Dietary Patterns of Bobolinks (*Dolichonyx oryzivorus)* During Fall Staging

by

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Abstract

Agricultural ecosystems present complex trade-offs between human and conservation priorities, especially for migratory or at-risk species whose life cycles depend on managed habitats. Many avian species can provide valuable ecosystem services in agroecosystems, such as pest and weed control, yet these roles are often poorly quantified, especially during energetically demanding periods such as prior to migration. The Bobolink (*Dolichonyx oryzivorus*) is a long-distance migratory grassland passerine and species-at-risk with a distinct knowledge gap surrounding its dietary function within ecosystems, as well as its dietary patterns during pre-migratory staging. My study examines Bobolink diet during the post-breeding, pre-migratory staging period of July and August. I collected fecal samples across eight agricultural sites in the Annapolis Valley of Nova Scotia, Canada. Using DNA metabarcoding of fecal samples, I identified the dietary composition of plants and insects over time, between age groups, and in relation to plant origin and insect pest status. My results indicate that the Bobolink consumes a diverse dietary community of both plants and insects, including weeds and pests. This composition shifted significantly over time as migration approached, and insect dietary composition differed significantly between adults and juveniles. This study provides the first DNA metabarcoding analysis of the Bobolink, as well as the first fine-scale identification of the fall staging diet of the Bobolink. Together, these findings indicate that the Bobolink may contribute ecosystem services within agroecosystems while simultaneously shifting its diet to meet the energetic demands of migratory staging. By contributing to the knowledge of the Bobolink during a potentially limiting period of the life cycle, I hope to improve conservation management techniques by identifying

important prey sources and emphasizing the value of this species-at-risk in agricultural ecosystems.

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Chapter 1. General Introduction

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1.1 Migration

Migration is a biological phenomenon occurring across all major groups of the animal kingdom, where animals undertake movements essential to their life history and ecological niche (Dingle & Drake 2007). Birds migrate the furthest among migratory taxa; bird migration has long captivated humans as an extreme physiological feat (McWilliams & Karasov 2005). The avian ability to combine physical vigour , metabolization, navigation, sleep deprivation, lack of food and water for long periods, and fuel economization is extraordinary among animals (Jenni-Eiermann et al. 2002; Guglielmo 2010; Klaassen et al. 2012).

While migration allows birds to maximize the exploitation of seasonal resources (Dingle 2014), it also represents a calculated risk, given that avian mortality rates can be 6-15 times higher than the non-migratory period (Sillett & Holmes 2002). Physiological preparation is crucial, especially for long-distance avian migrants, as the breeding and wintering grounds may be thousands of kilometres apart (Jenni & Jenni-Eirmann 1988). This preparation period often coincides with a shift in diet selection to prepare adequately both physiologically and morphologically (Bairlein 1998; Tsuru & Tonra 2026). This pre-migratory preparation is also known as “staging” and is a period when birds significantly increase energy reserves and undergo physiological changes using substantial and predictable resources (Warnock 2010).

Birds rapidly accumulate fat during staging, a phenomenon known as pre-migratory hyperphagia (Newton 2023). Hyperphagia occurs where birds can congregate with enough resources available to allow adequate preparation for migration (Warnock 2010). The time required for staging is species-specific and, in some cases, staging for migration

may take weeks (Warnock 2010; Wright et al. 2018). The composition of fat stores changes seasonally in migratory birds, and this composition impacts migratory flight performance (Pierce & McWilliams 2005; Price 2010). For example, body condition and pre-migratory fat reserves are important predictors of migratory behaviour in songbirds, and higher fat deposition rates have been correlated with faster migratory speeds (Hedenström & Alerstam 1997; Fusani et al. 2009). Fat accumulation and deposition through dietary choices can be crucial to a successful migration, especially given the elevated risk of mortality while migrating (Sillett & Holmes 2002; Warnock 2010).

The pre-migratory stage in birds is understudied compared to other stages of the annual cycle (e.g., migration, breeding) that are of much longer duration (Vitz & Rodewald 2006; Van der Winden et al. 2010; Portugal et al. 2012), and even less is known about specifics of diet during staging (Odum 1960; McWilliams & Karasov 2005; Sarà et al. 2014). While diet during pre-migratory staging may be limiting for populations of migratory birds, it may be especially limiting for juvenile birds. Not only are juvenile birds preparing physiologically for their first migration, but they are also learning how to forage independently and developing bodily systems and body parts including their wings and bill (Chandler et al. 2012). While most avian conservation efforts focus on nestling production, more recent studies have focused on young birds preparing for their first migration (Rivera et al. 1998; Vitz & Rodewald 2006; Chandler et al. 2012). These studies highlight how mortality can be high during the post-fledging period (which coincides with the fall staging period) and that management focused on post-fledgling habitat could improve survival (Cox et al. 2012). Therefore, the fall staging period may be a pinchpoint of the avian life cycle for first year birds.

Staging in passerines appears to be comparatively less studied than staging in non-passerines (Leinaas & Ambrose 1999; Selman et al. 2005; Sarà et al. 2014; Fox & Abraham 2017). The understanding of passerine staging in agricultural landscapes is particularly limited, especially since this period has population-level implications for survival during migration (Warnock 2010). My study aims to focus on the pre-migratory preparation of one of North America's longest migrating passerines and grassland obligate bird, the Bobolink (*Dolichonyx oryzivorous*) (Renfrew et al. 2019, 2020).

1.2 Study species

Grassland birds across Canada, including the Bobolink, have shown stark population declines since 1970 (Birds Canada and Environment and Climate Change Canada 2024). This decline is attributed to several factors, including habitat degradation, agricultural activities, and pesticide use (Renfrew et al. 2020). Between 1970 and 2022, the Breeding Bird Survey revealed -2.9% annual declines of the Bobolink's Canadian breeding population (Smith et al. 2024). The Bobolink is listed as Threatened under the Species at Risk Act (SARA) (Government of Canada 2017) and was recently re-assessed as Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2022 (COSEWIC 2022). The Federal Recovery Strategy for Bobolinks in Canada (Environment and Climate Change Canada 2022) states there is inadequate information available on their staging and migration habitat. This knowledge gap is considerable, as the Bobolink travels approximately 20,000 kilometers each year to and from its South American overwintering grounds and its breeding grounds in the U.S. and Canada (Renfrew et al. 2019, 2020), which is an exceptional energetic demand requiring high dietary quality.

The Bobolink's population decline highlights the need for research on the ecology of the species, specifically to identify areas of priority for conservation. Staging habitat and diet quality during this crucial period may be limiting for the Bobolink's migratory survival and success, given the high risk of mortality during migration (Sillett & Holmes 2002). Understanding the diet of the Bobolink during the critical understudied time prior to migration will provide insight into energetic requirements and subsequent habitat use. This understanding will help close knowledge gaps and inform conservation decisions that could help combat the population decline of this species-at-risk and could provide the foundation for further post-breeding pre-migratory studies of other long-distance or grassland passerines.

