes of the wetland (area, shape, and depth), and availability of additional wetlands in the surrounding landscape. The first category of covariates comprised information about invertebrate prey availability. Invertebrate prey can constitute a majority of food consumed by spring-migrating lesser scaup and blue-winged teal during migration (Chapter 6, Appendix 1; Anteau and Afton 2008b, Hitchcock 2009, Tidwell et al. 2013), so we predicted improved refueling performance in individuals collected on wetlands with high prey abundance. We focused our analyses on 3 taxa — Amphipoda, Chironomidae, and Mollusca (including all Gastropoda and Sphaeriidae) — that were abundant in diets of lesser scaup and blue-winged teal in our study (Chapter 6, Appendix 1) and others (Anteau and Afton 2008b, Hitchcock 2009). We converted biomass of each taxa to densities (mg/m^2) by accounting for the area sampled by the sweep net based on depth of the water at the sampling location and the area of the net submersed at 1 cm depth increments (to account for the D-shape of the net in cases where the entire net was not submerged).

We included the relative density of SAV in the wetland as a covariate, expressed as the percentage of lake-rake tines obstructed by SAV across all samples in the wetland. Submersed aquatic vegetation can represent a prey item for spring-migrating lesser scaup and blue-winged teal because both species consume aquatic plant seeds (Anteau and Afton 2008b, Hitchcock 2009). It can also can confer improved habitat for invertebrate prey in wetlands (Murkin et al. 1991, Anteau et al. 2011). Therefore, we predicted that increased SAV could improve forage availability for both species and therefore positively impact refueling performance.

Fish have well-documented negative impacts on invertebrate and plant communities in prairie wetlands (Bouffard and Hanson 1997) so we predicted covariates related to fish presence would be negatively associated with the refueling index. The first covariate we included for fish was a binomial term indicating whether we had detected any fish in the wetland with either sampling gear. The second fish covariate we tested was the relative density of fathead minnows (*Pimephales promelas*) expressed as average catch-per-unit-effort (CPUE) of minnow traps on wetlands. We calculated CPUE as the mean count for a single trap on the wetland over a 24 hour period. Fathead minnows have strong impacts on prairie wetland ecology (Zimmer et al. 2002) and are ubiquitous throughout the upper Midwest because of their ecological tolerances for wetlands and dietary flexibility (Duffy 1998, Herwig and Zimmer 2007, Anteau and Afton 2008a). Therefore, we surmised their density may have the greatest effect on wetland attributes of concern for spring-migrating lesser scaup and blue-winged teal, perhaps more so or at least in addition to the binary variable indicating whether or not any fish were present in the wetland. Further, previous work in the region has indicated fathead minnow impacts are non-linear (Anteau et al. 2011), so we fit the covariate with a quadratic effect to allow for variable impacts between low-densities (main effect) and high densities (quadratic effect) of fathead minnows.

The final biotic covariate we evaluated was the relative density of conspecifics using the wetland during migration. This covariate allowed us to evaluate whether density of each species on a wetland during migration was associated with the refueling performance of the ducks. Understanding the relationship between refueling and densities could reveal interesting patterns related to factors influencing the spatial distribution of ducks in potential foraging patches during migration. Under an ideal-free distribution, we would expect there to be no variation in refueling performance with increasing conspecific density on the wetland (Fretwell and Lucas 1970). However, we also surmised that increased density of individuals on the wetland may convey advantages for foraging individuals with respect to trade-offs in foraging and vigilance times, which may be reflected with greater refueling performance in the presence of higher conspecific densities (Arzel et al. 2007, Guillemain et al. 2007). We expressed the relative density of conspecifics on each wetland based on the residuals of a regression on the square-root of the total number counted on the two survey occasions and the log of the area open water on the basin during the study year. This regression procedure corrected for variation in wetland size and served as a relative index of conspecific density on the wetland during migration.

The next category of covariates we examined described structural attributes of the wetland and included the maximum depth of the wetland recorded during sampling and the size and shape of the basin. To characterize the shape and size of the basin in one term, we used the perimeter to area ratio of open water. This ratio accounts for the availability of edge habitats, which are preferred by blue-winged teal and avoided by lesser scaup, and the total availability of water (Fairbairn and Dinsmore 2001). The ratio is negatively correlated with wetland area (Figure 1) and greater on wetlands with interspersed patches of open water and vegetation with more edge (i.e., hemimarsh; Weller and Spatcher 1965). Therefore, we predicted blue-winged teal would have improved refueling performance on wetlands with higher ratios (i.e., more interspersion of vegetation and water) and lesser scaup would have lower refueling performance on wetlands with lower ratios because it would represent less open-water foraging habitat availability.

The final category of covariates we examined represented the availability of potential foraging habitats within a 2 km radius (12.5 km^2). We included a covariate for the perimeter-to-area ratio of open water to index the total availability of open-water foraging locations for lesser scaup and edge habitats for blue-winged teal. As with the collection basin-specific perimeter-to-area ratio described above, we predicted blue-winged teal refueling performance would be positively and lesser scaup refueling performance would be negatively associated with the score. This index did not perfectly characterize water availability for blue-winged teal because it only pertained to open water patches (≥0.5 ha) of wetlands, whereas we observed blue-winged teal using small wetland patches and other surface water during our study. Therefore, we included a covariate for the total area of open water habitats and a covariate for total non-open water availability (including interspersed wetlands, small open water patches <0.5 ha, and river and streams with available surface water). We included the same open water availability covariate in the lesser scaup analysis, but did not include the term for non-open water because they were not used by lesser scaup. In the rare (3%) case where there were no open water patches around blue-winged teal collection locations we assigned the bird the highest value of the index score observed among birds with open water in the surrounding buffer. We used the highest score, rather than for example the median, because we assumed landscapes with no available open water were more similar to high perimeter-to-area landscapes than any other landscape (Figure 1).

We had no *a priori* predictions or explicit interest on how individual covariates may collectively impact the response variable together, but rather were interested in the average effect of each individual covariate on the refueling index. Therefore, we used a variable selection procedure to draw inferences on the average impact of each individual covariate on refueling performance. To do this, we tested all possible covariate combinations and evaluated their individual significance based on model-averaged regression coefficients and relative importance values from models that included the covariate (Burnham and Anderson 2002). We interpreted the significance of a parameter estimate based on whether 85% confidence intervals around the parameter excluded 0. We choose 85% confidence intervals to approximate the behavior of traditional model-selection procedures common in the literature (Arnold 2010). We used the methods described in Burnham and Anderson (2004) to estimate adjusted standard errors of the regression coefficients. We used generalized linear mixed effects models in the lme4 package in R to perform regression analyses. Mixed effects models were advantageous for our study design because they allowed us to control for dependency among individuals collected on the same study sites and in the same year with random intercept terms, and then allowed us to focus inferences on main effects associated with the wetland covariates of interest. We square-root transformed invertebrate densities, fathead minnow CPUE, and landscape wetland availability covariates to minimize the influence of extreme observations. We z-transformed all continuous covariates (all covariates except the fish presence/absence term) to facilitate direct-comparisons of covariate effects that were measured on widely varying scales and to improve model fit (Schielzeth 2010). We reported summary statistics on covariates to facilitate comparisons with other studies and standardizations (Table 1; Schielzeth 2010). We tested all z-transformed covariates for multicollinearity and removed one covariate from highly correlated ($|r| \geq 0.6$) pairs.

RESULTS

We included 120 female lesser scaup in the analysis (25 in 2013, 61 in 2014, 34 in 2015). We collected lesser scaup on 50 different wetland basins on 23 different study areas. Two covariate pairs were highly correlated in the lesser scaup data set: the collection basin perimeter to area ratio and the perimeter to area ratio within the 2 km buffer ($r = 0.68$) and the total open wetland area and the perimeter-to-area ratio of open water in the 2 km buffer and the total open water area in the 2 km buffer ($r = -0.71$). We therefore excluded the total open water area and the open water perimeter to area ratio of the basin from the analyses and retained the perimeter to area ratio within the 2 km buffer to draw inferences on the three related terms and reduce the number of variables in our analyses. Five of the 10 individual covariates we evaluated had 85% confidence intervals that did not contain 0 (Figure 2; Table 2). Chironomidae density, SAV density, maximum depth, perimeter-to-area ratio of the collection basin and open water patches within the 2 km buffer were all positively associated with the refueling index. The quadratic fathead minnow CPUE term was the only covariate with a significant negative association with the refueling index of lesser scaup and indicated that fathead minnow densities had negative impacts on refueling at high densities (Figure 2; Table 2).

We included 218 female blue-winged teal in the analysis (71 in 2013, 94 in 2014, 53 in 2015). We collected blue-winged teal on 29 different study areas and 111 unique wetland basins. Total open wetland area and perimeter-to-area ratio of water in the 2 km buffer were highly correlated in the blue-winged teal data set (*r* = -0.82) so we excluded the total open water term from the models. The quadratic fathead minnow CPUE covariate was the only covariate with 85% confidence intervals that did not contain 0 (Figure 2; Table 2) and indicated decreased refueling performance at higher fathead minnow densities.

DISCUSSION

The two wetland-foraging ducks we examined had variable physiological responses to conditions in or surrounding wetlands used during migration in our study area. Among blue-winged teal, our analyses showed equivocal relationships with key prey abundances and structural attributes of the wetlands. In contrast, lesser scaup refueling performance varied in association with all the covariate categories we examined, including biotic factors such as conspecific abundance and prey availability and abiotic factors associated with wetlands used during migration or surrounding those wetlands. Both species converged however in their negative response to high densities of fathead minnows in wetlands suggesting ecosystem alterations associated with high fathead minnow densities have impacts that extend beyond those demonstrated within the aquatic food web (Zimmer et al. 2002, Hanson et al. 2005). Together, these varied responses to wetland conditions and their convergence on fathead minnow densities offer compelling insights into the factors influencing lipid accumulation and metabolism during migration for each species and have implications for consideration in restoration or conservation of wetland habitats in the region.

The weak association with blue-winged teal and wetland attributes compared with lesser scaup could have resulted from their different behavior and habitat use patterns during migration. Blue-winged teal tended to use small, shallow wetlands or margins of larger wetlands, which together were comparatively more abundant than deeper water habitats used by lesser scaup (Anteau and Afton 2009b, Kahara and Chipps 2012). This may have resulted in higher movement rates of blue-winged teal among wetlands than lesser scaup. Regular switching among wetlands would mask variation in refueling performance in response to wetland conditions because it would become the sum product of multiple different wetlands in close proximity, rather than the single wetland on which the individual was collected. Field observations supported this behavioral difference, because blue-winged teal frequently switched among wetlands in response to disturbance, whereas lesser scaup tended to move to different areas on the same basin in response to disturbance. Therefore, we may expect lesser scaup refueling performance to reflect the wetland where we collected them and blue-winged teal refueling performance to be less reflective of individual basin attributes. This explanation would also fit with observed associations between blue-winged teal and fathead minnow densities, because fathead minnows would only occur on relatively larger wetlands (Herwig et al. 2010, Wiltermuth 2014), where perhaps blue-winged teal were less likely to leave in response to disturbance.

An alternative, or additional, factor leading to the contrasting results between blue-winged teal and lesser scaup refueling performance and wetland conditions is variation in migration and breeding nutrient allocation strategies used by females of each species. Blue-winged teal are a small-bodied temperate breeder, whereas lesser scaup are comparatively larger and have high breeding densities at more northern latitudes (Baldassarre 2014). Accordingly, lesser scaup would be predicted to maintain and accumulate more lipids during migration than blue-winged teal because their larger body size (Witter and Cuthill 1993) and their tendency to breed in comparatively shorter time-windows at northern latitudes. This prediction fits with observations of the interannual variation in lipid reserves of lesser scaup and the lack thereof among blue-winged teal discussed in Chapter 6. Blue-winged teal appear to be income-migrants, fueling migratory flights with nutrients acquired en route, without obtaining additional nutrients for breeding or future constraints during migration. This behavior is supported by work with similar-sized ducks in Europe that used a similar strategy (Guillemain et al. 2004, Arzel et al. 2007) and the tendency for female blue-winged teal to accumulate appreciable nutrients for reproduction after cessation of migration on the breeding grounds (Janke et al. 2015). In contrast, lesser scaup appear to accumulate or maintain supplemental lipid reserves during migration as conditions allow (Chapter 6) and these reserves have demonstrated importance for subsequent reproductive success (Afton and Ankney 1991, Esler et al. 2001). Therefore, in maintaining or accumulating additional reserves during migration, we may expect more spatial variation in refueling performance of lesser scaup as conditions allow, whereas blue-winged teal would demonstrate more stasis in refueling performance as they maintain base-line condition rather than opportunistically increasing nutrient intake.

Regardless of the underlying behavioral or life-history factors leading to variable responses between these two species, our results with lesser scaup indicated sensitivity to wetland conditions experienced during migration, which has been previously predicted from coarser-scale studies on wetlands and migrating lesser scaup in the region (Anteau and Afton 2009a;2011) and has implications for management and restoration of their habitats. The associations between refueling and significant covariates seemed to be categorized into 2 broad classes; those associated with the size of the wetland and surrounding wetlands and those associated with the biotic communities in wetlands.

Covariates describing the perimeter-to-area ratio of open water in the surrounding 12.5 km^2 landscape had a strong standardized effect on refueling performance. This term was also positively associated with wetland-specific perimeter-to-area ratios and collectively these terms indicated a positive association with higher perimeter-to-area ratios, which conflicted with our original prediction of increased refueling performance with more large open water areas (lower ratios). This result suggested landscapes with larger open water bodies (i.e., lakes) seem to provide relatively poorer refueling conditions for lesser scaup in eastern South Dakota. This result has implications for the changing landscapes in the southern PPR where recent studies have illustrated a tendency towards increased basin sizes because of increased connectivity and inputs associated with artificial drainage (Miller et al. 2011, McCauley et al. 2015, Vanderhoof and Alexander 2015). Low basin perimeter-to-area ratios can therefore be a symptom of concomitant biotic impacts of increasing basin size or permanency, namely fish colonization and persistence (Herwig et al. 2010), which have cascading negative impacts on aquatic invertebrate and wetland plant communities (Hanson and Riggs 1995, Anteau et al. 2011, Hanson et al. 2012, Maurer et al. 2014).

Four biotic covariates — Chironomidae biomass, submersed aquatic vegetation, conspecific density, and high densities of fathead minnows — all had significant impacts on the refueling index score. The positive association with the relative density of conspecifics on the wetland was an interesting result in revealing how lesser scaup may distribute during migration to improve individual fitness. Adherence to an ideal free distribution (*sensu* Fretwell and Lucas 1970) would predict no association with conspecific abundance and physiology, but rather that all individuals would be distributed in densities that conferred equivalent potential foraging success, and by extension equivalent refueling performance. Our results indicating a positive association suggests lesser scaup may distribute disproportionally into habitats that confer the best refueling performance, or that refueling performance in enhanced by the presence of more conspecifics, which could allow for more foraging and less vigilance time, favoring increased refueling (Arzel et al. 2007, Guillemain et al. 2007). Regardless of the mechanism of these slightly positive effects of conspecific abundance, the result suggests density of lesser scaup in wetlands during migration is arguably a suitable indicator of the realized quality of those wetlands for refueling (*sensu* Van Horne 1983, Jones 2001).