1.3 Current dietary knowledge

Research on Bobolink diet has focused on the breeding, nonbreeding, and migratory periods; except for Toner's (2025) study on isotopic composition and Beal's (1900) study on Bobolink stomach contents, there has been no assessment (to my knowledge) of their diet during staging and no Bobolink study during any stage that used high-resolution techniques to examine diet. During the breeding season, the Bobolink's diet consists primarily of grain and weed seeds as well as larval insects, including orders Coleoptera, Lepidoptera, Hymenoptera, and Orthoptera (Beal 1900; Nocera et al. 2007). The dependent young are fed almost exclusively invertebrates during the nestling and fledgling stages (Renfrew et al. 2020). While migrating, the Bobolink is highly granivorous, converting carbohydrate-rich seeds of coastal rice (*Oryza sativa*) into fat (Renfrew et al. 2017, 2020). In their South American nonbreeding grounds, Bobolinks remain in large flocks while foraging primarily on rice (Renfrew et al. 2017, 2020).

No studies have used high-resolution techniques, such as DNA metabarcoding, to examine Bobolink diet. Therefore, many Bobolink dietary studies remain at the relatively coarse order or family level (Renfrew et al. 2020). This lack of fine-scale data limits interpretation of the dietary community composition of the Bobolink to a broad taxonomic scale. To rectify this, I use DNA metabarcoding analysis to better explore the prey items of the Bobolink during staging and the potential implications for conservation. In addition to gaining a better understanding of staging, I examine if the Bobolink could be contributing ecosystem services (defined as the benefits that humans obtain from ecosystems; Zhang et al. 2007) to its breeding season hayfields through pest or weed control. Wild fauna, including birds, can provide crucial ecosystem services in agroecosystems such as the consumption of weed seeds and pest control (Garcia et al. 2020). I seek to identify if the Bobolink is consuming plants deemed weeds or insects considered pests to hayfields. These results could create a foundation for future studies quantifying ecosystem services of the Bobolink and create incentives for farmers to support Bobolink conservation management.

1.4 Research Objectives

The goal of my research is to fill the knowledge gap of what Bobolinks eat during the post-breeding, pre-migratory stage in July and August. I aim to expand the knowledge of dietary composition and quality of diet during this critical period. The overall objective of this study is to quantify the Bobolink's diet during the post-breeding, pre-migratory period of July and August, particularly the dietary community composition. To do so, I conducted a DNA metabarcoding analysis on fecal samples from birds captured from 1 July – 15 August 2025 using mist netting capture techniques (Renfrew et al. 2020). I used

fecal samples to determine dietary composition because they can be collected easily and in large quantities with minimal disturbance to the bird compared to other methods (Rytkönen et al. 2019). This analysis compares the DNA to a reference library, allowing prey identification to the finest possible taxonomic level (Rytkönen et al. 2019). While this method cannot represent the abundance of what the Bobolink is consuming, it demonstrates what taxonomic groups are present in their diet (Deagle et al. 2019). I examined differences in prey consumption temporally, as well as differences between animal and plant matter and differences between adult and juvenile Bobolinks. I also analyzed contributions to diet from native and non-native plants and pest and non-pest insects.

Specifically, I answer the following questions:

What taxa make up the Bobolink's diet during the staging period of July and August?

Given the lack of knowledge on the fall staging period of the Bobolink (Environment and Climate Change Canada 2022), and specifically their diet during this time (Beal 1900), I aim to provide a quantitative description of the taxa being consumed by the Bobolink in July and August. I endeavour to determine the dietary composition of the Bobolink to the finest possible taxonomic-level and compare this with other Bobolink diet studies across different spatial and temporal scales.

How does the dietary community composition of Bobolinks change over time?

Due to the energetic requirements during staging (Warnock 2010), **I hypothesize that Bobolink diet transitions in the frequency of occurrence (FOO) of prey items as migration approaches.** I will test this hypothesis by comparing the results of the DNA

metabarcoding analysis over the sampling timeframe. To do this, I will create Jaccard's dissimilarity matrices for the community composition of each sample, then compare this to Ordinal Date using a distance-based redundancy analysis, and model differences using unconstrained principal coordinate analyses. I predict that the dietary community composition of the Bobolink will show separation along ordination axes through time.

How do the diets of hatch year (juvenile) and after hatch year (adult) Bobolinks differ?

I compare and contrast the diet of adult and juvenile Bobolinks to provide insight into their foraging choices when juveniles are developing and preparing for their first migration. This knowledge is especially important in a conservation context given the increased focus on the importance of juvenile survivorship during the post-breeding season (Chandler et al. 2012). **I hypothesize that adults and juveniles differ in their diet while preparing for fall migration, due to the higher demand from their growing bodies and the development of foraging skills, which would prevent the successful capture of insects, but not plants (Chandler et al. 2012).** If this is true, I predict that juvenile Bobolinks will consume a significantly different community composition of insects than adults, occupying different regions of multivariate dietary space.

Is the Bobolink contributing ecosystem services through weed or pest control?

I will compare the contributions of plant prey based on origin (native and non-native) and insect prey based on pest status (pest or non-pest). **I hypothesize that Bobolink are consuming a diverse array of prey items including pests, non-pests, native, and non-native plants.** This hypothesis aligns with the results of Beal's (1900)

study which identified several different taxonomic groups of plants and insects in the diet of the Bobolink during a similar period. Thus, my prediction is that the prey item groupings will show no statistically significant differences, indicating no strong preference for certain groups. I will test this prediction by categorizing prey items into groups based on literature and analyzing the effect of these groupings on the presence and absence of genera using generalized linear mixed models. This approach will allow for an initial evaluation of whether the Bobolink is contributing ecosystem services through weed or pest control.

My study aims to expand on the very limited knowledge of the Bobolink's diet during the post-breeding, pre-migratory period. The results of this research will provide information on dietary community composition over time, differences between demographic groups (adults and juveniles), and the types of prey items the Bobolink is consuming (native, non-native, pest, and non-pest). These results will have implications for conservation and management decisions associated with the fall staging period of the Bobolink and will create a foundation for future studies of staging in other grassland or long-distance migratory passerines.

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Chapter 2: DNA Metabarcoding Analysis Reveals Dietary Composition of Bobolinks During Fall Staging

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Abstract

Migration is an energetically costly trade-off that allows species to exploit resources in seasonal patches. For avian migrants who cover especially far distances, the pre-migratory preparation period (staging) is critical. My study focuses on how one of North America's longest-migrating passerines, the Bobolink (*Dolichonyx oryzivorus*), dietarily prepares for fall migration. My study, which took place across eight agricultural sites in the Annapolis Valley of Nova Scotia, Canada, examined the dietary composition of the Bobolink as it transitions from breeding, in early July, to its departure from its nesting fields in mid-August. Using DNA metabarcoding analysis, I found that Bobolink consume a diverse community of both plant (most commonly genera *Phalaroides, Alopecurus,* and *Symphyotrichum*) and insect prey (most commonly genus *Mythimna*). Both plant and insect dietary community composition in Bobolink diet changed significantly over time as fall migration approached with samples collected later in the season occupying different regions of multivariate dietary space than earlier samples. Insect dietary community composition differed significantly between adult and juvenile Bobolink, with juvenile samples occupying different regions of multivariate dietary space than adult samples. This research provides the first taxonomic description of Bobolink diet during fall staging in over 100 years and is the first Bobolink dietary study to use DNA metabarcoding analysis. My results contribute to filling a recognized knowledge gap and have implications for Bobolink conservation, by highlighting the need to maintain access to these prey sources during a crucial life stage.

2.1 Introduction

Many species migrate, including those of insects, mammals, reptiles, and fish, but none migrate as far and wide across the globe as birds (Newton 2023). Although mortality is highest during migration for most avian species (Sillett & Holmes 2002; Schmaljohann et al. 2022), migration allows birds to maximize the exploitation of seasonal resources in widely separated locations (Jenni & Jenni-Eiermann 1998). Movement between these locations requires pre-migratory fuelling, a critical period in the avian life cycle where energy reserves are accumulated, which includes fuelling during migration and a period leading up to migration known as pre-migratory fuelling or "staging" (Alerstam & Bäckman 2018; Schmaljohann et al. 2022).

Ideal staging sites provide substantial and predictable resources that allow birds to prepare for migration by significantly increasing their energy reserves and undergoing physiological changes (Warnock 2010). This period includes pre-migratory hyperphagia, a rapid increase of food consumption and accumulation of fat stores (Bianchini & Morrissey 2018). The amount of fat acquired is species-specific and depends on refuelling opportunities, predation risk, and migratory distance (Piersma 1998; DeLong 2006; Hedenström 2008). Higher fat deposition rates are correlated with faster migration speed (Hedenström & Alerstam 1997), and body condition and pre-migratory fat reserves are important predictors of migratory behaviour in songbirds (Fusani et al. 2009). Because of the elevated risk of mortality during migration and the benefits of successful migration, efficient pre-migratory fuelling is crucial for the survival and success of migratory birds (Sillett & Holmes 2002; Warnock 2010). Staging becomes increasingly important to successful outcomes as migration distance increases.

Bobolinks (*Dolichonyx oryzivorus*) are one of North America's longest-migrating passerines, making a roundtrip journey of 20,000 kilometres each year from the U.S. and Canada to Bolivia, Paraguay, and northern Argentina (Renfrew et al. 2019, 2020). From 1970 to 2022, the Bobolink's Canadian breeding population declined by 2.9% annually (Smith et al. 2024). Among the steeper regional declines is a decrease of over 3.0% annually in Nova Scotia (Birds Canada and Environment and Climate Change Canada 2024). The Bobolink is currently listed as Threatened under the Species at Risk Act (SARA) (Government of Canada 2017) but was recently re-assessed as Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2022 (COSEWIC 2022). The reasons for this population decline are multiple and interacting and include mortality risks on migration (Renfrew et al. 2020). The annual distance Bobolinks travel makes them an ideal species for studying migration and migratory preparation.

Many migratory birds change their diets seasonally, due to resource availability or physiological constraints (McWilliams & Karasov 2005). To date, research on Bobolink's diet has focused on the breeding, nonbreeding, and migratory periods. During the breeding season, the Bobolink's diet consists primarily of grain and weed seeds as well as larval insects (Beal 1900; Nocera et al. 2007), with the dependent young being fed almost exclusively invertebrates during the nestling and fledgling stages (Renfrew et al. 2020). While migrating, the Bobolink is highly granivorous, converting carbohydrate-rich seeds of coastal rice (*Oryza sativa*) fields into fat (Renfrew et al. 2017, 2020).

There have been only two coarse assessments of Bobolink diet during staging. Toner (2025) detected a significant decrease in $\delta^{13}C$ in the Bobolink's diet from July to

August in New Brunswick (Canada), indicating a potential dietary shift during fall staging, more consistent with C_3-photosynethic pathway plant communities than C_4 plants. Beal (1900) examined Bobolink diet in nineteen northern states of the United States of America. This study took place over a century ago and there have been dramatic changes in agricultural practices, insect populations, and Bobolink population since then (Herkert 1997, Ethier and Nudds 2017, Smith and Edwards 2020). Thus, there is a distinct knowledge gap around the Bobolink's diet during fall staging, emphasized by the federal Recovery Strategy for Bobolinks in Canada (Environment and Climate Change Canada 2022), which identified there is inadequate information available on their staging and migration habitat.

The objective of my research is to quantify the Bobolink's diet during the post-breeding, pre-migration staging period. To do so, I obtained fecal samples from captured Bobolinks from the end of breeding (start of July) until the Bobolinks departed on fall migration (mid-August) (Renfrew et al. 2020). The fecal samples underwent a DNA metabarcoding analysis to determine (down to the genus level) the dietary composition of the Bobolink. My study asks: 1) What taxa (invertebrate or plant matter) make up the Bobolink's diet during the staging period of July and August, 2) How does this composition change as migration approaches, and 3) Are there significant dietary differences between adults and juveniles?

I hypothesized that Bobolinks prepare for migration by altering their diet during the post-breeding pre-migratory period. If true, then the dietary community composition of prey items, ascertained by their frequency of occurrence (FOO), will change significantly from July to August. I further hypothesized that dietary composition during

staging differs between adults and juveniles and therefore predict that juvenile Bobolinks should have a different FOO of invertebrates in their diet when compared to adults. I predict this result as juveniles have additional energy requirements during growth and are undergoing behavioural skill development, making moving prey items such as invertebrates more difficult to capture (Marchetti & Price 1989; Chandler et al. 2012). Testing these hypotheses and predictions will fill a long-standing knowledge gap, contribute to a better understanding of this species at risk, and further the collective knowledge of avian migration and the staging period.

2.2 Methods

Study area

I selected eight sites (Figure 1) within the western Annapolis Valley of Nova Scotia (44.8018° N, 65.4024° W), an area of abundant agricultural land, with many privately-owned hayfields. I chose large hayfields (>6.0 hectares (ha)) with a higher density and height of vegetation, as well as a higher ratio of grass to alfalfa (*Medicago sativa*), as Bobolinks prefer these attributes (Bollinger 1988; Nocera et al. 2007). Bobolinks also prefer a mixture of grasses and forbs, as well as high litter cover and little bare soil, which I prioritized when selecting sites (Diemer & Nocera 2014; Renfrew et al. 2020). Departure timing from breeding grounds varies among populations, with some Bobolinks leaving the breeding areas from late July to early August (Renfrew et al. 2020). To ensure comprehensive sampling that would represent the transition from end of breeding until migration, I sampled from 1 July 2025 until the flocks departed the nesting fields (latest date = 15 August 2025). Sites were visited on a rotating, weather-dependent basis, with each site visited every 7-10 days from the beginning of the season. When a

study hayfield was cut and the Bobolinks had departed the nesting fields, that site was removed from the rotation, shortening the rotation and causing the remaining sites to be visited to as often as 1-3 days by the end of the season.