If indeed densities are a suitable indicator of realized habitat quality for lesser scaup during migration, this conclusion helps interpret other patterns we observed in our biotic covariates in the context of previous work on abundance of migrating lesser scaup. Our analyses showed there to be no association between refueling performance and densities of Amphipoda, despite the purported importance of Amphipoda in lesser scaup diets during migration (Anteau and Afton 2006). Amphipoda densities were predictive of lesser scaup abundance during migration in our study region (Anteau and Afton 2009b), and we found a similar pattern of higher mean Amphipoda densities on occupied wetlands compared to unoccupied wetlands surveyed during our study (unpublished data). Thus, selection for Amphipoda densities may be occurring at broader landscape scales such that within used wetlands, variation in Amphipoda densities were inconsequential. Another consideration in interpreting the lack of an impact of Amphipoda in refueling performance is that other work has indicated Amphipoda densities are depressed throughout the upper Midwest (Anteau and Afton 2008a) perhaps because of factors leading to increased persistence and occurrence of fathead minnows in wetlands (Anteau et al. 2011). This interpretation is consistent with low consumption of Amphipoda by lesser scaup during our study (Chapter 6, Appendix 1) and with other biotic covariates that collectively implicate wetland conditions associated with colonization and perpetuation of fathead minnows as the driving factor in lesser scaup, and to some extent blue-winged teal, refueling performance in our study.

Fathead minnows are omnivorous and can therefore directly compete with waterfowl for invertebrate prey (Duffy 1998, Herwig and Zimmer 2007). However, their primary route of impacts are through trophic alterations in aquatic ecosystems from consumption of smaller invertebrates (i.e., zooplankton) and detritus, and associated changes in turbidity and light attenuation that inhibits submersed aquatic vegetation growth and decreases invertebrate densities (Figure 3; Hanson et al. 2005, Zimmer et al. 2006, Herwig and Zimmer 2007, Maurer et al. 2014). Such trophic impacts of fathead minnow densities have been well-established in shallow lakes and wetlands in the Upper Midwest and potential to impact waterfowl has been inferred (Bouffard and Hanson 1997, Cox et al. 1998, Anteau and Afton 2009a;2011). Our results however, are the first to explicitly link a key physiological parameter to the trophic alterations associated with fathead minnows. If fathead minnows (or comparable fish communities) are abundant in wetlands at broad scales in southern the PPR, as reported in other studies (Anteau and Afton 2008a, Herwig et al. 2010, Wiltermuth 2014), our results provide a mechanism that could lead to cross-seasonal effects of fathead minnow densities on reproduction in lesser scaup breeding at higher latitudes. Further, wide-spread nutrient deficiencies in the southern PPR due to fathead-minnow induced reduction in lipid acquisition could lead to cross-ecosystem interactions, because nutrient deficits accrued in the PPR would need to be compensated for by increased nutrient acquisition on breeding areas.

Our results could be useful in surmising prescriptive wetland conditions most-favorable to refueling by spring migrating lesser scaup, and to a reduced extent blue-winged teal. However, we recognize the primary limitation of our study is that our inferences were constrained to available wetlands and to wetlands used by lesser scaup and blue-winged teal. The initial constraint is relevant because we may have not been able to sample across a range of potentially suitable landscapes that were under-represented because of widespread modifications to this landscape associated with wetland drainage and upland cultivation (Dahl 1990, Oslund et al. 2010, McCauley et al. 2015). This distinction seems particularly relevant in the context of the Amphipoda results. Amphipoda could provide forage that increases refueling performance for migrating ducks in the absence of negative effects of fathead minnows; however, in the current landscape it seems that Amphipoda densities may be constrained by artificially inflated densities of fathead minnows. A second important distinction for our results is the constraint imposed by our design of collecting ducks *in situ*. Many wetland and landscape factors likely drive wetland use by both species, and those underlying constraints had to be met in order for us to be able to sample a duck on a wetland. Once those constraints were met however, our results reflect factors associated with improved refueling performance of spring migrating ducks. In general, relatively small wetlands, free of fathead minnows and associated tropic impacts on SAV and invertebrates, provided the best refueling habitat for lesser scaup. Restoration and management in the region could first focus on precluding wetland alterations that facilitate fathead minnow colonization and persistence wherever possible (i.e., consolidation drainage; Figure 3; Wiltermuth 2014, McCauley et al. 2015, Vanderhoof and Alexander 2015). In cases where fathead minnows are present, restoration methods to restore hydrology of remaining wetlands could treat the root cause (e.g., Anteau 2012), or biomanipulation techniques could help alleviate some symptoms (e.g., Potthoff et al. 2008), yielding potential improvements in wetland foraging habitats for lesser scaup and other spring-migrating ducks.

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Table 1. Summary statistics on response variable (refueling index) and covariates used in regression analyses evaluating the impacts of wetland covariates on blue-winged teal and lesser scaup refueling performance during spring migration in eastern South Dakota 2013-2015. Variable definitions and explanations can be found in the text.

	Blue-winged teal				Lesser scaup			
		Percentiles				Percentiles		
Variable	Mean (SD)	25%	50%	75%	Mean (SD)	25%	50%	75%
Refueling index	0.81 (3.31)	-1.13	0.72	2.51	2.53 (3.62)	0.39	2.58	4.80
Conspecific abundance index	0.00 (3.02)	-2.02	-0.36	1.31	0.00 (4.83)	-2.53	-0.20	3.08
Amphipoda density (mg/m^2)[a]	0.71 (1.74)	0.00	0.00	0.77	3.99 (5.00)	0.00	2.79	6.02
Chironomidae density (mg/m^2)[a]	2.02 (2.02)	0.00	1.80	3.02	7.71 (5.13)	3.73	6.62	11.64
Mollusca density (mg/m^2)[a]	4.98 (7.4)	0.00	2.25	6.91	15.36 (21.52)	1.46	6.12	17.79
Fathead minnow CPUE[a]	1.19 (2.90)	0.00	0.00	0.00	2.44 (4.46)	0.00	0.00	2.11
Submersed aquatic vegetation density (%)	5.33 (12.32)	0.00	0.00	2.11	9.83 (15.44)	0.00	2.78	12.81
Maximum depth (m)	0.67 (0.56)	0.30	0.55	0.80	1.58 (0.86)	0.90	1.33	2.00
Basin perimeter:area (m/m^2)[a]	0.24 (0.12)	0.16	0.22	0.30	0.12 (0.04)	0.10	0.12	0.14

Surrounding non-open water (m^2/ha)[a]	7.85 (3.27)	5.88	7.09	9.27	---	---	---	---
Surrounding open water perimeter:area (m/m^2)[a]	0.17 (0.06)	0.13	0.16	0.21	0.13 (0.03)	0.10	0.13	0.14

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[a] Indicates mean, sd, and percentiles are for square-root transformed values

Table 2. Model-averaged regression coefficients, associated standard errors, and relative importance values for covariates included in an analysis on factors influencing refueling performance of spring-migrating lesser scaup and blue-winged teal collected in eastern South Dakota during 2013-2015.

	Blue-winged teal			Lesser scaup		
Variable	RI^{a}	b^{a}	SE^{a}	RI^{a}	b^{a}	SE^{a}
Intercept	---	1.318	0.875	---	3.419	0.828
Conspecific abundance index	0.192	-0.096	0.238	0.546	0.628	0.399
Amphipoda density	0.198	-0.113	0.239	0.292	-0.136	0.431
Chironomidae density	0.205	-0.117	0.252	0.858	0.996	0.378
Mollusca density	0.221	-0.178	0.231	0.437	0.498	0.402
Fish presence/absence	0.456	-0.047	0.901	0.547	-0.840	0.880
Fathead minnow CPUE	0.951	0.911	0.806	0.850	0.833	0.851
Fathead minnow CPUE quadratic	0.951	-0.643	0.292	0.850	-0.692	0.328
Submersed aquatic vegetation density	0.195	-0.126	0.223	0.832	0.910	0.397
Maximum depth	0.192	0.053	0.256	0.467	0.557	0.458

Basin perimeter:area	0.257	0.226	0.258	---	---	---
Surrounding non-open water	0.220	-0.097	0.293	---	---	---
Surrounding open water perimeter:area	0.261	0.222	0.301	0.738	0.844	0.392

[a] RI = relative importance (sum of model weights including variable); *b* = model-averaged regression coefficient; SE = model-averaged regression coefficient standard error

Figure 1. Relationship between square-root transformed basin area and open water perimeter-to-area ratio of wetlands on which female lesser scaup were collected during spring migration in eastern South Dakota during spring 2013 to 2015.

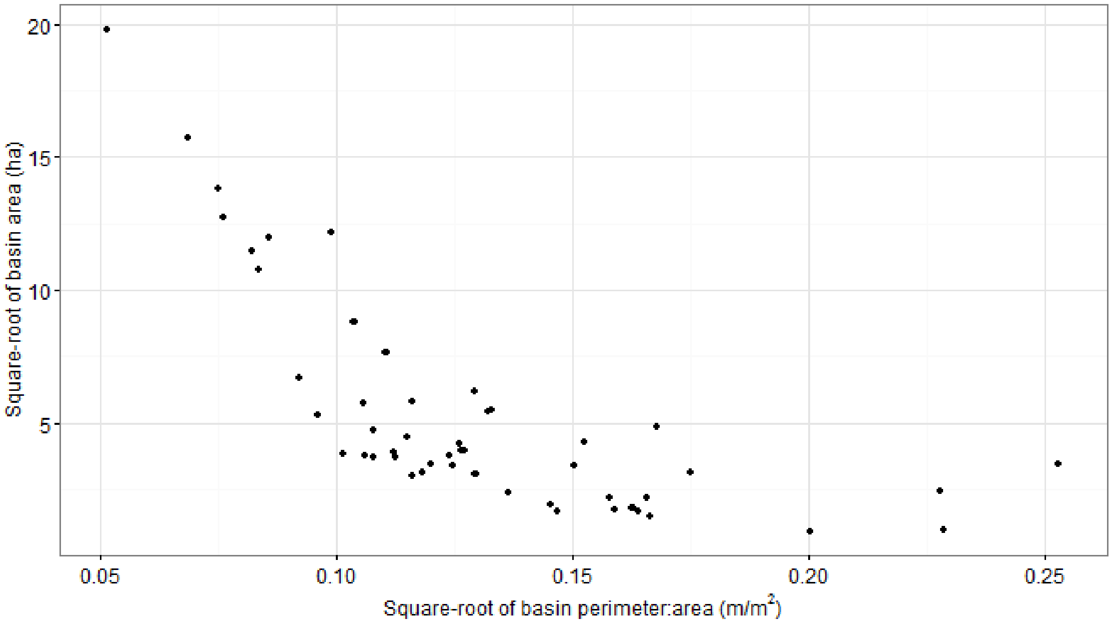


Figure 2. Model-averaged regression coefficient estimates (±85% confidence intervals) for wetland covariates predicted to influence refueling performance measured with concentrations of key lipid metabolites circulating in plasma of lesser scaup and blue-winged teal collected on wetlands in eastern South Dakota during spring migration 2013-2015. All covariate effects are directly comparable within each species except for the binomial fish presence-absence (Fish P/A) term. Gray bars indicate parameters that have 85% CI's that contain 0, whereas black bars indicate significant effects. See text for description of individual covariates.

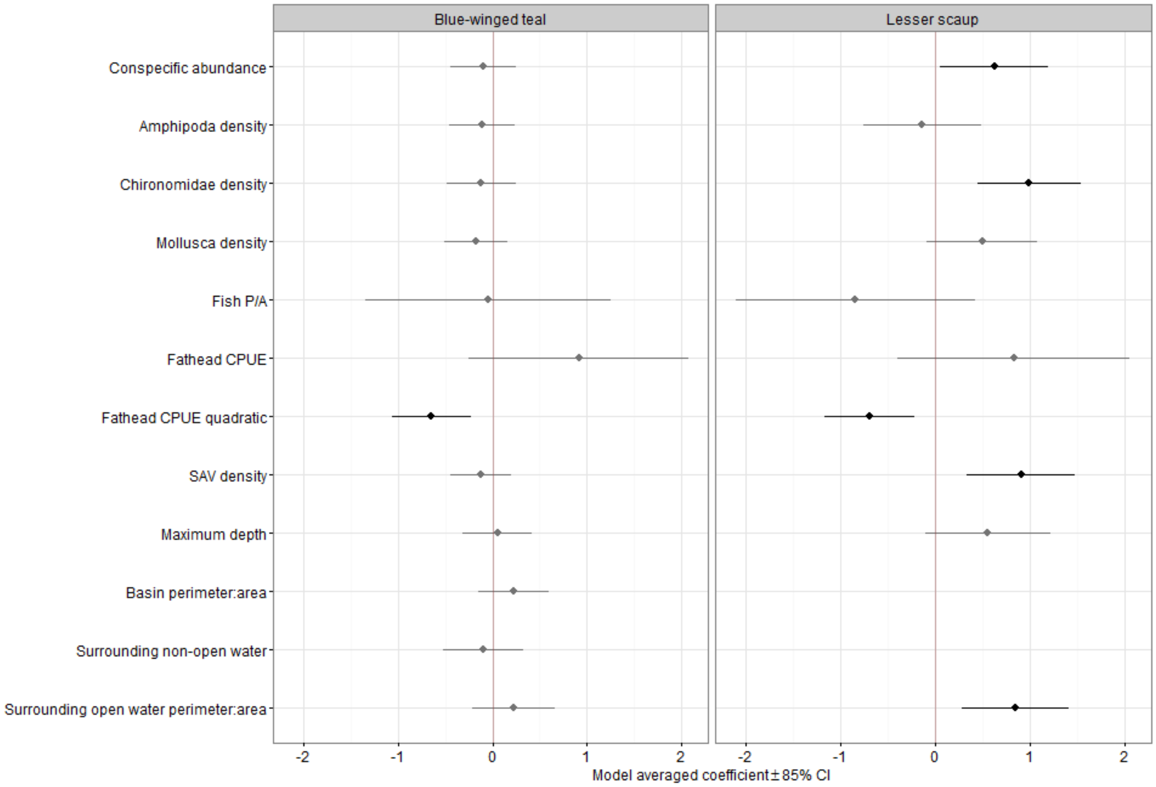
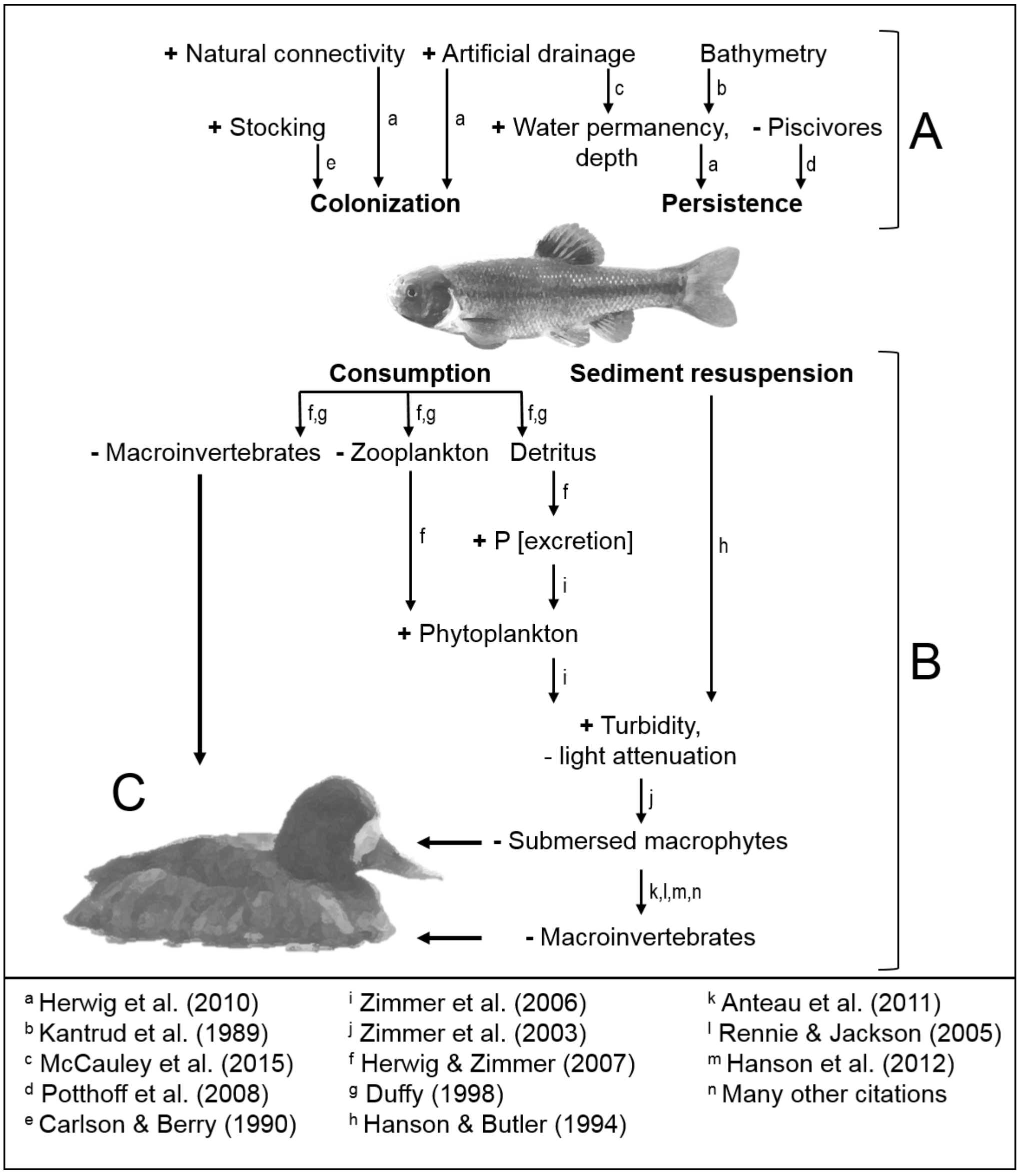


Figure 3. Conceptual model of pathways that influence the colonization and persistence of fathead minnows (A) and pathways their presence influences biotic communities in wetlands and shallow lakes (B). Light arrows with lower case letters indicate mechanisms explored in previous work, with a representative citation provided. The dark bold arrows (C) are the likely pathways in which fathead minnow densities influenced refueling performance of spring-migrating lesser scaup and blue-winged teal in our study.