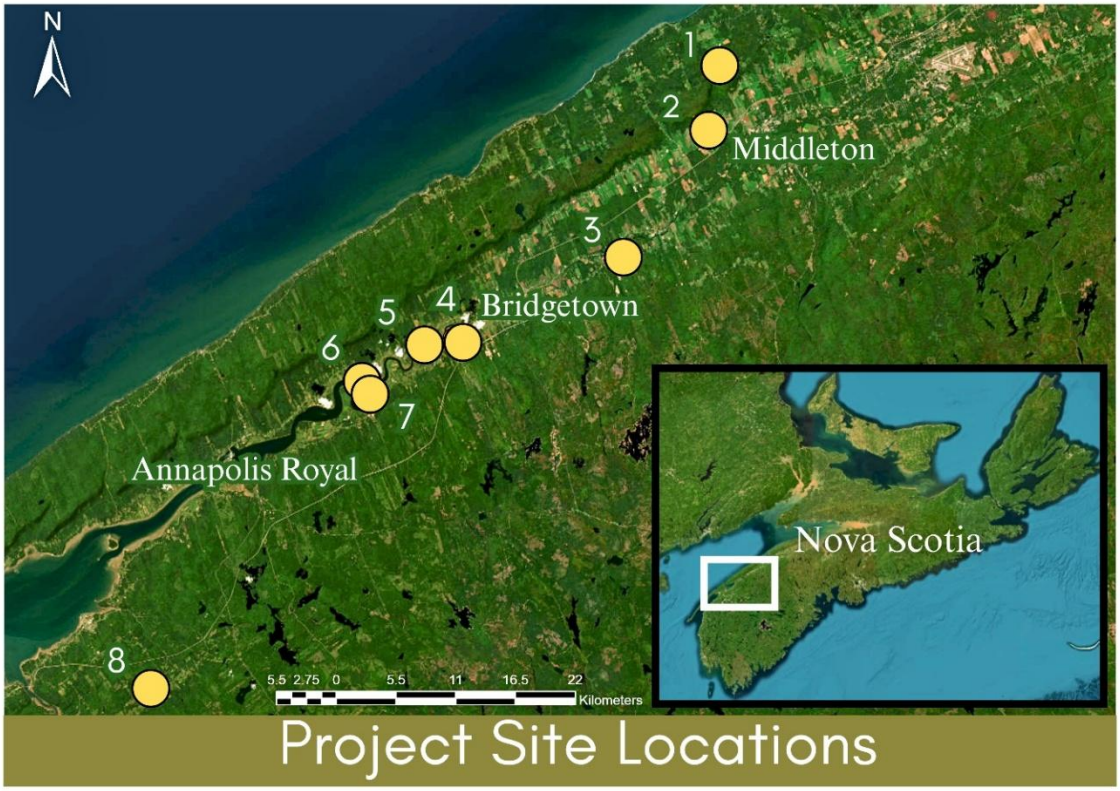


Figure 1. Project site locations in the Annapolis Valley, Nova Scotia, Canada (44.8018° N, 65.4024° W). Eight sites are identified using a yellow circle and a unique number.

Capture and fecal collection

To determine the dietary composition of Bobolink during the staging period, I collected and analyzed fecal samples. I chose to sample feces because adequate quantities can be collected easily and provide and an accurate representation of diet, and it is less invasive for the bird than other diet sampling methods, such as regurgitation or dissection (Lorenzón et al. 2019; Ando et al. 2020). Bird capture methods followed the guidelines as described by Fair et al. (2010). Banding was conducted under Master Banding permit

#10801 and in accordance with UNB animal care protocol # 202500111, and numeric Canadian Wildlife Service-issued aluminum bird bands were used. Mist nets (mesh size 36mm; length of 6m or 12m) were placed on 2.5m tall poles in areas of frequent use by Bobolinks. Banding was conducted when Bobolinks are most active: from sunrise until approximately 1000h (Robbins 1981), meaning that the results will not be indicative of potential dietary changes throughout the day.

To collect fecal samples, I followed a non-invasive method like that described by Knutie & Gotanda (2018). This method uses a flat-bottomed paper bag for fecal collection. Following removal from the net, individual birds were placed in a bag for five minutes or until defecation. After the fecal sample had been collected, each bird was banded and measured. Given the similar plumage of the juvenile and adult females during the post-fledging period, Bobolinks were determined to be hatch years (juveniles) or after hatch years (adults) by assessing skull ossification (Renfrew et al. 2020). Juveniles have not completed pneumatization as of July and August, and this can be seen by separating the feathers on the rear of the head and gently moving the skin to determine skull ossification (Stewart 1972). The bag containing the fecal matter was labelled with the band number of the individual and the date and put in a resealable bag. The captured birds were released away from the nets following data collection. The collected fecal samples were frozen at -4°C until they could be analyzed.

DNA metabarcoding analysis

Metabarcoding analysis uses the sequencing of prey DNA from fecal or gut samples of predators, which allows prey identification using a reference library (Rytkönen et al. 2019). The frozen samples were placed in individually labelled

centrifuge tubes and covered with 95% ethanol for preservation. The samples were then sent to the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph, Canada to be analyzed, where the samples were thawed, homogenized, and dried at 50°C (Rytkönen et al. 2019).

Samples were centrifuged at 10,000 rotations per minute for one minute. Following centrifuging, ethanol was removed and a sterilized steel bead was added to each tube. Samples were lysed in 500 microlitres (µL) of invertebrate lysis buffer containing 2 milligrams per millilitre (mg/mL) Proteinase K, after which homogenization was performed using a Bead Ruptor Elite. DNA was then extracted from 50 µL of each homogenate using a validated glass fiber, plate-based method (Ivanova et al. 2006). DNA was amplified using the arthropod-specific primer ZBJ ArtF1c_M13/ZBJ-ArtR2_M13 (ZBJ) which targets a 157 base-pair (bp) fragment of the cytochrome c oxidase subunit I (COI) gene (Zeale et al. 2011), and the plant-specific primer ITS-3p62plF1_t1/ITS-p4R2_t1 (ITS2), which targets a 350 bp fragment of the second internal transcribed spacer from nuclear ribosomal DNA (Kolter & Gemeinholzer 2021). For each sample, three polymerase chain reaction (PCR) replicates were performed. Sequencing was performed on an Illumina NextSeq 1000/2000 platform. The ZBJ library was run on a P1 flow cell using 300 cycles with 2 × 166 bp paired end chemistry, while the ITS2 library was run on a P1 flow cell using 600 cycles with 2 × 319 bp paired-end chemistry.

The resulting sequence reads were processed using MAP, an in-house bioinformatics pipeline (N. Nguyen, personal communication, 25 February 2026), in conjunction with a comprehensive Barcode of Life Data System (BOLD) reference library (Ratnasingham et al. 2024). Sequences were demultiplexed into separate FASTQ

files for each sample amplified with the ZBJ and ITS2 primer sets. Primer and adapter sequences were removed using cutadapt and only reads falling within a user-defined size threshold were retained (100–400 bp for ZBJ and 100-600bp for ITS2) and a minimum size filter was applied (157 bp for ZBJ and 350 bp for ITS2). Processed reads were clustered into ASVs (amplicon sequence variants) using vsearch --cluster_fast, and only ASVs meeting a minimum read depth threshold (≥2) and a minimum identity of 99% for ZBJ and 95% for ITS2 were retained. Denoising was completed using the identification and removal of potential contaminant ASVs from each sample. To distinguish contamination from genuine low-level detections, the CCDB calculated a background noise threshold by examining ASV distributions in negative control wells relative to sample wells (N. Nguyen, personal communication, 25 February 2026).

DNA metabarcoding inadvertently minimizes the importance of certain prey items and cannot represent abundance (Deagle et al. 2019; Ruvck & Koper 2024). Summaries of occurrences are less accurate when samples are pooled (Deagle et al. 2019), and therefore my samples were kept separated by individual. I chose to analyze dietary items at the genus level due to the short length of the molecular marker used for identification and the limited sequence divergence among closely related plant species (N. Nguyen, personal communication, 25 February 2026). Analyzing at the genus level allowed me to make inferences at a finer scale than the family or order level and better use the detail provided by the DNA metabarcoding (De Ruyck & Koper 2024). However, I chose to avoid the species-level to be conservative and to reduce potential misidentification. I considered a detection confident if the genus had >100 reads in each of at least two out of three PCR replicates. I considered a taxon rare if it was present in ≤5% of samples, and

these were excluded from statistical analyses as they would be less important to Bobolink diet.

Statistical analysis

Statistical analyses were conducted using the R v4.5.2 (R Core Team 2025). Prior to analysis, I removed individuals of unknown age to avoid complications with building Jaccard dissimilarity matrices. From the DNA metabarcoding analysis, I determined the FOO for prey genera, which I calculated using the number of consumer species samples where a specific taxon was present, then divided by the total number of samples from the consumer species (Bowser et al. 2013). I chose to use FOO (instead of relative read abundance or weighted percent of occurrence; Deagle et al. 2019), as it quantifies diet effectively without using proportion and it allows comparison with other dietary studies (Bowser et al. 2013). A FOO of 1.0 indicates a taxon was detected in the diet of all Bobolinks sampled, and a FOO of 0.5 indicates that a taxon was detected in the diets of half of the Bobolinks sampled (Deagle et al. 2019). FOO was visualized using the package *ggplot2* (Wickham 2016).

I calculated the presence and absence of each confident, non-rare genus for every sample. I separated these presence and absence data into insect genera, plant genera, and both plant and insect genera combined. I then created Jaccard dissimilarity matrices using the *vegdist* function from the *vegan* package for each of these groupings (Oksanen et al. 2025). Each site in the community matrix represented an independent sample. To assess the effect of Date and Age on diet dissimilarity, I used distance-based redundancy analyses (dbRDA) using the *dbrda* function in the *vegan* package (Legendre & Anderson 1999; Oksanen et al. 2025). The function *vif.cca* in the *vegan* package (Oksanen et al.

2025) was used to test collinearity between variables. Site was excluded as a fixed effect due to high multicollinearity with other predictors. The *adonis2* function in the *vegan* package (Oksanen et al. 2025) was used to test whether dietary community composition differed by site using a PERMANOVA.

I assessed the significance of each term in my models using permutation tests in the *anova.cca* function in the *vegan* package (Oksanen et al. 2025). A correction constant, *lingoes*, was added to avoid negative eigenvalues (Legendre & Anderson 1999). I conducted model selection using stepwise forward permutation tests using the function *ordiR2step* from the package *vegan* (Oksanen et al. 2025). I visualized the dietary community composition of insect genera, plant genera, and both insects and plants combined using unconstrained principal coordinates analysis (PCoA) based on Jaccard dissimilarities. The PCoAs were conducted using the *cmdscale* function from the *vegan* package (Oksanen et al. 2025), and plots were then generated to visualize the relationships in two-dimensional space using the package *ggplot2* (Wickham 2016). Species accumulation curves were calculated using the *specaccum* function in *vegan* (Oksanen et al. 2025) and visualized using *ggplot2* (Wickham 2016) for plant and insect genera (De Ruyck & Koper 2024).

2.3 Results

I caught 155 Bobolinks from 1 July-15 August 2025 with no recaptures. I obtained fecal samples from 136 of those Bobolinks, and those samples were sent to the CCDB resulting in 408 PCR replicates analyzed. The NextSeq run and sequencing produced approximately 110 million read pairs in total for the insect primers, and approximately 150 million read pairs in total for the plant primers. After additional

processing, including quality filtering, primer/adaptor trimming, and size selection, 81,365,388 reads remained for taxonomic analysis from the ZBJ primers, and 108,815,892 reads remained for taxonomic analysis from the ITS2 primers.

Once correcting for a minimum of >100 reads in two of the three PCR replicates, all 136 samples had at least one insect or plant genus present. The sample size was reduced to 132 by removing birds of unknown age, resulting in 60 samples from juveniles and 72 samples from adults. Across my samples, 54 unique genera of plants were confidently detected, with 17 of those present in >5% of samples; 133 unique genera of insects were confidently detected across samples, and 8 of those present in >5% of samples. Of the remaining 132 samples, 131 of the samples had at least one plant genus present, and 94 of the samples had at least one insect genus present. Species accumulation curves (Figure 2) reflect the insect and plant genera commonly (>5%) consumed by the sampled Bobolink. Both curves reach a well-defined asymptote, indicating sufficient sample size to estimate plant and insect diversity of the community consumed (De Ruyck & Koper 2024).

The most frequent plant genera consumed by Bobolink were *Phalaroides, Alopecurus, Symphyotrichum, Trifolium,* and *Anthoxanthum*, which occurred more frequently in the diets of Bobolinks than any insect genera (Table 1, Figure 3). Insect genera *Mythimna, Cisseps,* and *Olibrus* occurred most frequently out of the insect genera present (Table 1, Figure 3). Between age groups, the genus *Mythimna* was consumed at the highest frequency among adult Bobolink, and both genera *Mythimna* and *Olibrus* were consumed at the highest frequency among juvenile Bobolink (Table 1, Figure 4). Among plants, the genus *Phalaroides* was consumed at the highest frequency among

juvenile Bobolink, and *Phalaroides* and *Alopecurus* were consumed at the highest frequency among adult Bobolink (Table 1, Figure 4). I visualized FOO over time in Figure 5.