CHAPTER 6: INTERANNUAL PHYSIOLOGICAL AND DIETARY VARIABILITY IN SPRING-MIGRATING DUCKS

ABSTRACT

Environmental stochasticity encountered during spring migration can have negative demographic consequences on individuals and populations through direct reductions in survival or cross-seasonal impacts associated with nutrient reserves necessary for breeding or successful migration. We took advantage of substantial interannual variation in the timing and progression of spring migration conditions over four years to examine annual variation in the physiology and diets of two wetland-foraging obligate ducks during spring migration. We collected female lesser scaup (*Aythya affinis*) and blue-winged teal (*Anas discors*) during spring migration in wetlands in eastern South Dakota and measured annual variation in lipid and protein reserves, an index of recent lipid metabolism based on concentrations of lipid metabolites in plasma, and diets. We found interannual variation among these metrics in both species, contrasting mainly between the warmest, earliest spring and the latest, coldest spring. Lesser scaup had reduced lipid and protein reserves in the coldest year, showed no interannual variation in the index of lipid metabolism, and seemed to reduce consumption of energy-rich prey in the cold, late spring. Blue-winged teal similarly had reduced protein reserves in the cold, late spring, but maintained constant lipid reserves among years, perhaps facilitated by increased consumption of energy-dense seeds as reflected in diets and the index of lipid metabolism. These contrasting responses to interannual variability suggest lesser scaup may have been caught in inclement spring conditions directly leading to reductions in lipid reserves that could not be compensated for with

increase dietary intake of lipid-rich prey as seen with blue-winged teal. Our results provided insights into impacts of environmental stochasticity on these species and have implications for consideration of factors influencing annual recruitment following variable spring migration conditions or degradation of spring migration habitats.

INTRODUCTION

Environmental stochasticity during migration poses direct threats to migratory birds (Newton 2007) and has potential to erode physiological condition in a way that impedes migration or constrains reproduction upon arrival on breeding grounds (Drent et al. 2006, Newton 2006). Accordingly, migratory birds have evolved diverse physiological and behavioral mechanisms to anticipate environmental stochasticity and minimize its effects on individual fitness. During migration, birds make energetically costly long-distance flights, often over inhospitable terrain to escape environmental stochasticity characteristic of seasonal breeding habitats. Although migration strategies are timed to minimize exposure to seasonality, time constraints for breeding at mid-to-high latitudes often lead migrants to experience environmental stochasticity during transition from wintering to breeding which could strongly influence individual fitness and population productivity.

Early breeding is adaptive among migratory birds because improvements conferred to offspring fitness (Blums et al. 2005, Drent et al. 2006). Therefore, individuals transitioning into the breeding season often push the limits of their ecological tolerances to arrive relatively early. Environmental stochasticity during this period can delay reproduction directly by inhibiting movement to the breeding grounds (Richardson 1978) or indirectly by altering foraging conditions that delay the tempo of migration

(Lindstrom and Alerstam 1992, Bauer et al. 2008) or acquisition of nutrients necessary for breeding (Drent et al. 2007). Delays may result in later nest initiation dates and reduced reproduction or reproductive failure (Skinner et al. 1998, Krapu et al. 2000, Alisauskas 2002, Devries et al. 2008).

The ultimate factor controlling progression of migration in many populations is the accrual and use of nutrient reserves (namely lipids) to fuel migration (Jenni and Jenni-Eiermann 1998) and for subsequent allocation to reproduction after arrival (Drent et al. 2006). Impediments to nutrient accumulation or maintenance at stopover locations can slow progression and delay arrival (Lindstrom and Alerstam 1992, Drent et al. 2003, Smith and McWilliams 2014). Faster migration and earlier arrival confers energetic advantages for birds limited by nutrient acquisition prior to breeding (i.e., capital breeders *sensu* Drent and Daan 1980), because it allows time to acquire nutrients for use in clutch formation (Klaassen et al. 2006) or territory establishment and maintenance (Krapu 1981, Smith and Moore 2005). Thus, nutrient reserve balances during the transition to breeding among many birds, and particularly waterfowl (Ankney et al. 1991), are strong predictors of onset of reproduction and ultimate productivity (Esler and Grand 1994, Ebbinge and Spaans 1995, Alisauskas 2002, Devries et al. 2008).

Considerable empirical research has been conducted on long-distance migratory geese across the Holarctic regions, to understand potential impacts of interannual climatic variability on migration ecology. However, these populations are unique with respect to their capacity to store nutrients, because their large body size and near-universal tendency to exploit anthropogenic foods from agricultural (e.g., Krapu et al. 1995, Madsen 1995, Alisauskas 2002, Jefferies et al. 2004, Fox et al. 2005). Most research on inter- and intraannual variation in nutrient reserves of smaller-bodied ducks has been conducted on the breeding grounds, after spring migration. These studies have clearly shown the importance of nutrient reserves accumulated either en route to the breeding areas (Krapu 1981, Esler and Grand 1994, Esler et al. 2001, Devries et al. 2008) or on the breeding grounds after arrival (Alisauskas and Ankney 1994, Cutting et al. 2011, Janke et al. 2015). However, few studies have examined how and when ducks accumulate nutrient reserves during spring migration and most research in North America has been on ducks that can forage in terrestrial environments during migration (e.g., Lagrange and Dinsmore 1988, Pearse et al. 2011). Understanding factors affecting nutrient reserve dynamics among ducks across of range of environmental conditions could help explain patterns in annual productivity, improve allocation of conservation efforts for those species (Arzel et al. 2006), and anticipate the consequences of changing migration conditions in future climate scenarios (Marra et al. 2005).

We evaluated annual variation in physiology and diets of two species of spring-migrating, wetland-foraging ducks — lesser scaup (*Aythya affinis*) and blue-winged teal (*Anas discors*) — in a key migration area at the southern edge of each species core breeding range. These two species make for interesting contrasts to previous migration research and with each other. They are both wetland-foraging obligates during migration, meaning all nutrients acquired are derived from plant or animal prey in wetlands, rather than supplemented by terrestrial food sources (e.g., Abraham et al. 2005, Pearse et al. 2011). They also occupy two extremes of a continuum of foraging strategies and habitat use: lesser scaup are diving ducks that use large permanent and semi-permanent wetlands (Anteau and Afton 2009b, Kahara and Chipps 2012), whereas blue-winged teal are surface-feeding ducks that show considerable flexibility in wetland use, ranging from ephemeral to permanent wetlands (Baldassarre 2014). Both species demonstrate potential for substantial dietary plasticity during migration (Anteau and Afton 2006, Hitchcock 2009), but transition to nearly exclusive animal-based diets during breeding (Swanson et al. 1974, Afton and Hier 1991). Finally they differ with respect to the timing of their arrival on our study areas, with lesser scaup generally arriving soon after ice-out on semipermanent or permanent wetlands (Austin et al. 2002), and blue-winged teal arriving relatively late among waterfowl. We conducted our study during four highly-variable springs with respect to weather that comprised the range of temperature variability observed over the previous 30 years. Our comparisons of the migration ecology of wetland-foraging ducks provided insights into factors affecting nutrient reserves of each species and the consequences of environmental stochasticity encountered during migration.

METHODS

Study area

We conducted our study in the Prairie Pothole Region (PPR) of eastern South Dakota, comprising most of the land east the Missouri River in the state. The region was described in detail by Johnson and Higgins (1997) who reported that wetlands comprised 9.8% of the land area in eastern South Dakota. Upland habitats were a mix of row crop agriculture (primarily corn and soybeans) and grasslands (Naugle et al. 2001). The region was mostly privately owned with scattered small (generally < 260 ha) parcels in public ownership that is managed for production of waterfowl and other wildlife. We conducted our research on 6 (2012 and 2015) or 12 (2013 and 2014) circular focal areas in the region each year. During 2012, we sampled on publicly owned lands in 8-km radius focal areas systematically placed in regions of high public land ownership. During 2013 through 2015 we worked on both privately and publicly owned lands in randomly-selected 4 km-radius focal areas selected for a concurrent study. Focal areas changed annually but were always distributed throughout the study region (Figure 1) and stratified across a gradient of agricultural land-use intensity (Chapter 4). Although repeated observations on the same study areas during each year of our study may have been advantageous for drawing annual comparisons, we assumed our spatial stratification strategy and relatively large annual sample sizes guarded against site-specific biases. This assumption was generally supported by other analyses with these data that indicated low variation in response to broad land cover patterns (Chapter 4) or latitude, and the high interannual variation reported here.

Spring climate

We calculated a seasonal climate index for each study year to inform interpretation of our results in the context of temperature variation relative to long-term conditions (30 year). The climate index was the cumulative mean daily temperature for all days during 1 March through 20 May each year. Temperature may be viewed as a proxy for the progression of spring-thaw and migration, which has been shown to influence the timing and migration ecology of lesser scaup (Austin et al. 2002, Finger 2013), waterfowl (Murphy-Klassen et al. 2005), and migratory birds in general (Marra et al. 2005, Swanson and Palmer 2009). We developed an index for each year during 1985 through 2015 by extracting daily temperatures for the 30 study areas we sampled during 2013 through 2015 from the Parameter-elevation Regressions on Independent Slopes

Model (PRISM) daily mean temperature rasters available for the continental U.S. (PRISM Climate Group, Oregon State University). The sites at which we extracted temperature values were evenly distributed throughout the study area, and therefore their average provided a suitable approximation of temperatures observed across the study region in each year (Figure 1).

The long-term average cumulative mean temperature on 20 May was 5.5 C (95% CI = 4.8-6.2) over the 30 year time series. The cumulative mean temperatures on 20 May during our study ranged from 1.4 C to 10.1 C and included the coldest (2013) and warmest (2012) observed values in the 30 year time series (Figure 2, Table 1). The other 2 years in the study fell near the 25th (2014) and 75th (2015) percentile of the cumulative mean temperature observations in the last 30 years (Figure 2). Thus, the 4 years of our study comprised the whole range of variability in spring temperatures observed in the study area over the last 30 years.

Duck collections

To ensure we collected across the entire study region in each year, we attempted to collect ca. 10 individuals of each species on each focal area, though in some instances lesser scaup were not abundant enough on a site to meet this criteria. We began collections on a site once surface water was approximately ≥60% ice-free. In later springs, this may have precluded sampling the vanguard of the migration because they could arrive while some larger wetlands and lakes were still ice-covered. However, we attempted to conduct our collections during peak migration in each year, which consistently occurred after the ice-out criteria was met, so any bias should be small and consistent among years. All collections were done ≥6 hours after sunrise to allow individuals to forage in the study area before being collected. We targeted foraging individuals, but opportunistically collected non-foraging birds if they were encountered. In all cases, we noted whether the individual was observed foraging or with foraging conspecifics. All collections were done with shotguns and non-toxic shot (Envrion-metal, Inc., Sweet Home, OR) from shore or small boats without the use of decoys to avoid potential biases (Pace and Afton 1999). Immediately after collection, we drew a 0.5-1 mL sample of whole blood via cardiac puncture with a 16-21 ga. heparinized needle and 3 mL heparinized syringe. We transferred whole blood from the syringe to a heparinized 1.5 mL microcentrifuge tube and then stored the sample in a cooler away from direct-contact with ice until centrifuging. We slowly injected a small volume (3-5 mL) of 10% buffered formaldehyde solution into the upper gastrointestinal (GI) tract to stop postmortem digestion and placed a small rubber band around the bill to retain upper GI contents. We uniquely labeled each bird and blood sample and placed the bird in a freezer bag and stored it at -20 C until further processing. We centrifuged blood samples in the field at 4,000-6,000 rpm for 5-10 minutes and transferred plasma to a 1.5 mL microcentrifuge tube and froze it at -20 C. Plasma samples were transferred to -80 C freezer within 1 month of collecting and stored at that temperature until processing within 8 months.

Laboratory analyses

Necropsies—We conducted detailed necropsies on all individuals following Janke et al. (2015) and Afton and Ankney (1991). We measured wet mass of the thawed bird to nearest gram and recorded the following morphometrics: total length from tip of the bill to the end of the longest retrix, length of the longest retrix, wing chord length, keel length, and tarsus length. We excised one breast muscle, including the supracoracoideus and pectoralis, and one leg and thigh muscle, removed all visible fat and bones, and recorded the wet mass. We removed all visible fat deposits in the abdominal cavity, including discrete omental fat deposits and all fat associated with the heart, gizzard, and intestines and recorded its total wet mass (hereafter abdominal fat). We recorded the wet mass of the heart. We removed the gizzard and recorded the wet mass with all contents and then rinsed it to remove the contents and recorded the wet mass. We repeated the same procedure with intestinal contents and mass to account for the mass of ingesta in the lower GI. Finally, we rinsed and froze contents of the esophagus and proventriculus to evaluate diets.

We attempted to time collections to coincide with peak migration but we occasionally incidentally collected individuals that had transitioned into breeding. Our strategy to gauge migration progression and commence collections immediately after ice melt ensured that most collections occurred before transition into breeding, particularly after the pilot year in 2012. During 2012 however, we attempted to collect more individuals on each site and continued collections into the early breeding season, particularly for blue-winged teal (Janke et al. 2015). We collected ducks over 45 days in 2012, whereas we never exceeded 33 days for lesser scaup, and never exceeded 26 days for blue-winged teal in the other 3 years. Therefore, to maintain consistency among years, we excluded all birds collected after 30 April in 2012 to avoid including birds that were transitioning into breeding status (see Janke et al. 2015) and to focus inferences on peak-migration timing in that year. We further restricted our analysis to migrants by excluding individuals had commenced rapid follicle growth (RFG), which we defined among blue-winged teal as having mean size of the 3 largest ovarian follicles >5 mm (Janke et al. 2015). No evidence of RFG was noted among scaup.