Distance-based redundancy analyses using Jaccard's dissimilarities (final models presented in Table 2) revealed the final model for insect genera (Date + Age) explained 5.90% of total variation in insect dietary community composition. Two canonical axes were significant ($F_1 = 3.9$, $df_1 = 1$, $p_1 = 0.001$; $F_2 = 1.8$, $df_2 = 1$, $p_2 = 0.028$; Figures 6 and 7). Insect genera composition differed significantly between age groups and explained a small proportion of total variation (1.36%, $p = 0.032$, Table 2), meanwhile date accounted for a larger portion of the variation (2.99%, $p = 0.001$, Table 2). Ordination showed substantial overlap between adult and juvenile samples, indicating that dietary community composition is broadly similar. However, the two groups were slightly separated along the two principal coordinate axes, supporting the subtle but significant differences between age classes found in the distance-based redundancy analysis. Additionally, juvenile samples appeared more dispersed than adult samples (Figure 6), indicating additional differences. Ordination revealed a gradient of samples along the first principal coordinate axis associated with date, suggesting differences in dietary insect community composition throughout the sample period (Figure 7).

The final model for plant genera (Date) explained 3.28% of total plant dietary community composition and one canonical axis was significant ($F = 4.4$, $df = 1$, $p = 0.001$; Figure 8). The final model for both plant and insect genera combined (Date) explained 4.00% of total variation, with one significant canonical axis ($F = 5.4$, $df = 1$, $p = 0.001$; Figure 9). Ordinations for both plant dietary community composition and plant

and insect dietary community composition also displayed a distinct gradient of samples along the first principal coordinate axis associated with date (Figure 8, Figure 9). This supports the findings of the distance-based redundancy analysis, indicating that diet changes temporally throughout the late breeding season.

PERMANOVA indicated that dietary community composition differed significantly among sites for insects ($R^2 = 0.115$, $F = 1.88$, $p = 0.001$), plants ($R^2 = 0.090$, $F = 2.04$, $p = 0.001$), and both insects and plants combined ($R^2 = 0.088$, $F = 2.00$, $p = 0.001$), which has been visualized using PCoA ordinations (Figures 6, 7, and 8). Ordination displayed substantial overlap in dietary community composition across sites, with no clear separation among site groupings, indicating that diet is not strongly structured by site.

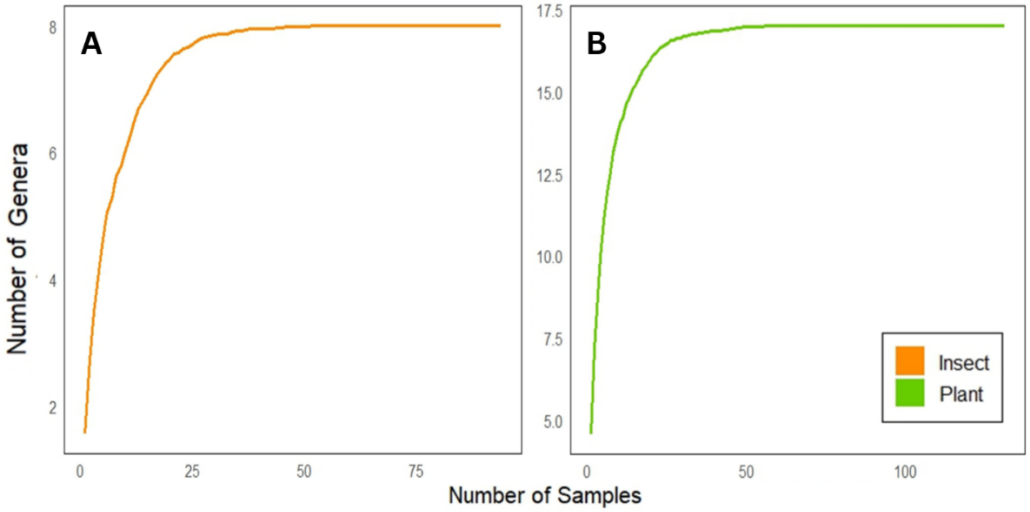


Figure 2. Species accumulation curves comparing number of genera identified in every Bobolink diet by number of samples. Insect genera are indicated in orange, and plant genera are indicated with light green.

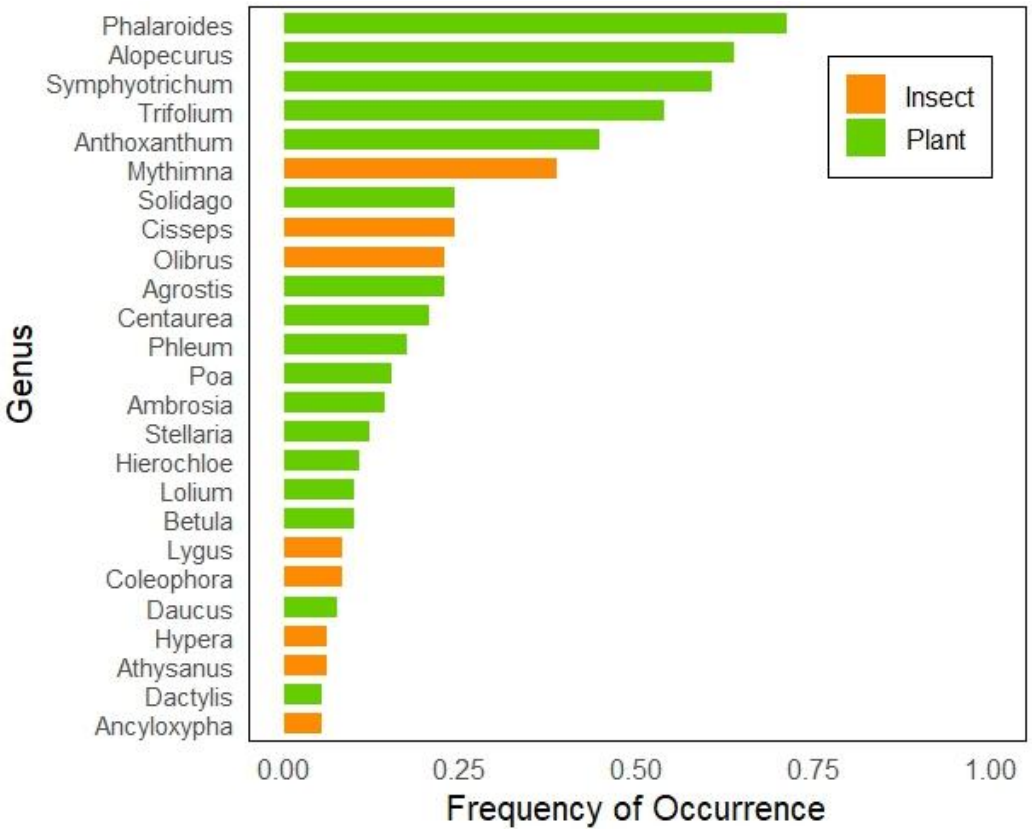


Figure 3. Frequency of occurrence (FOO) of insect and plant genera detected across 132 Bobolink fecal samples via DNA metabarcoding. Insect genera are identified with orange, and plant genera are identified with light green.

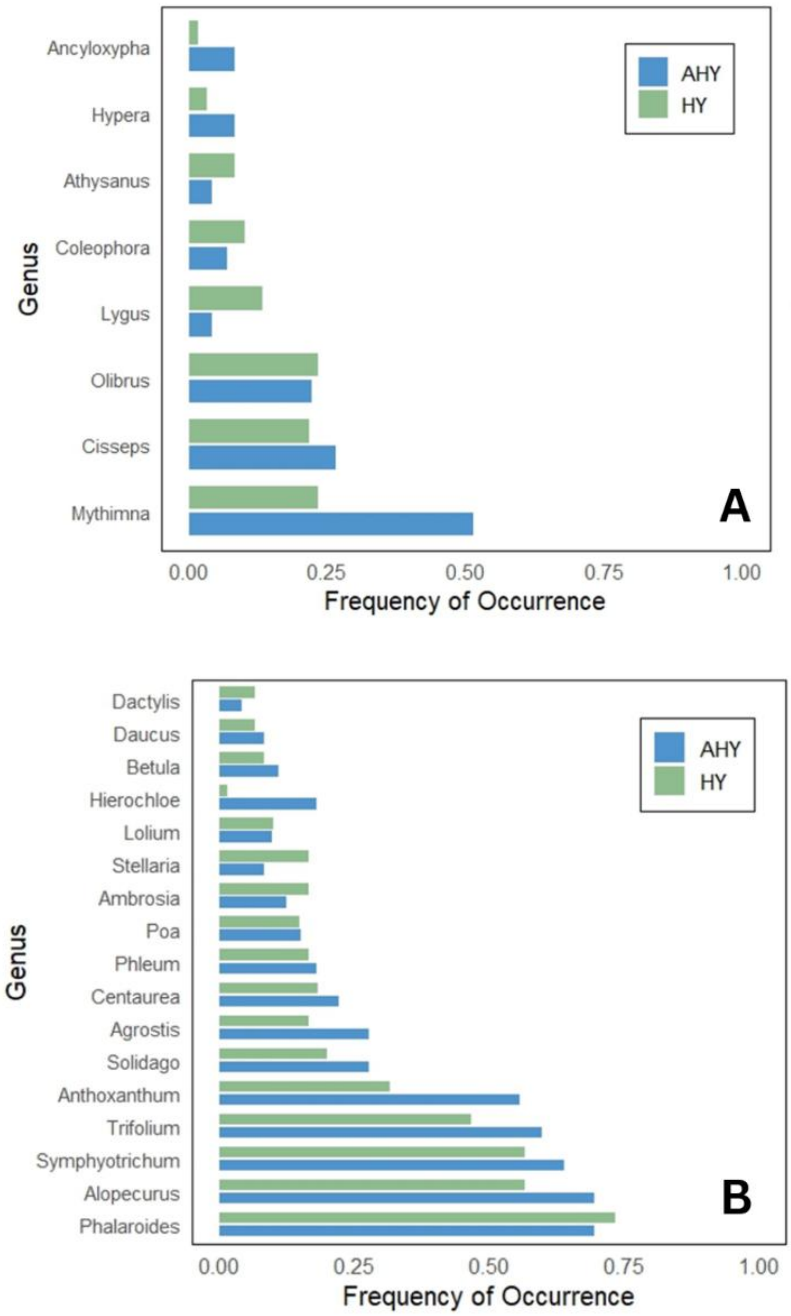


Figure 4. Frequency of occurrence (FOO) of insect genera (panel A) detected across 53 adult/after hatch year (AHY; blue) Bobolink and 41 juvenile/hatch year (HY; green) Bobolink, and plant genera (panel B) detected across 72 AHY and 59 HY Bobolink, via DNA metabarcoding. The genera are ordered from lowest overall FOO to highest. Among insects, the genus *Mythimna* was consumed at the highest frequency among AHY, and both genera *Mythimna* and *Olibrus* were consumed at the highest frequency among HY Bobolink. Among plants, the genus *Phalaroides* was consumed at the highest frequency among HY Bobolink, and both genera *Phalaroides* and *Alopecurus* were consumed at the highest frequency among AHY Bobolink