Proximate analysis—We used proximate analysis to quantitatively determine whole-body lipids for a systematic subsample of individuals of each species to build a predictive relationship between abdominal fat and whole-body lipids (Devink et al. 2008). We used a systematic random sample to select 15 individuals of each species across the range of abdominal fat measurements recorded in the necropsy, and included the individual with the largest and smallest abdominal fat measurements (total n = 17 individuals/species). We homogenized ingesta-free tissue with all organs, fat, and feathers in a Hobart industrial meat grinder (Model 4146, Hobart Corporation, Troy, Ohio) and further homogenized the wet sample with a food processor (Model QB900, SharkNinja Operating LLC, Newton, Massachusetts). We then took a ca. 100 g subsample of the wet homogenate and dried it to a constant mass at 80 C for 24-36 hours. We reweighed the dried sample to calculate sample dry mass, and further homogenized the sample with a high-speed rotor mill (Retsch Ultra Centrifugal Mill ZM 200, Retsch GmbH, Haan Germany) so it could pass through a 2 mm sieve. We submitted a ca. 10 g subsample of the homogenate to SGS Laboratories in Brookings, South Dakota to perform petroleum ether lipid extraction in a modified Soxhlet apparatus. We determined the mass of lipids in the sample by multiplying the proportion of lipids in the subsample by the sample dry mass (Afton and Ankney 1991).

We used simple linear regression to predict total somatic lipids (g) in the sample from the mass of abdominal fat (g) recorded during necropsy. We square-root transformed abdominal fat to improve model fit (Devink et al. 2008) and evaluated if ingesta-free mass (weight of ingesta from gizzard and intestines subtracted from wet mass) improved model fit. We excluded one sample with the highest abdominal fat mass among the blue-winged teal because it was a substantial outlier. The final model predicted this individual to have 57.8 g of lipids, but the laboratory procedures determined it to have 17.8 g. We had noted the presence of conspicuously irregular structures of abdominal fat during the necropsy of this bird and therefore feel this exclusion was justified. Ingesta-free mass improved model fit for blue-winged teal (P = 0.003), but not lesser scaup (P = 0.712), so we included it in the former model, but not the latter. The final equations to predict somatic lipid reserves of each species were:

$$\text{Blue-winged teal somatic lipids (g)} = -55.89 + 15.04 * \sqrt{ABF} + 0.17 * IFM$$

$$\text{Lesser scaup somatic lipids (g)} = -27.24 + 35.98 * \sqrt{ABF},$$

where ABF is abdominal fat in grams and IFM is ingesta-free mass in grams. Both equations had strong model fit (blue-winged teal adjusted r^2 = 0.955, lesser scaup adjusted r^2 = 0.931) and were used to predict somatic lipids for all individuals in the analysis.

Diet processing—We thawed diets and sorted and identified food items. We identified invertebrates into families, except we identified Amphipoda to genus and non-Amphipoda crustaceans to order (i.e., Copepoda, Ostracoda, Cladacera). We identified fish to species. We distinguished between agricultural (i.e., corn) and non-agricultural (natural) seeds. We dried contents in each category to a constant mass in a 60 C oven and to record dry mass (±0.0001 g).

Metabolite assays—We measured concentrations of key lipid metabolites in plasma samples to index the recent trajectory of lipid reserves of each individual in each year. Metabolites respond relatively quickly (i.e., hours to 1 day) to changes in metabolic state (Jenni-Eiermann and Jenni 1998, Guglielmo et al. 2005), and therefore allowed us to evaluate if, on average, birds migrating through the study area in each year were accumulating or catabolizing lipids at different rates. We focused on 2 key lipid metabolites – triglycerides (TRIG) and β-hydroxybutyrate (BUTY) – which each reflect contrasting trajectories of either lipid accumulation (TRIG) or catabolism (BUTY). We measured concentrations in plasma samples using commercially available kits and standards typical in other lipid metabolite studies (Appendix 2).

Statistical analyses

Collection weather—We used Analysis of Variation (ANOVA) to test if mean temperatures over the span of collection dates in each year differed in the same manner that cumulative mean temperatures differed among the 4 years. This allowed us to ask if observed variation in diets or physiology among years was most-likely associated with variation in temperatures during migration or associated with other factors each spring, such as timing of migration within the year or the phenology of wetlands.

Physiological condition—We tested for differences in mean somatic lipid and protein reserves among the 4 years for each species. We used predicted total somatic lipid mass from the proximate analysis equations to test differences in lipid reserves. We calculated an index of protein reserves by summing 2 times wet mass of breast and leg muscles and wet mass of the heart. Muscle mass and heart mass were correlated in both species (lesser scaup $r = 0.73$, blue-winged teal $r = 0.43$). Breast and leg muscles represent important protein pools in waterfowl (Alisauskas et al. 1990, Janke et al. 2015) and heart mass is indicative of improved overall physiological condition in migrants

(Driedzic et al. 1993). We calculated an index of body size by using the first axis from a Principal Components Analysis (PCA) that combined 4 correlated morphometrics: body length (total length minus retrix length), wing length, keel length, and tarsus length (Afton and Ankney 1991). The first principal component for scaup loaded all terms from 0.18 to 0.69 and explained 34.4% of the variation in the 4 morphometrics. The first principle component for blue-winged teal loaded all terms from 0.47 to 0.54 and explained 41.6% of the variation in the 4 morphometrics. We used Analysis of Covariance (ANCOVA) with this covariate to test for annual differences in nutrient reserves of each species, controlling for structural size variation (Afton and Ankney 1991). The PCA was significant ($P < 0.001$) in all analyses except for the lesser scaup fat analysis ($P = 0.050$) but was left in the analysis for consistency.

We combined concentrations of TRIG and BUTY into a composite refueling index for each individual. This refueling index offered advantages over interpreting concentrations of individual lipid metabolites separately because it accounts for the latent correlation structure between the two metabolites and expresses values in a composite index of refueling performance, with higher values indicating relatively improved lipid accumulation (Chapter 3). Higher mean values in years indicated birds collected in that year were, on average, accumulating more lipids than birds in years with lower mean scores. We used ANOVA to test for variation in mean refueling indices among years for each species. Before compositing the indices we conducted exploratory analyses on metabolite concentrations to ensure there was not bias associated with collection timing within the day, latitude of collection, or pair or foraging status of the individual noted at the time of collection. We did not find any biases in these metrics, so we continued with raw metabolite concentrations, excluding highly hemolyzed samples (plasma hemoglobin concentration >1 g/dL; Chapter 2).

We tested for potential biases of only sampling on publicly owned lands in the first year of the study by testing for additive impacts of land ownership (public vs. private) with each individual response variable described above in the latter 3 years of the study. The land-ownership term was insignificant in all models ($P \geq 0.208$), so we assumed there was no bias among study years and reported results from models without the land ownership term.

Finally, we were interested in direct comparisons of physiological condition among years between the two species, to compare potentially contrasting nutrient strategies or physiological impairments among years. To facilitate direct comparisons between the two species, we calculated the ratio of lipids to ingesta-free body mass in each year and tested for differences between species and years with an ANCOVA. This ANCOVA used PC1 as a covariate, which was interacted with species to mimic the species-specific models used above. Significant species-specific effects in the ratio would indicate that the two species contrasted in the ratio of lipid reserves to whole body mass among years, perhaps indicating different responses to interannual variation in migration conditions.

Diets—We excluded all diet samples with <2 mg (dry weight) of food in the upper GI and considered those samples empty to avoid averaging aggregate percentages across small samples. We conducted 3 statistical tests on diet data for each year. First we used a Chi-square test to evaluate if there was a difference in the number of individuals collected in each year that had <2 mg food in their upper GI, as an index of feeding success or frequency for each species among years (Anteau and Afton 2008b). Second, we used ANOVA to test for differences among years in the proportion of the diet samples composed of animal material (fish and invertebrates) to evaluate annual variation in the relative contribution of animal or plant-based foods (seeds and vegetation) to diets of each species. Finally, we used Multivariate Analysis of Variation (MANOVA) to test for annual variation in the composition of key taxonomic groups of food items in the diets. We categorized food items into 7 categories based on previous research that demonstrated the relative importance of key food items for lesser scaup and blue-winged teal (e.g., Dirschl 1969, Swanson et al. 1974, Anteau and Afton 2008b, Hitchcock 2009, Tidwell 2010). We focused on 3 specific categories of invertebrates that both species consume in the Northern Plains or during migration — Amphipoda, Chironomidae, and Mollusca (including Sphaeriidae and gastropods) — and grouped remaining invertebrate taxa into a category called other invertebrates. We examined seed consumption separately from other vegetation (algae, tubers, leaves, etc.) to evaluate relative seed consumption among years, which is variable in both species (e.g., Hitchcock 2009). We used MANOVA to test for annual variation in the proportion of diets comprising the 4 main diet items in our analysis; Amphipoda, Chironomidae, Mollusca, and seeds. In excluding other invertebrates, vegetation, and fish, we focused our evaluation on the key diet items in the study and also reduced dependency among the proportions in the analysis. We used a randomization procedure to guard against violations of the multivariate normality assumption of MANOVA by randomly reordering the year factor in the data frame and calculating Wilk's Λ for 10,000 simulations (Chipps and Garvey 2007). We then compared our test statistic to the distribution of the randomization procedure test statistics to evaluate whether it was in the bottom 5th percentile or lower, indicating significance at an $\alpha = 0.05$. We used a Tukey Honest Significant Difference test to make post-hoc multiple comparisons of all ANCOVA and ANOVA analyses and assessed significance at $\alpha = 0.05$.

RESULTS

We included 232 lesser scaup and 322 blue-winged teal in the nutrient reserve analyses (Table 1). Collection dates among years varied with weather: for example, 75% of the scaup collected during the two warmest years, 2012 and 2015, were collected before all but 1 individual in the coldest spring of 2013 was collected (Table 1). Although there was substantial inter-annual variation in cumulative mean temperatures during the study (Figure 2, Table 1), temperatures during collecting did not differ significantly for blue-winged teal ($F_{3,90} = 0.515$, $P = 0.673$) or lesser scaup ($F_{3,108} = 1.091$, $P = 0.356$; Figure 3).

The index of protein reserves of lesser scaup varied significantly among years ($F_{3,227} = 11.692$, $P < 0.001$; Figure 4) and the multiple comparisons test revealed that 2013 had a lower mean and all other years were similar (Figure 4). Blue-winged teal protein masses followed the same results, illustrating significant interannual variation ($F_{3,317} = 9.306$, $P < 0.001$; Figure 4) and the only paired significant difference was a lower mean during 2013 (Figure 4). There was also significant interannual variation in lipid masses of lesser scaup among years ($F_{3,227} = 11.3195$, $P < 0.001$; Figure 4) and the multiple comparisons test revealed variable differences among years (Figure 4). The ANCOVA on lipid masses of blue-winged teal was significant ($F_{3,317} = 3.226$, $P =$

0.0228; Figure 4) but the multiple comparisons test revealed no significant differences among years (Figure 4).

We included 181 lesser scaup in the metabolite assays after excluding highly hemolyzed samples (range = 28 to 75 samples included annually). There was no significant variation in the refueling index among years for lesser scaup ($F_{3,176}$ = 1.797, P = 0.149; Figure 4). We included 285 blue-winged teal samples in the metabolites analysis (range = 48 to 94 samples included annually). There was significant annual variation in the refueling index ($F_{3,227}$ = 10.941, $P < 0.001$; Figure 4), and the multiple comparisons test revealed variable differences among years.

The analysis on the ratio of lipids to ingesta-free body mass revealed significant differences among years ($F_{3,544}$ = 6.347, $P < 0.001$), species ($F_{1,544}$ = 152.331, $P < 0.001$), and their interaction ($F_{3,544}$ = 10.497, $P < 0.001$). The multiple comparisons procedure revealed that lesser scaup had significantly higher ratios than blue-winged teal during 2012, 2014, and 2015 (mean difference ± SE = 5.17 ± 0.68, 4.71 ± 0.54, and 4.4 ± 0.7, respectively; $P < 0.001$) but did not differ during 2013 (0.29 ± 0.82, P = 0.684; Figure 5).

We recovered usable diet samples from 228 lesser scaup. Of these, 29 individuals (12.7%) had <2 mg of food and were excluded. There was no annual variation in the proportion of diet samples containing ≥2 mg of food (X^2 = 5.352, P = 0.148) and there was also no annual variation in the proportion of the diet comprising animal material ($F_{3,195}$ = 1.057, P = 0.368; Figure 6). There was evidence for significant annual variation in the composition of diets ($Wilks\ \Lambda$ = 0.8954, 4.6th percentile of simulations, P = 0.045; Figure 7). The post-hoc ANOVA of individual diet items included in the MANOVA indicated that only Mollusca varied significantly among years (P = 0.013). We recovered usable diet samples from 314 blue-winged teal and found 95 individuals (30.2%) had <2 mg of food. There was no annual variation in the proportion of diet samples containing ≥2 mg of food ($X^2 = 0.625$, $P = 0.8907$) and there was significant variation in the proportion of the diet comprising animal material among years ($F_{3,215} = 13.232$, $P < 0.001$; Figure 6). There was also significant interannual variation in the composition of diets ($Wilks\ \Lambda = 0.754$, <1st percentile of simulations, $P < 0.001$; Figure 6). The post-hoc ANOVA of diet composition indicated Chironomidae ($P < 0.001$) and seeds ($P < 0.001$) varied significantly among years.

DISCUSSION

We sampled during 4 disparate springs and observed notable interannual variation in the physiology of two sympatric wetland-foraging ducks. The similar temperatures we observed during collections in each year, despite the month-wide span of collection dates among years, suggests variation in physiology and diets was related to factors other than the direct effect of ambient temperature experienced by ducks at the time of collections. Rather, it seems variation in diets and key physiological metrics of these migrants changed in association with climatic variability in each year, which influenced the timing of migration and perhaps the phenology of wetland foraging habitats. Such interannual variation has implications for understanding factors affecting successful migration and associated carry-over effects into breeding among ducks (Sedinger and Alisauskas 2014).

Springs 2012 and 2013 provided stark contrasts and allow for interesting comparisons of 2 extremes of spring migration conditions in our study area. Among the metrics we investigated that demonstrated annual variation, 2012 and 2013 always contrasted, whereas patterns across the more moderate years of 2014 and 2015 tended to be inconsistent in their relation to cumulative mean temperature or separation from one another. The tendency for comparable results between 2014 and 2015 suggests migrant physiology is perhaps uninfluenced by conditions in our study areas during average years and only varies in response to exceptionally divergent conditions. Indeed, previous research on spring migrating ducks in the U.S. has reported generally low interannual variation (Strand 2005, Anteau and Afton 2009a, Tidwell 2010). Failure to observe physiological variation in more moderate seasons may also be due to confounding factors that influence physiological condition across broadly-varying spatial and temporal scales beyond those experienced during migration. Many studies have reported variation in waterfowl condition within and among wintering areas (e.g., Baldassarre et al. 1986, Miller 1986, Whyte et al. 1986, Thompson and Baldassarre 1990, Lovvorn 1994) and some studies have linked condition on wintering areas to those on breeding areas (Heitmeyer and Fredrickson 1981, Raveling and Heitmeyer 1989, Tamisier et al. 1995, Guillemain et al. 2008). Therefore, that we observed variation in our study in concordance with weather variability suggests migration conditions in our study area exceeded competing influences from distant and diverse wintering areas. In this context, we contend it may be appropriate to interpret the two extremes observed in our study as representative of a range of likely responses of spring-migrating lesser scaup and blue-winged teal to annual weather variation that is otherwise difficult to detect in less-extreme years.

Mean lipid mass of lesser scaup during 2013 was conspicuously lower than in the other 3 years and was almost unprecedented in the literature, particularly among migration studies. Among more than 30 lipid reserve estimates we found in the published literature for lesser scaup, only 1 study reported lipid masses lower than our mean value from 2013, and that study (Austin and Fredrickson 1987) was conducted on post-breeding lesser scaup undergoing remigial molt in late summer. Gammonley and Heitmeyer (1990) reported a comparably low (53 g) mean for females they collected during spring migration in California but they had a very small sample size (*n* = 5) and high variability (SE = 27). Anteau and Afton (2009a) collected migrating females throughout upper Midwest over 3 years and never reported a least-squared mean <70 g. We found fewer studies reporting lipid masses of blue-winged teal, but our values were functionally identical to those recently reported for spring-migrating blue-winged teal in Nebraska (26 g; Tidwell 2010). Therefore, it seems lipid reserves were typical of migrant blue-winged teal among the 4 years of our study but sensitive to annual variation among lesser scaup.

Arzel et al. (2007) described spring-migrating Eurasian green-winged teal (*Anas crecca*) as 'income migrants' meaning they fueled migration as they progressed to breeding grounds, rather than by accumulating reserves at stopovers for future use. This strategy appears consistent with our observation that blue-winged teal maintained functionally constant levels of lipid reserves annually and had variable annual refueling indices. Although we noted a decline in protein reserves among blue-winged teal during 2013, the consequence of that decline for migrating birds is likely minimal, because protein does not constitute an important fuel source (Jenni and Jenni-Eiermann 1998) and can be easily recouped by blue-winged teal on their breeding grounds (Ankney et al. 1991). Annual variation in diet composition and the refueling index also fit this pattern: in the year in which lesser scaup lipid reserves declined substantially, blue-winged teal increased intake of energy-rich foods (seeds) and had the highest refueling index score. Such a diet shift could explain the maintenance of lipids in that year and the concomitant decline in protein, because high dietary intake of invertebrate-rich diets has been associated with increases in protein masses in ducks (Alisauskas et al. 1990, Barzen and Serie 1990, Alisauskas and Ankney 1994). By extension, we would expect decreases in mean protein masses in years with reduced dietary intake of invertebrates.

Lesser scaup, in contrast, seem more likely to adopt a ‘capital migrant’ strategy where they at least maintain nutrient reserves accumulated on wintering or major staging areas (Anteau and Afton 2004, Herring and Collazo 2006), and possibly accumulate additional reserves en route to the breeding grounds for clutch formation (Afton and Ankney 1991, Esler et al. 2001, Anteau and Afton 2009a). Therefore, in contrasting blue-winged teal and lesser scaup, we would expect to observe relatively high ratios of lipid reserves to ingesta-free mass in lesser scaup and static refueling indices as they attempt to gain or maintain lipids during migration (Anteau and Afton 2011). We did observe this pattern during all 4 years with the refueling index, indicating the same accumulation or catabolism in each year, but lipid reserves regressed to low ratios during the 2013 season, comparable to those seen in blue-winged teal. Further, it appeared during 2013, the year with lowest lipid reserves in lesser scaup, that they decreased intake of dietary energy, because consumption of Amphipoda — an energy-rich food (Jorde and Owen 1988, Sherfy 1999) — declined (from 12.7% to 0.3% between 2012 and 2013) and consumption of mollusks (mainly gastropods) — an energy-deficient food (Jorde and Owen 1988, Sherfy 1999, Ballard et al. 2004) — increased (from 10.1% to 26.7% between 2012 and 2013). The significance of this protein-focused diet when lipid reserves were low is unclear, but could reflect a focus on maintenance of protein accumulation during migration (Anteau and Afton 2009a) or repair of muscle damage sustained during flight or starvation in that extreme year (Suter and Van Eerden 1992, Guglielmo et al. 2001). These dietary shifts, without concomitant increases in refueling performance, may also suggest lipid-rich prey were unavailable in sufficient quantities for lesser scaup to recover from lipid deficits accrued before peak migration through our study area. This interpretation would be consistent with a large body of work that suggested foraging conditions for lesser scaup are degraded in a way that inhibits lipid accumulation or maintenance during migration (Anteau and Afton 2008a; 2008b; 2009a; 2011).

Differences in the timing and tempo of migration between the two species could also help interpret patterns of nutrient reserve variation. Birds that arrive later in the season on average have less inter-annual variation in arrival dates (Murphy-Klassen et al. 2005), likely because they have less exposure to environmental stochasticity during transition from winter to spring. Such a risk-averse migration strategy may explain the different patterns we observed between blue-winged teal and lesser scaup. Blue-winged teal are comparatively late migrants and therefore likely arrived after the energetic burdens of late winter conditions subsided. In contrast, lesser scaup arrived earlier and therefore risk exposure to environmental stochasticity. Field observations during 2013 confirmed this pattern: we observed substantial numbers of migrating scaup prior to the onset of a cold-weather pattern during mid-April 2013, whereas we did not observe any blue-winged teal until after the pattern had subsided in late April and early May. Therefore, the lower mean lipid reserves among lesser scaup in 2013 could have manifested from a combination of unanticipated energetic demands (Suter and Van Eerden 1992, Lovvorn 1994), such as increased costs of thermogenesis associated with cold temperatures, decreased food availability because of ice cover (Lovvorn 1989), or potential energetic costs of reverse migration out of the study area (Richardson 1978). This hypothesis coincides with the pattern of lesser scaup lipid dynamics in our study, where accumulation (or catabolism) did not vary systematically among years, based on the refueling index, but lipid reserves varied considerably, perhaps further supporting the notation of degraded refueling habitat for migrant scaup in the region.

Regardless of the mechanisms driving annual variation in lipid reserves among lesser scaup, we would expect opposite results. That is, in warm years, we should expect that arriving on the breeding grounds with high lipid reserves is unimportant, because time schedules for breeding (Gurney et al. 2011) would allow time to recruit sufficient nutrients for use in clutch formation and incubation on the breeding grounds (Bromley and Jarvis 1993, Klaassen et al. 2006, Janke et al. 2015). In contrast, shortened time windows between arrival on the breeding grounds and clutch formation during late springs would afford less opportunity for local nutrient acquisition and therefore add value to nutrient reserves acquired away from breeding grounds (Klaassen et al. 2006). But our results for lesser scaup suggest individuals migrating through cold, late springs in the northern prairies have energetic burdens to recoup in a short time window before onset of clutch formation after arrival at breeding areas. Failure to recover from nutrient deficiencies incurred during migration could result in reduced population productivity in those years. The same would be true of migrating blue-winged teal, which still experienced a delay in arrival during late springs, and therefore have less time to recruit nutrients for early clutch formation.

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Table 1. Mean average temperature, sample size and collection dates of female lesser scaup and blue-winged teal collected during spring migration in eastern South Dakota during March through May of 2012 through 2015.

	Mean temp.		Collection dates			
Year	(°C)	*n*	First	25th percentile	75th percentile	Last
Lesser scaup						
2012	10.1	61	5-Apr	12-Apr	22-Apr	29-Apr
2013	1.4	38	23-Apr[b]	24-Apr	8-May	16-May
2014	3.0	88	11-Apr	23-Apr	29-Apr	13-May
2015	6.7	45	23-Mar	3-Apr	15-Apr	21-Apr
Blue-winged teal						
2012	10.1	60	1-Apr	9-Apr	24-Apr	30-Apr
2013	1.4	102	29-Apr	3-May	13-May	16-May
2014	3.0	101	21-Apr	24-Apr	5-May	12-May
2015	6.7	59	7-Apr	15-Apr	22-Apr	30-Apr

[a] Mean daily temperature from 1 March to 20 May

[b] One individual was collected on 8-April, prior to the onset of a severe storm that re-froze wetlands and delayed additional collections and migration until 23-April.

Figure 1. Location of focal regions used to study spring-migrating lesser scaup and blue-winged teal in eastern South Dakota during spring migration (March through May) of 2012 through 2015.

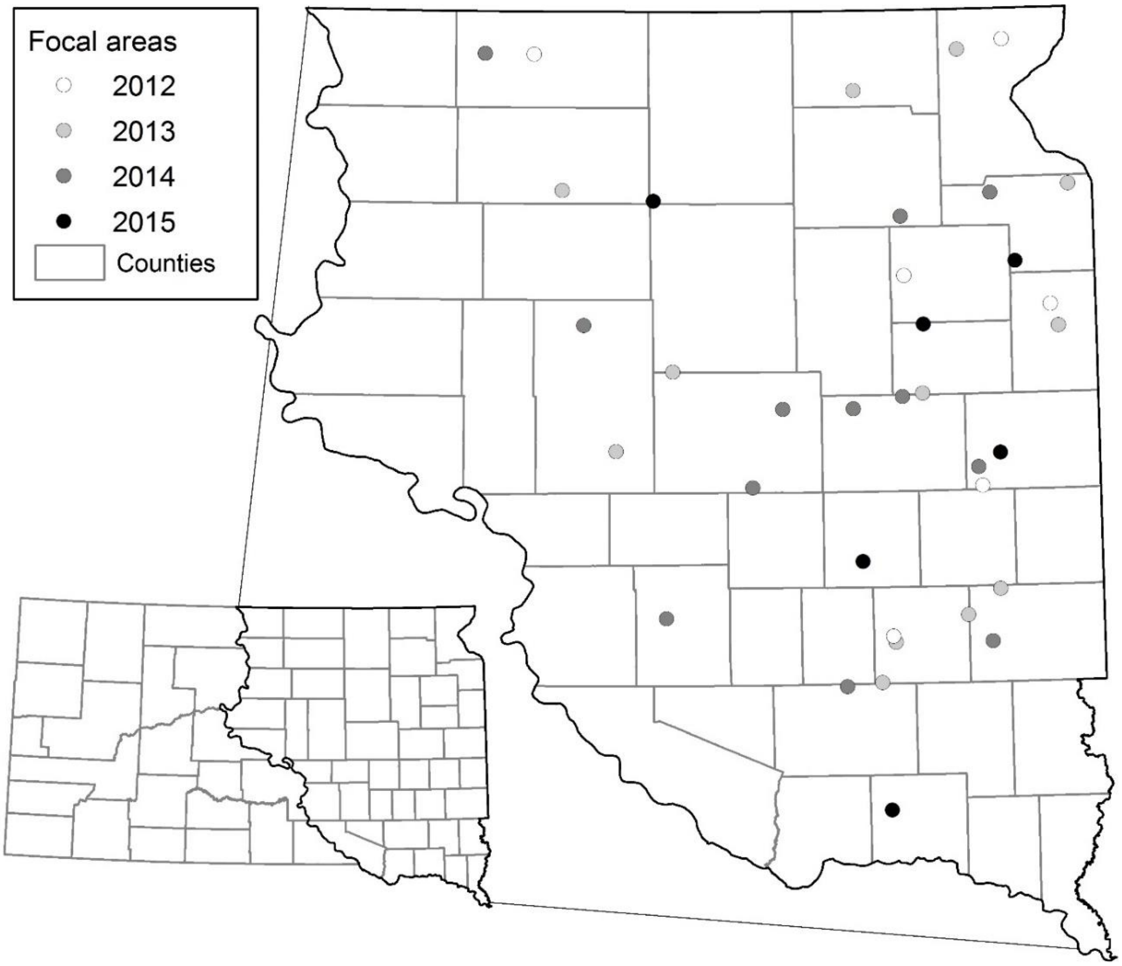


Figure 2. Cumulative spring temperatures in eastern South Dakota over 4 years (2012 – 2015, black lines) during which we collected spring-migrating female lesser scaup and blue-winged teal. Cumulative spring temperatures were a rolling average of all daily mean temperatures recorded from the observation date to 1 March in that year. Long-term interquartile range (IQR; dark shaded region) and long-term mean (light shaded region) were based on daily observations of the cumulative spring temperatures recorded over 30 years.

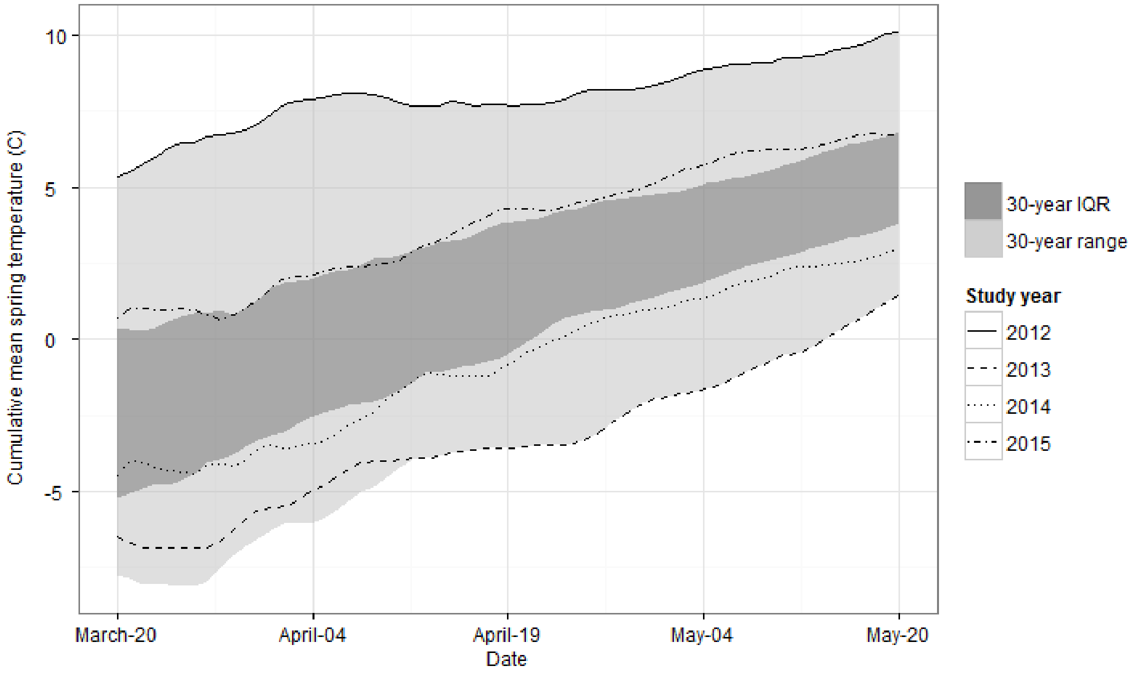


Figure 3. Boxplot of the range of daily temperature values observed in eastern South Dakota across the range of collection dates for spring-migrating blue-winged teal and lesser scaup during March through May 2012—2015. The relative constancy of the box plots for each species illustrates that although spring temperatures leading up to collection periods for each species were highly variable, temperatures during peak migration were similar among years.

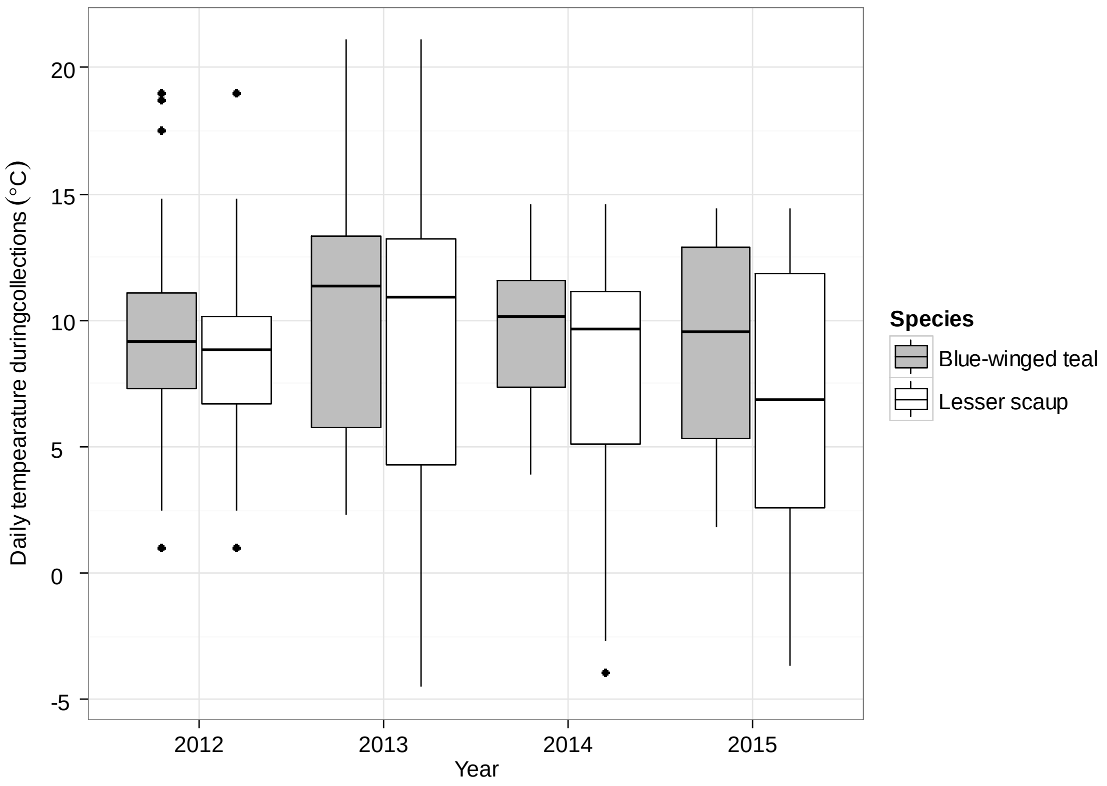


Figure 4. Annual variation in key physiological metrics measured in migrating female lesser scaup and blue-winged teal collected throughout eastern South Dakota during spring migration (March through May) of 2012 through 2015. Unique letters on figures indicate significant differences among years within the species based on a Tukey Kramer post-hoc multiple comparisons test (α = 0.05). Means for protein and lipid mass are Least-Squared means after controlling for variation in body size.

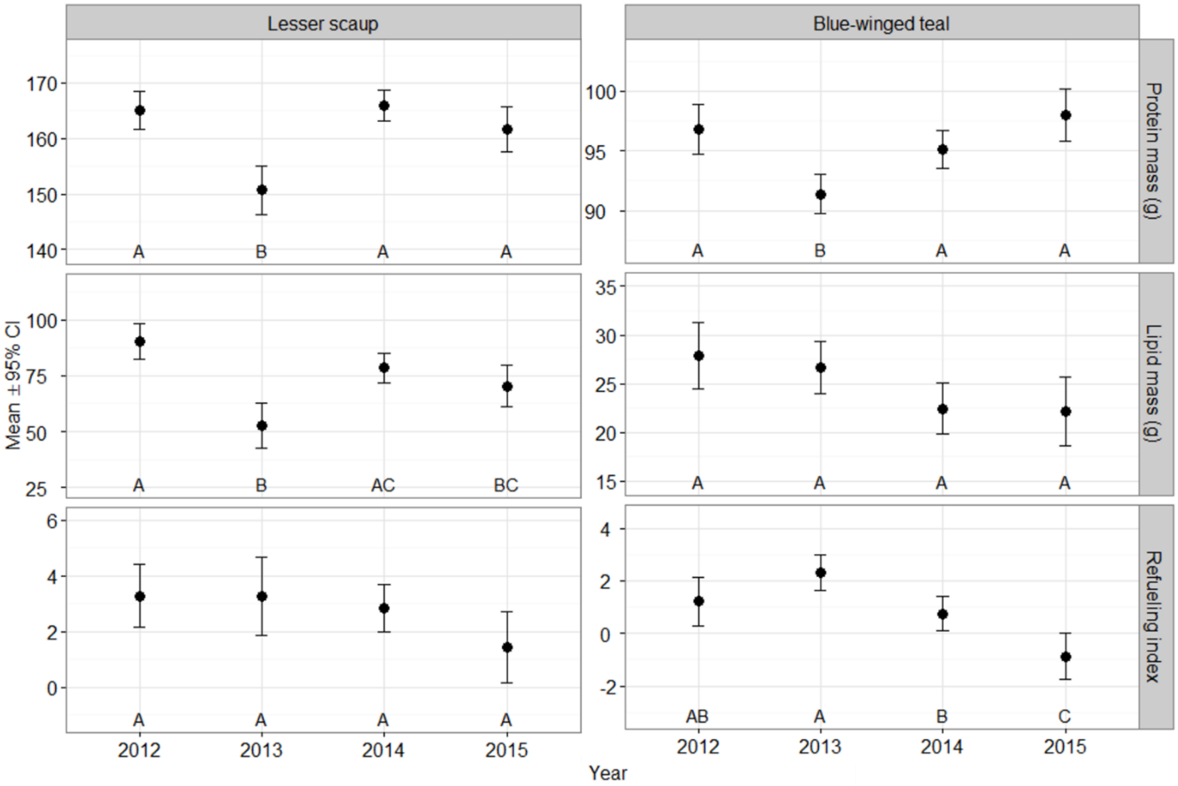


Figure 5. Annual variation in the ratio of lipid mass to ingesta-free body mass of migrating female lesser scaup (hollow circles) and blue-winged teal (filled circles) collected throughout eastern South Dakota during spring migration (March through May) of 2012 through 2015. Asterisks indicate significant species differences within each year based on a Tukey Kramer post-hoc multiple comparisons test (α = 0.05).

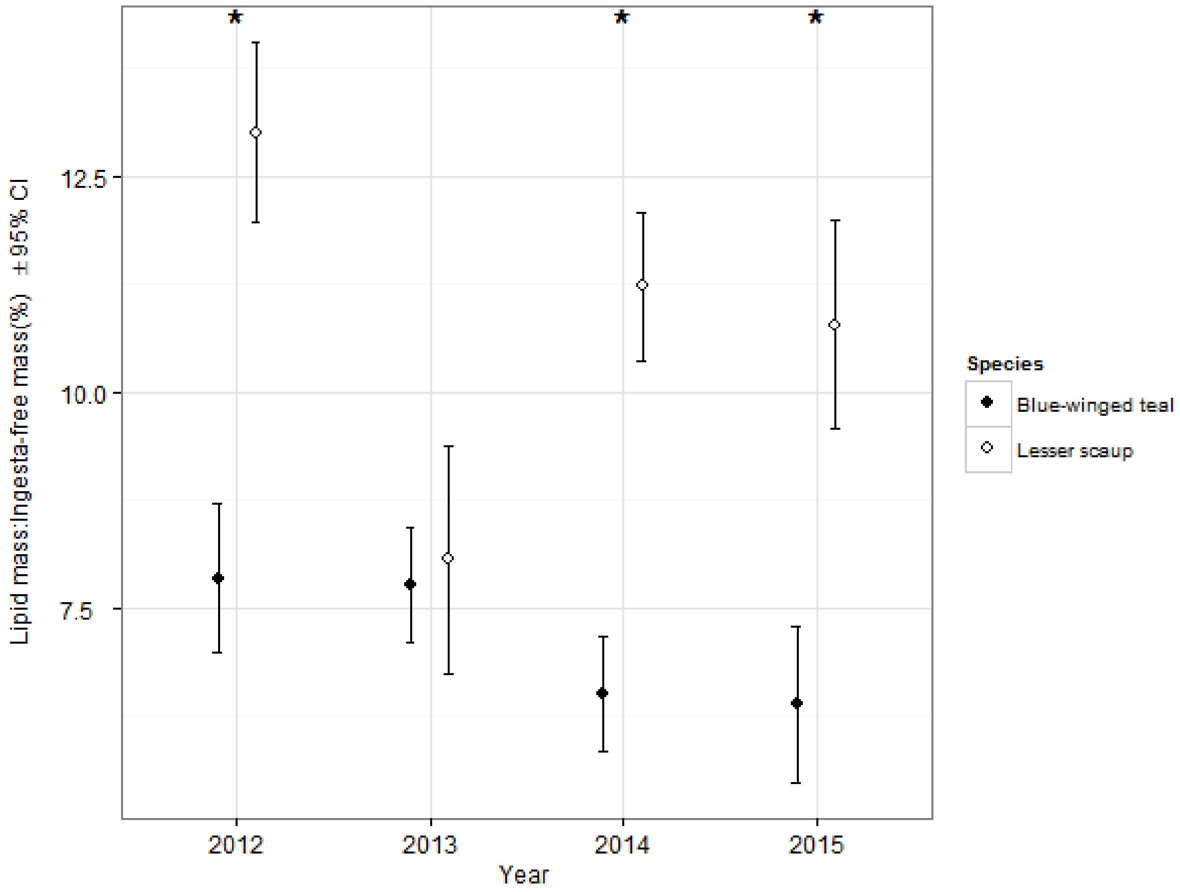


Figure 6. Annual variation in the proportion of food material in the upper GI that was animal matter in migrating female lesser scaup and blue-winged teal collected throughout eastern South Dakota during spring migration (March through May) of 2012 through 2015. Unique letters on figures indicate significant differences among years within the species based on a Tukey Kramer post-hoc multiple comparisons test (α = 0.05).

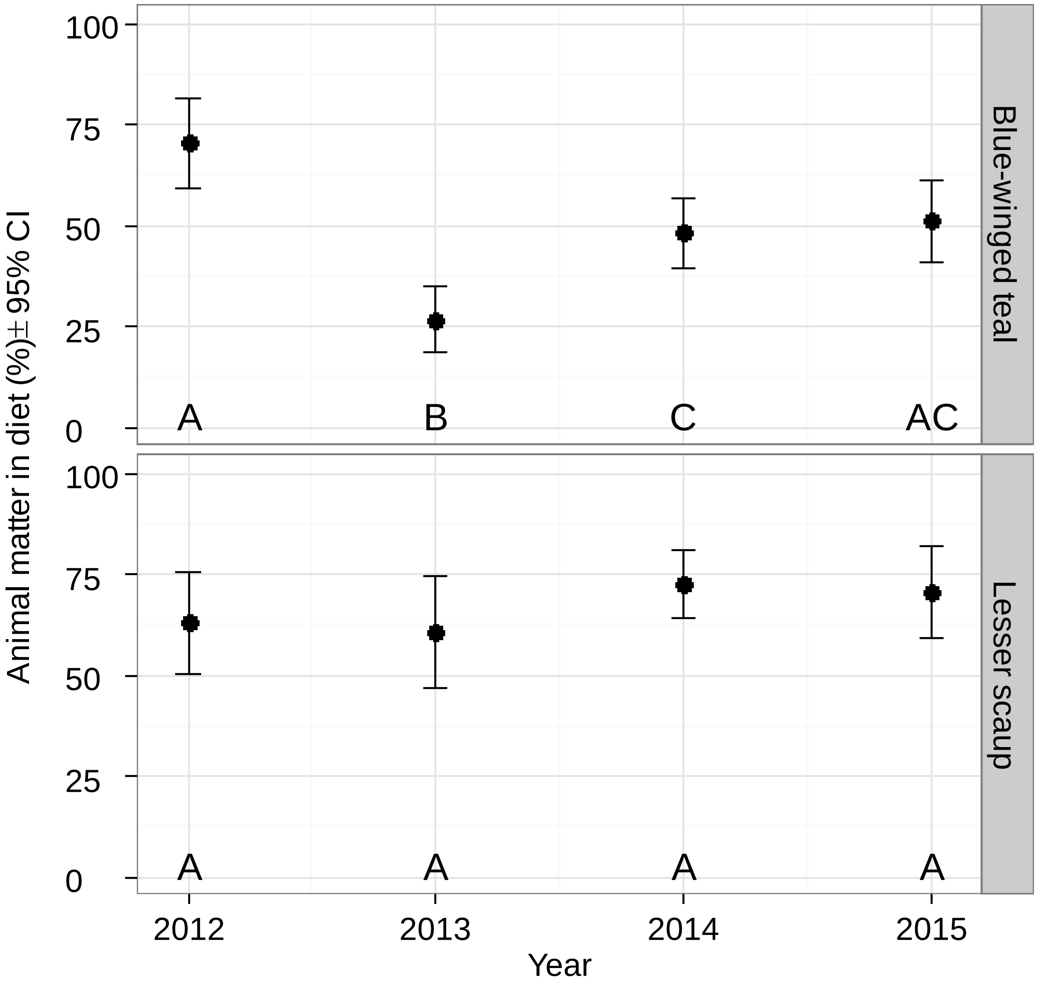
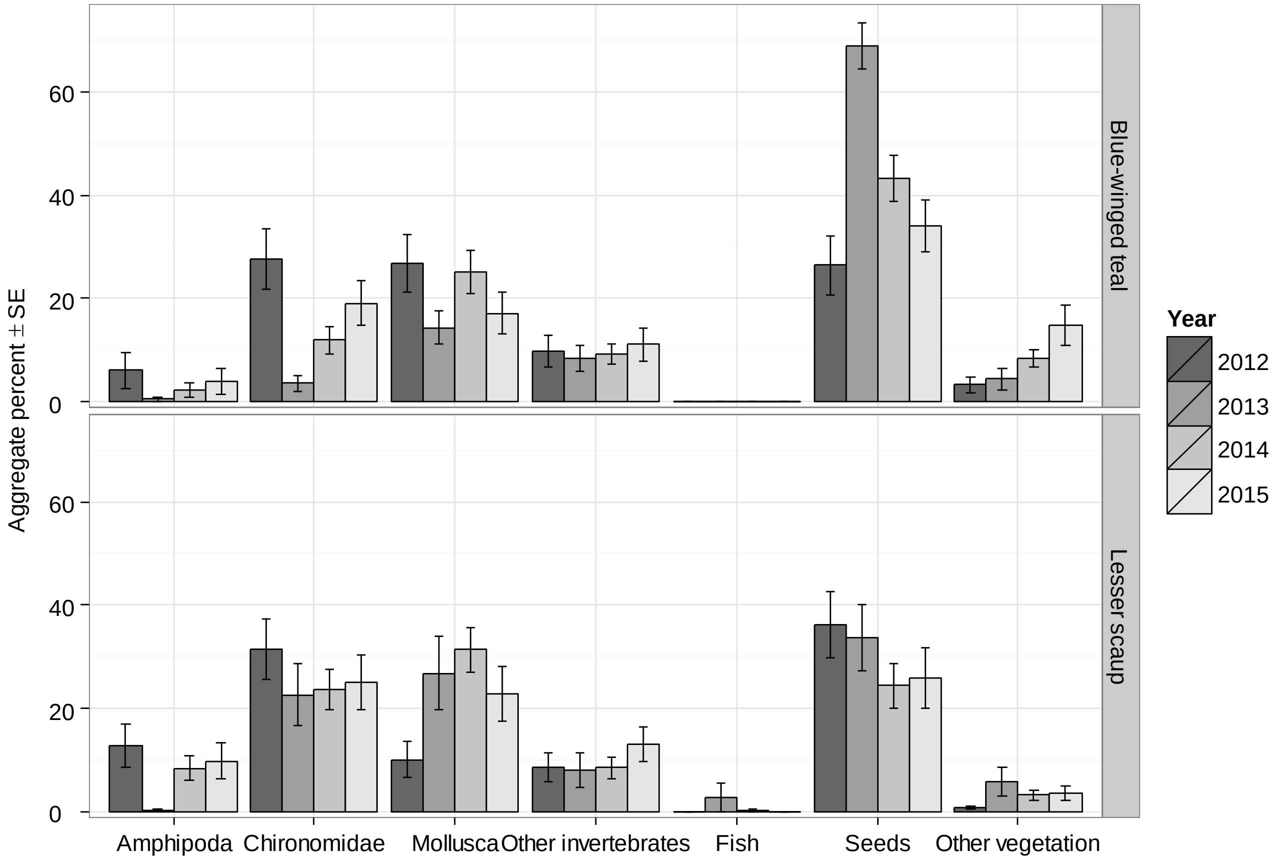


Figure 7. Annual variation in the aggregate percentages of different food items in the upper gastrointestinal tract of migrating female lesser scaup and blue-winged teal collected throughout eastern South Dakota during spring migration (March through May) of 2012 through 2015.



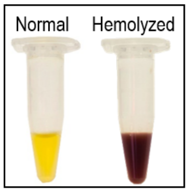
CHAPTER 7: ON CULTIVATION, CONSERVATION, AND MIGRATION IN NORTH AMERICA'S DUCK FACTORY: SUMMARY AND MANAGEMENT IMPLICATIONS

Although the utility of the Prairie Pothole Region (PPR) is traditionally assessed by its annual capacity to produce a significant proportion of the iconic fall-flights of North American waterfowl, a perceptive, comprehensive view of the region can, and arguably should, extend beyond its ability to produce ducks. Indeed, North America's Duck Factory is also the gateway to the rest of the duck factories in Canada and Alaska and therefore annually plays an important role in the pre-fabrication step – to extend the metaphor – of breeding waterfowl, as they acquire nutrients to fuel migration and subsequent breeding efforts during their vernal trek northward across the continent. Migrants encounter challenging environments during this annual return, chancing encounters with brief returns of winter conditions and foraging exclusively on the left-overs of last growing season's diminishing prey base. It is in this season, with these constraints, in which my dissertation research was conducted, and for those interested in understanding the availability and quality of habitats encountered by migrants in the southern PPR, my research offers a few new insights and raises new questions.

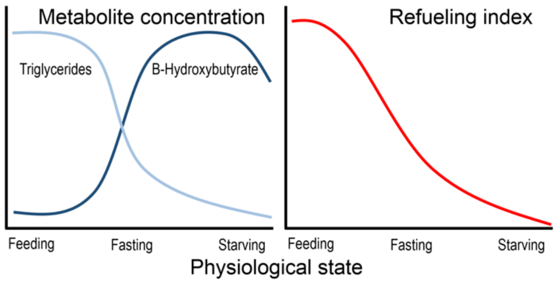
The central question for my dissertation research laid out in the introduction was; what is the current condition of prairie wetlands in agricultural landscapes with respect to their potential contributions to spring-migrating ducks? I started my dissertation by examining a few methodological questions that were only necessary precursors to addressing this central question. I decided not to summarize those results explicitly here, though a brief summary of the main conclusions and implications is found in Box 1.

Similarly, in Chapter 6 I addressed questions tangential to my central research question and showed that my two study species were indeed foraging extensively on aquatic invertebrates and that interannual variability in condition of migrants exists, clearly adding to the possible constraints imposed on waterfowl during spring migration.

BOX 1 – RECOMMENDATIONS FOR PLASMA METABOLITES RESEARCH



Plasma samples with a dark-red hue indicate red blood cells have ruptured at some point during sampling, a process called hemolysis. My analyses and review of the biomedical literature revealed that highly-hemolyzed samples, like the one shown here, should be excluded from avian plasma metabolites studies to avoid biased concentration estimates.



The two most-commonly used lipid metabolites used in migration studies are β-hydroxybutyrate (BUTY) and triglycerides (TRIG), which each index contrasting metabolic states of accumulation or loss of lipid reserves. I developed a composite index to combine the redundant information contained in both of these metabolites into a single index of relative mass change or loss. The resulting equation was predictive of mass change in independent data and corresponded well with other predictive models using one metabolite at a time.

$$Refueling\ Index = \\ -0.162 + 2.700\ \times TRIG\ - 8.207\ \times\ \ln(BUTY + 0.5)$$

The index can be a useful means to express relative refueling performance of migrants, particularly when comparisons among contrasting habitats are the main focus of the study. Comparisons among species or study areas with different diets is inappropriate however because the way dietary intake can change baseline concentrations of metabolites.

Chapter 4 is where I addressed my main research question and found that after controlling for inter-wetland variability, wetlands in intensively farmed landscapes in eastern South Dakota were providing at least the same contributions, and perhaps improved conditions, as those found in less intensively farmed landscapes across the three metrics I examined – invertebrate prey availability, duck abundance, and physiology – for blue-winged teal and lesser scaup. This result was surprising in light of the well-documented pathways for intensive upland cultivation to negatively impact surrounding wetland ecosystems, and raises an important question about the mechanism leading to similarities in wetland conditions across the landscape gradient I sampled, which I will revisit in my discussion of additional research questions below. The apparent conclusion from these results is that regardless of the mechanisms giving rise to the similarities, wetlands in South Dakota's intensively farmed landscapes have the capacity to make substantial contributions to spring-migrating ducks.

The similarity of contributions of wetlands to ducks across the upland cultivation gradient I measured does not of course imply agricultural-associated land use changes, namely those related to wetland drainage and modifications, do not impact ducks and wetlands. Analyses in Chapter 5, where I examined wetland-specific factors influencing the physiology of blue-winged teal and lesser scaup, showed evidence to the contrary. In that analysis, I found that variation in refueling performance in lesser scaup, and to a reduced degree blue-winged teal, was associated with variation in wetland attributes. Lesser scaup were performing better on relatively small, interspersed wetlands that were not degraded because of high fathead minnow densities. Extensive and thorough work in a diverse body of literature on shallow lakes in Minnesota and elsewhere has shown the complex impacts fathead minnow have in altering food webs and therefore whole aquatic communities in wetlands[a]. These pathways are outlined in Box 2. My results are the first to directly link food web impacts of fathead minnows to physiological impacts of ducks passing through on migration. Thus, fathead minnows in prairie wetlands introduce potential cross-seasonal and cross-ecosystem effects because migrant ducks accruing nutrient deficits on wetlands with high fathead minnow densities will then have to offset those deficits in other aquatic systems along their migration route or after arriving on

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[a] Hanson et al. (2005) *Wetlands* 25, 764-775

their breeding grounds. These findings also relate back to the central question about agricultural landscapes in my dissertation, because research has shown fathead minnows benefit from increased water permanency and connectivity[b], and both of these factors are favored by wetland drainage practices typical in intensively farmed landscapes[c].

IMPLICATIONS FOR POLICY AND MANAGEMENT

To state the obvious, migrating ducks don't use fully drained wetlands. Less obvious however, but implicit in my results, is that the drainage of those wetlands has impacts that reach beyond the loss of an individual stopover location. Combining my results with others, we can begin to see the clear trophic impacts that widespread wetland drainage can have in altering an entire wetland landscape: by draining small basins into larger basins, the hydrology of the landscape changes, which manifests in changes to the biotic communities in wetlands, and ultimately in physiological impairments to spring-migrating ducks. This mechanism was central to the articulation of the Spring Condition Hypothesis for explaining lesser scaup declines[d] and proposed as a likely mechanism in coarse-scale work[e,f] but never definitively established until now. If indeed fathead minnows, or other fish with comparable effects like black bullheads[g] or carp are sufficiently abundant in the modern prairie wetland landscape, they clearly could be having negative physiological consequences for spring-migrating wetland-foraging ducks in the region at a large scale. If indeed this is the case, policy makers should consider the implications of drainage practices that increase water permanency in remaining prairie

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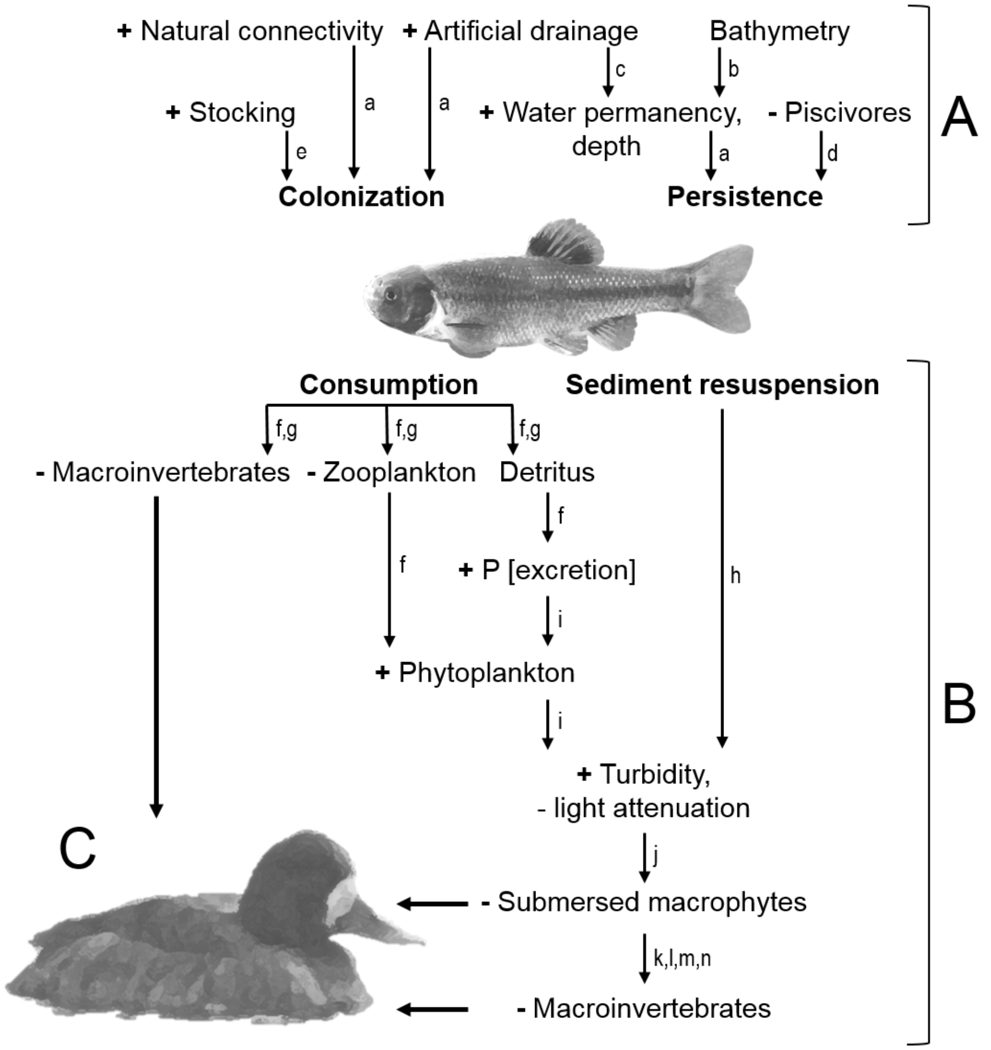
[b] Herwig et al. (2010) *Wetlands* 30, 609-619
[c] McCauley et al. (2015) *Ecosphere* 6, 1-22
[d] Anteau & Afton. (2004) *Auk* 121, 917-929
[e] Anteau & Afton. (2009) *Auk* 126, 873-883
[f] Anteau & Afton. (2011) *PLoS One* 6, e16029
[g] Maurer et al. (2014) *Wetlands* 34, 735-745

BOX 2 – FATHEAD MINNOWS AND PRAIRIE WETLANDS

Conceptual model of the factors influencing the colonization and persistence of fathead minnows in prairie wetlands (A), their impacts in prairie wetlands (B), and the resulting impacts on spring-migrating ducks (C) based on previous research and results presented in this dissertation. Lower case letters next to each line identify a citation for each step shown in previous research. + and - signs next to each step indicate the direction of the effect and absence of a sign indicates variable directional effects.



[a] Herwig et al. (2010) Wetlands 30, 609-619
[b] Kantrud et al. (1989) USFWS Biological Report 85
[c] McCauley et al. (2015) Ecosphere 6
[d] Potthoff et al. (2008) J. Appl. Ecol. 45, 1170-1179
[e] Carlson & Berry. (1990) Prairie Naturalist 22, 119-128
[f] Herwig & Zimmer. (2007) Ecol. Freshw. Fish 16, 282-294
[g] Duffy. (1998) Can. J. Fish. Aquat. Sci. 55, 15-27
[h] Hanson & Butler. (1994) Hydrobiologia 280, 457-466
[i] Zimmer et al. (2006) Limnol. Oceanogr. 51, 197-207
[j] Zimmer et al. (2003) Wetlands 23, 717-728
[k] Anteau et al. (2011) Hydrobiologia 664, 69-80
[l] Rennie & Jackson. (2005) Can. J. Fish. Aquat. Sci. 62, 2088-2099
[m] Hanson et al. (2012) PLoS One 7, e44644
[n] Many other citations

wetlands. Managers can focus on precluding factors that favor colonization and persistence of fathead minnows in semipermanent wetlands and, in cases where fathead minnows or other benthivorous fish already exist in high densities, consider management options to eliminate them.

Barring the negative consequences of altered hydrologic regimes of wetlands associated with drainage practices in agricultural landscapes, my results suggest that such wetlands still have capacity to make meaningful contributions to spring-migrating ducks. This finding has implications for policy makers in considering wetland-protection strategies or wetland restoration practices in agricultural landscapes. Whether these wetlands offer utility for breeding ducks likely depends primarily on the availability of surrounding upland nesting cover. That comparable densities of aquatic invertebrates can be found across the entire cultivation intensity gradient however, certainly implies that there is capacity to support reproduction in intensively farmed landscapes if constraints on nesting habitats are relaxed.

ADDITIONAL RESEARCH QUESTIONS

I have eluded to a number of potentially important follow-up questions to be addressed throughout my dissertation, but I wanted to explicitly highlight the three primary questions that I think are central to interpreting my results and understanding the potential contributions of wetlands in agricultural landscapes to waterfowl in the southern PPR. These questions are:

1. How prevalent are fathead minnows and other benthivorous fish species in semipermanent wetlands throughout the southern PPR and what factors facilitate their colonization and persistence? Answering this question is central

to defining the scope of the potential problem posed by benthivorous fish and the trophic modifications they impart, and could help improve wetland management strategies or in setting wetland drainage policies that reduce potential impacts. Important sub-questions for this research avenue include,

   a. Does agricultural drainage, both surface and subsurface, influence fathead minnow or other fish persistence and abundance?
   b. How has the recent wet-period on the U.S. prairies influenced the distribution and abundance of fathead minnows and how will a return to drier conditions affect their abundance?
   c. What management strategies can be used to reduce the impacts of fathead minnows and other fish on wetlands with extant populations?

2. What underlying mechanisms gave rise to the apparent similarities between wetlands in intensively farmed landscapes and those in less disturbed landscapes? This question should seek to address whether wetlands in agricultural landscapes have been degraded to their current condition or if they simply show resiliency to agricultural land uses. Important sub-questions for this research avenue include,
   a. What land-use factors favor improved wetland productivity for migrants, such as grassed buffers or drainage practices?
   b. What restoration efforts are feasible or merited in intensively farmed landscapes, if degradation has indeed occurred?

   c. Is there a threshold effect of agricultural land uses, namely nutrient enrichment, from which it is difficult for wetlands to recover, as commonly seen in shallow lakes[h]?

3. In light of the evidence in my research for relatively abundant invertebrate prey in wetlands in agricultural landscapes during spring, what is the potential capacity for these landscapes to support breeding ducks? Important sub-questions for this research avenue include,
   a. What factors are associated with breeding duck densities in these landscapes?
   b. Are the purported increases in predation pressure in these fragmented landscapes[i] too much to result in contributions to annual recruitment?
   c. What is the overall potential for reproduction across the expansive agricultural landscapes in the southern U.S. PPR?

I started the introduction to this dissertation with a quote from the 1936 North American Wildlife and Natural Resources conference to provide context and insights from the rich legacy of conservation in North America. I thought it fitting then to conclude the document with another quote from a conservation icon that spoke at that meeting. In his brief address on the importance of private lands for wildlife conservation, Aldo Leopold[j] remarked, “Few people as yet understand that wildlife is best produced as a thin crop. To get either quantity or variety you have to spread it over large areas.” This philosophy seems especially key today, as the footprint of anthropogenic land uses, and

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[h] Zimmer et al. (2009) *Ecosystems* 12, 843-852

[i] Cowardin et al. (1985) *Wildlife Monographs*, 3-37

[j] U. S. Government Printing Office (1936) *Proceedings of the North American Wildlife Conference*, 156-158

specifically agriculture in the mid-continent regions important for waterfowl, has grown in the last century, finding places for conservation on a large scale are increasingly challenging. In these landscapes, the difficult work to conserve and protect remaining native, unmodified grassland and wetland ecosystems are invaluable. But relics alone will not suffice. Through scientific understanding of factors influencing ecosystem stability and functioning, perhaps the sum contribution of many small parts offers a solution. This seems true for the most intensively farmed regions of the eastern and southern PPR in which I have shown there remains promise for waterfowl when wetlands are unaffected by drainage. In these landscapes, we can still find important wetland habitats serving their role in facilitating successful reproduction of waterfowl and providing a diversity of ecological services. With a little luck, good science, and sound conservation policies they can hopefully continue to do so for many generations to come.

APPENDIX 1: DETAILED DIET SUMMARIES

Detailed taxonomic summary of diets of female lesser scaup (*n* = 199) and blue-winged teal (*n* = 219) collected in eastern South Dakota during spring migration (March – May) of 2012 – 2015. Aggregate percentages are based on dry weight of each taxa.

	Lesser scaup			Blue-winged teal		
	Aggregate percent			Aggregate percent		
Organism	Mean	SE	Freq. of occurrence	Mean	SE	Freq. of occurrence
Crustacea						
Amphipoda						
Gammarus	0.773	0.470	3.015	0.000	0.000	0.000
Hyalella	7.471	1.426	32.161	2.638	0.878	10.046
Branchiopoda						
Cladocera	0.893	0.616	5.025	0.643	0.440	10.959
Cladocera resting eggs	0.051	0.026	8.040	0.145	0.065	4.566
Laevicaudata	0.001	0.001	1.005	0.255	0.252	0.913
Copepoda	0.000	0.000	0.000	0.001	0.001	0.457
Isopoda	0.000	0.000	0.000	0.003	0.003	0.457
Ostracoda	1.434	0.646	13.568	1.953	0.530	18.721
Mollusca						
Bivalvia						
Sphaeriidae	0.965	0.678	3.518	0.000	0.000	0.457
Gastropoda						
Hydrobiidae	1.361	0.784	1.508	0.145	0.145	0.457
Lymnaeidae	4.611	1.198	13.065	6.449	1.305	18.265
Physidae	3.685	0.888	20.603	2.360	0.769	10.502
Planorbidae	8.568	1.374	44.221	8.048	1.199	40.183
Valvatidae	3.913	1.276	7.035	0.417	0.417	0.457
Gastropod fragments	0.825	0.500	7.538	2.920	0.639	21.005
Clitellata						
Hirudinea						
Erpobdellidae	0.710	0.296	9.045	0.733	0.458	2.283
Glossiphoniidae	0.013	0.009	1.508	0.007	0.007	0.457
Insecta						
Arachnida						
Acarina	0.042	0.011	12.563	0.075	0.036	5.936

Araneae	0.000	0.000	0.000	0.129	0.100	2.283
Coleoptera						
Chrysomelidae	0.002	0.002	0.503	0.000	0.000	0.000
Curculionidae	0.000	0.000	0.000	0.010	0.007	0.913
Dytiscidae	0.042	0.027	2.010	0.183	0.086	6.849
Elmidae	0.000	0.000	0.000	0.004	0.004	0.457
Gyrinidae	0.000	0.000	0.000	0.008	0.008	0.457
Haliplidae	0.731	0.302	17.588	0.224	0.094	7.306
Helophoridae	0.000	0.000	0.000	0.001	0.001	0.457
Hydraenidae	0.000	0.000	0.000	0.000	0.000	0.000
Hydrophilidae	0.000	0.000	0.000	0.369	0.179	7.763
Staphylinidae	0.000	0.000	0.000	0.001	0.001	0.457
Coleoptera fragments	0.002	0.002	0.503	0.260	0.198	1.370
Diptera						
Ceratopogonidae	0.019	0.012	3.015	0.209	0.097	5.936
Chaoboridae	0.018	0.011	3.518	0.000	0.000	0.000
Chironomidae	25.617	2.515	57.286	13.232	1.679	40.183
Culicidae	0.000	0.000	0.000	0.001	0.001	0.913
Dixidae	0.000	0.000	0.000	0.004	0.004	0.457
Dolichopodidae	0.001	0.001	0.503	0.005	0.005	0.457
Empididae	0.000	0.000	0.000	0.001	0.001	0.913
Ephydridae	0.000	0.000	0.000	0.128	0.128	0.457
Psychodidae	0.000	0.000	0.000	0.040	0.027	1.370
Stratiomyidae	0.000	0.000	0.000	0.847	0.387	7.306
Syrphidae	0.000	0.000	0.000	0.221	0.221	0.457
Tabanidae	0.001	0.001	0.503	0.060	0.043	0.913
Tipulidae	0.000	0.000	0.000	0.010	0.008	0.913
Ephemeroptera						
Baetidae	0.248	0.116	8.543	0.026	0.026	0.457
Caenidae	0.185	0.072	12.060	0.038	0.032	2.283
Hemiptera						
Corixidae	0.451	0.213	9.548	0.677	0.381	4.566
Hebridae	0.000	0.000	0.000	0.002	0.002	0.913
Notonectidae	0.046	0.046	0.503	0.001	0.001	0.457
Pleidae	0.002	0.001	1.005	0.175	0.121	3.196
Lepidoptera						
Pyralidae	0.001	0.000	2.010	0.000	0.000	0.000
Odonata						
Aeshnidae	0.038	0.038	0.503	0.000	0.000	0.000
Coenagrionidae	1.793	0.438	26.633	0.067	0.037	3.653

Lestidae	0.011	0.011	1.005	0.000	0.000	0.000
Trichoptera	0.759	0.339	13.568	0.137	0.072	2.283
Miscellaneous invertebrates						
Terrestrial insect	0.837	0.594	2.010	1.056	0.513	7.763
Unidentifiable invertebrate	0.002	0.002	1.005	0.099	0.099	0.457
Aquatic insect fragments	0.512	0.276	7.538	0.477	0.228	10.046
Invertebrate eggs	0.537	0.503	3.015	0.085	0.047	2.283
Nematoda	0.000	0.000	0.503	0.000	0.000	0.000
Oligochaeta	0.000	0.000	0.503	0.010	0.007	0.913
Fish						
Pimephales promelas	0.612	0.492	1.508	0.000	0.000	0.000
Culaea inconstans	0.005	0.005	0.503	0.000	0.000	0.000
Vegetation						
Agricultural seeds	0.000	0.000	0.000	1.818	0.862	2.283
Natural seeds	29.033	2.759	75.377	45.138	2.668	89.954
Algae	0.001	0.001	0.503	0.858	0.596	1.826
Tubers	0.365	0.253	2.010	0.722	0.519	1.370
Miscellaneous vegetation	2.816	0.629	45.226	5.876	0.924	46.119

APPENDIX 2: ASSAY PROTOCOLS

Protocols for assays used to measure concentrations of metabolites or plasma hemoglobin. Metabolite assays were adapted from protocols originally developed and provided by Christopher Guglielmo at the University of Western Ontario.

TRIGLYCERIDE AND FREE GLYCEROL

Required supplies:

Triglyceride Reagent (Sigma – T2449-10ML)- Reconstituted with 10 mL dH_20
Glycerol Reagent (Sigma – F6428-40ML)- Reconstituted with 40 mL dH_20
Glycerol Standard Solution (2.82 mmol/L glycerol; Sigma G7793-5ML)
Supplies notes: Order equal number of bottles of Triglyceride and Glycerol reagents; Serum Triglyceride Determination Kit from Sigma (TR0100) contains both product but in unequal quantities. Each bottle can do about 125 wells.

Standard curve:

Create a serial dilution of the standard solution from 2.82-0.176 and include a well with dH_20. Make a higher standard of 5.64 by adding 2 volumes of standard to the well (10 µl).
Final serial dilution concentrations (mmol/L): 5.64, 2.82, 1.41, 0.705, 0.3525, 0.

Sample preparation:

Dilute samples 3-fold with 0.9% Sodium Chloride (Saline) if necessary for small volumes.

Procedure:

Load 5 µl of standard or sample to each well in duplicate; ensure that no samples have both duplicates on the edge of the plate - Vortex all samples before adding to wells
Load 240µl of the Glycerol Reagent to each well prewarmed to 37 C
Load plate into reader, prewarmed to 37 C
Incubate at 37 C 10 minutes
Shake low 10 seconds at 37 C
Read absorbance at 540nm and secondary (background) at 750nm
Eject the plate and add 60µl of the Triglyceride Reagent prewarmed to 37 C to each well
Load plate into reader, prewarmed to 37 C
Incubate at 37 C 10 minutes
Shake low 10 seconds at 37 C
Read absorbance at 540nm and secondary (background) at 750nm

Calculations:

Glycerol estimated by taking reference wavelength minus secondary wavelength
Total Triglycerides estimated by taking reference wavelength minus secondary wavelength
Multiply all concentration estimates by dilution factor – 3 fold dilution (2 parts dH_20 1 part sample) is multiplied by 3.

Calculate True Triglyceride by subtracting Glycerol from Total Triglyceride

Procedural notes:

Inspect results and rerun samples where CV ((SD/mean)*100) is greater than 15%

Dilute and rerun any samples that were estimated outside the standard curve

URIC ACID

Required supplies:

Uric Acid Reagent Set (Teco Diagnostics U580-240) – reconstituted with 12 ml dH_2O – 1 kit does 1200 wells.

Sodium Hydroxide (NaOH; Sigma 415413)

Uric Acid (Sigma U0881)

Glycine Free Base (Sigma G7126)

Supplies notes: The standard that comes with the reagent set is too low for concentrations observed in birds, which is why additional supplies are ordered to mix a custom standard.

Standard curve:

Create a serial dilution of the standard solution from 2.97-0.3713 and include a well with dH_20.

Final serial dilution concentrations (mmol/L): 2.97, 1.485, 0.7425, 0.3713, 0.

Sample preparation:

Dilute samples 3-fold with 0.9% Sodium Chloride (Saline) if necessary for small volumes.

Procedure:

Standard preparation:

First make a 0.1 M glycine buffer at 9.3 pH: Add 0.7507 glycine to 100 ml dH_20 and adjust pH to 9.3 with NaOH.

Make a 0.5 mg/ml standard by mixing 0.0250 g (25 mg) Uric Acid powder with 50 ml of the glycine buffer.

The standard solution will be stable at room temperature for a few days

Load 5 µl of standard or sample to each well in duplicate; ensure that no samples have both duplicates on the edge of the plate – Vortex all samples before adding to wells

Load 200µl of the Uric Acid Reagent to each well

Load plate into reader, prewarmed to 37 C

Incubate at 37 C 10 minutes

Shake low 10 seconds at 37 C

Read absorbance at 520nm and secondary (background) at 700nm

Calculations:

Uric Acid concentration estimated by taking reference wavelength minus secondary wavelength

Multiply all concentration estimates by dilution factor – 3 fold dilution (2 parts dH_20 1 part sample) is multiplied by 3.

Procedural notes:

Inspect results and rerun samples where CV ((SD/mean)*100) is greater than 15%

Dilute and rerun any samples that were estimated outside the standard curve

B-HYDROXYBUTYRATE

Required supplies:

β-Hydroxybutryrate Linearity Standards (Stanbio-2450; order from Fisher)

D-3 Hydroxybutryic Acid Assay Kit (R-Biopharm – 10 907 979 035) – One box can do 4 trays with 41 samples in duplicate (Solution 2 is the limiting factor)

Reconstitute Solution 2 with 2.5 mL dH_2O
Reconstitute Solution 3 with 6 ml dH_2O

Standard curve:

Create a serial dilution by adding the following concentrations of standards from the standards kit:

1. 5 µl Standard 1 – 0.0 mmol/L
2. 2.5 µl Standard 2 – 0.25 mmol/L
3. 5 µl Standard 2 – 0.5 mmol/L
4. 5 µl Standard 3 – 1.0 mmol/L
5. 7.5 µl Standard 3 – 1.5 mmol/L
6. 5 µl Standard 4 – 2.0 mmol/L
7. 7.5 µl Standard 4 – 3 mmol/L

Sample preparation:

Dilute samples 3-fold with 0.9% Sodium Chloride (Saline) if necessary for small volumes.

Procedure:

Prepare a 22.5 ml working solution to be used immediately by gently mixing the following parts:

15 ml dH2O
4.5 ml Solution 1 pre-warmed to 20-25 C
1.5 ml Solution 2 pre-warmed to 20-25 C
1.5 ml Solution 3 pre-warmed to 20-25 C

Load 5 µl of standard or sample to each well in duplicate; ensure that no samples have both duplicates on the edge of the plate - Vortex all samples before adding to wells

Load 200µl of the Working Solution to each well

Incubate at 20-25 C for 2 minutes

Quickly add 4 µl Solution 4 (activator enzyme) to each well- this has to be done very quickly and is a source of error in the assay.

Gently tap the plate on the bench to ensure the activator enzyme dropped into each well

Shake med-high 10 seconds

Read absorption at 492 nm immediately (t=0) and every minute for 40 minutes (n=41 readings)

Calculations:

Subtract 3 times the change in absorbance between t=30 and t=40 from the change in absorbance between t=0 and t=30; net absorbance = $(t_{30}-t_0)-3(t_{40}-t_{30})$

Procedural notes:

Inspect results and rerun samples where CV ((SD/mean)*100) is greater than 15%

HEMOGLOBIN

Required supplies:

Hemoglobin Colorimetric Assay Kit (700540 from Cayman Chemical-Reconstitute detector and buffer (if needed for dilutions) following kit instructions.

Supplies notes: Each kit can do slightly more than 3 plates with 40 samples/plate in duplicate.

Standard curve:

Follow kit instructions to create a serial dilution of the standard. You can double the volumes to allow for doing more than one plate at a time.

Mixed standard solutions are stable at room temperature for 12 hours.

Final serial dilution concentrations (g/dL): 0, 0.016, 0.040, 0.080, 0.160, 0.240, 0.320, and 0.400.

Sample preparation:

Ideally samples should not be diluted because hemoglobin is naturally low in plasma. However, if sample volumes are low, dilutions can be done using the kit-provided Hemoglobin Sample Buffer (reconstituted with 90mL dH20).

Procedure:

All standards and reagents should be equilibrated with room temperature before adding to the wells.

Add 200 µl of each standard solution to the plate in duplicate.

Load 20 µl of sample to wells in duplicate.

Load 180 µl of the detector *only* to the sample wells (it's already mixed with the standards).

Ensure no bubbles remain at the top of the wells. Don't expel the last drop into the well and lightly blow on the plate before loading.

Incubate at 20-25C for 15 minutes

Read absorbance at 575 nm (between 560-590, per kit).

Calculations:

Calculated corrected absorbance for each individual plate by subtracting the mean absorbance of standard 1 (0.00 g/dL) from all wells.

Calculate a standard curve from corrected absorbance to predict the concentration of Hemoglobin in the wells.

The final Hemoglobin concentration (g/dL) is 10 x's the well concentration to account for the dilution in the wells.

Procedural notes:

Inspect results and rerun samples where CV of absorbance ((SD/mean)*100) is greater than 15%

Dilute and rerun any samples that were estimated outside the standard curve