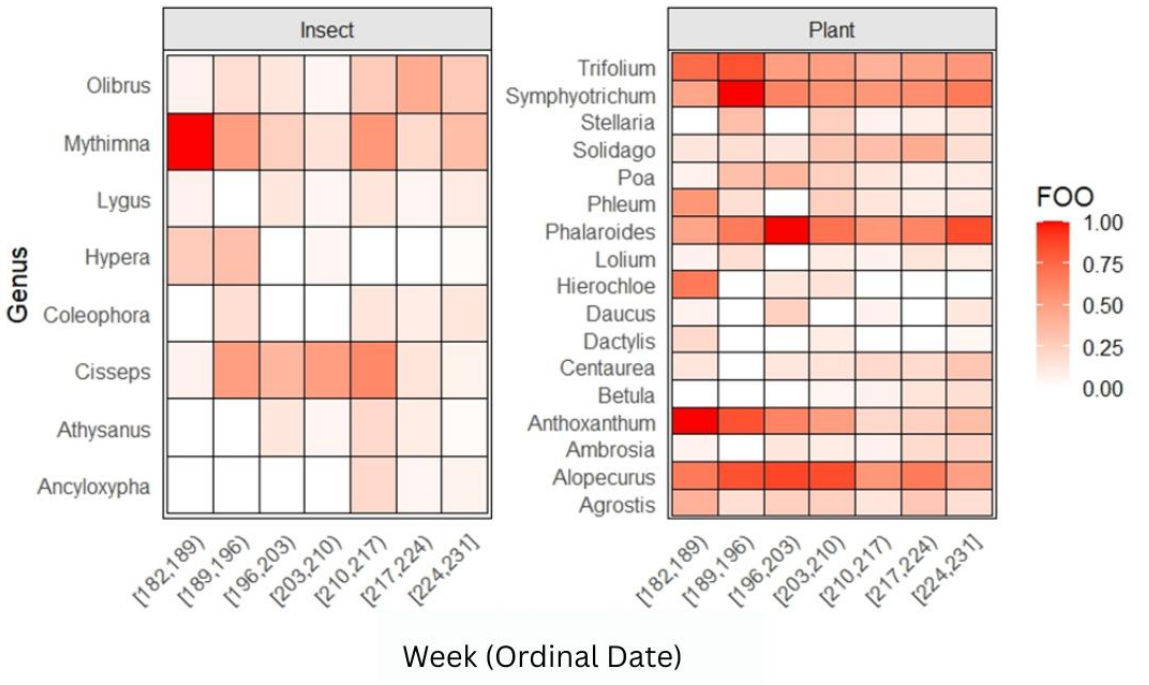


Figure 5. Frequency of occurrence (FOO) of insect and plant genera detected across 132 Bobolink fecal samples via DNA metabarcoding. The FOO of genera is grouped by week, beginning on 1 July 2025 (Ordinal Date 182) and ending on 19 August 2025 (Ordinal Date 231), although sampling ended on 15 August 2025 (Ordinal Date 227). The genera are arranged in reverse alphabetical order. A FOO of 1.0 indicates that the genus was detected in the diets of all the samples and is indicated with the darkest red; a FOO of 0.0 indicates that the genus was detected in none of the samples and is indicated with white.

Table 1. Frequency of occurrence (FOO) of insect and plant genera detected across 132 Bobolink fecal samples via DNA metabarcoding. Adult/after hatch year (AHY) Bobolink are compared to juveniles/hatch year (HY) Bobolink. The five most frequently consumed by Bobolinks in my study are *Phalaroides, Alopecurus, Symphyotrichum, Trifolium,* and *Anthoxanthum*.

Genus	FOO (overall)	FOO (AHY)	FOO (HY)	Type	Common Name
Phalaroides	0.712	0.694	0.733	Plant	Canary grass
Alopecurus	0.636	0.694	0.567	Plant	Foxtail grass
Symphyotrichum	0.606	0.639	0.567	Plant	Aster
Trifolium	0.538	0.597	0.467	Plant	Clover
Anthoxanthum	0.447	0.556	0.317	Plant	Vernal grass
Mythimna	0.386	0.514	0.233	Insect	True army worm
Cisseps	0.242	0.264	0.217	Insect	Tiger moth
Solidago	0.242	0.278	0.200	Plant	Goldenrod
Olibrus	0.227	0.222	0.233	Insect	Shining flower beetle
Agrostis	0.227	0.278	0.167	Plant	Bentgrass
Centaurea	0.205	0.222	0.183	Plant	Knapweed
Phleum	0.174	0.181	0.167	Plant	Timothy
Poa	0.152	0.153	0.150	Plant	Meadow grass
Ambrosia	0.144	0.125	0.167	Plant	Ragweed
Stellaria	0.121	0.083	0.167	Plant	Chickweed
Hierochloe	0.106	0.181	0.017	Plant	Sweetgrass
Betula	0.098	0.111	0.083	Plant	Birch
Lolium	0.098	0.097	0.100	Plant	Ryegrass
Coleophora	0.083	0.069	0.100	Insect	Seed casebearer
Lygus	0.083	0.042	0.133	Insect	Lygus bug
Daucus	0.076	0.083	0.067	Plant	Carrot
Athysanus	0.061	0.042	0.083	Insect	Leafhopper
Hypera	0.061	0.083	0.033	Insect	Alfalfa weevil
Dactylis	0.053	0.042	0.067	Plant	Bluegrass
Ancyloxypha	0.053	0.083	0.017	Insect	Skipper butterfly

Table 2. Results from distance-based redundancy analysis (Jaccard's dissimilarity). Models were selected via stepwise forward selection based on *p*-values and R^2. Significant *p*-values (<0.05) are shown in bold.

Model	Constrained	Unconstrained	Adjusted R^2	F	df	*p*
Insect Genera Dissimilarity ~ Date + Age	5.9%	94.1%	0.03834	2.854	2	**0.001**
+ Date			0.0299	3.900	1	**0.001**
+ Age			0.0136	1.808	1	**0.032**
Plant Genera Dissimilarity ~ Date	3.3%	96.7%	0.02528	4.371	1	**0.001**
Plant and Insect Genera Dissimilarity ~ Date	4.0%	96.0%	0.03262	5.417	1	**0.001**

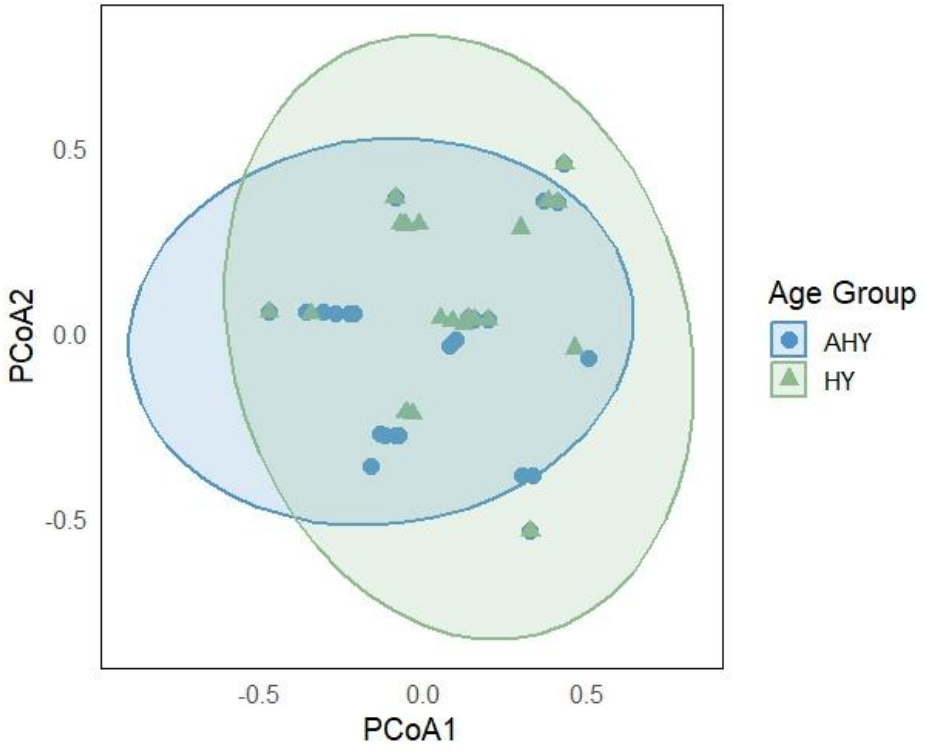


Figure 6. Principal coordinate analysis of the Jaccard dissimilarity matrix of insect genera detected in Bobolink fecal samples via DNA metabarcoding. Adult/after hatch year (AHY) Bobolink are represented by blue circles, while juvenile/hatch year (HY) Bobolink are represented by pale green triangles. Ellipses represent 95% confidence intervals.

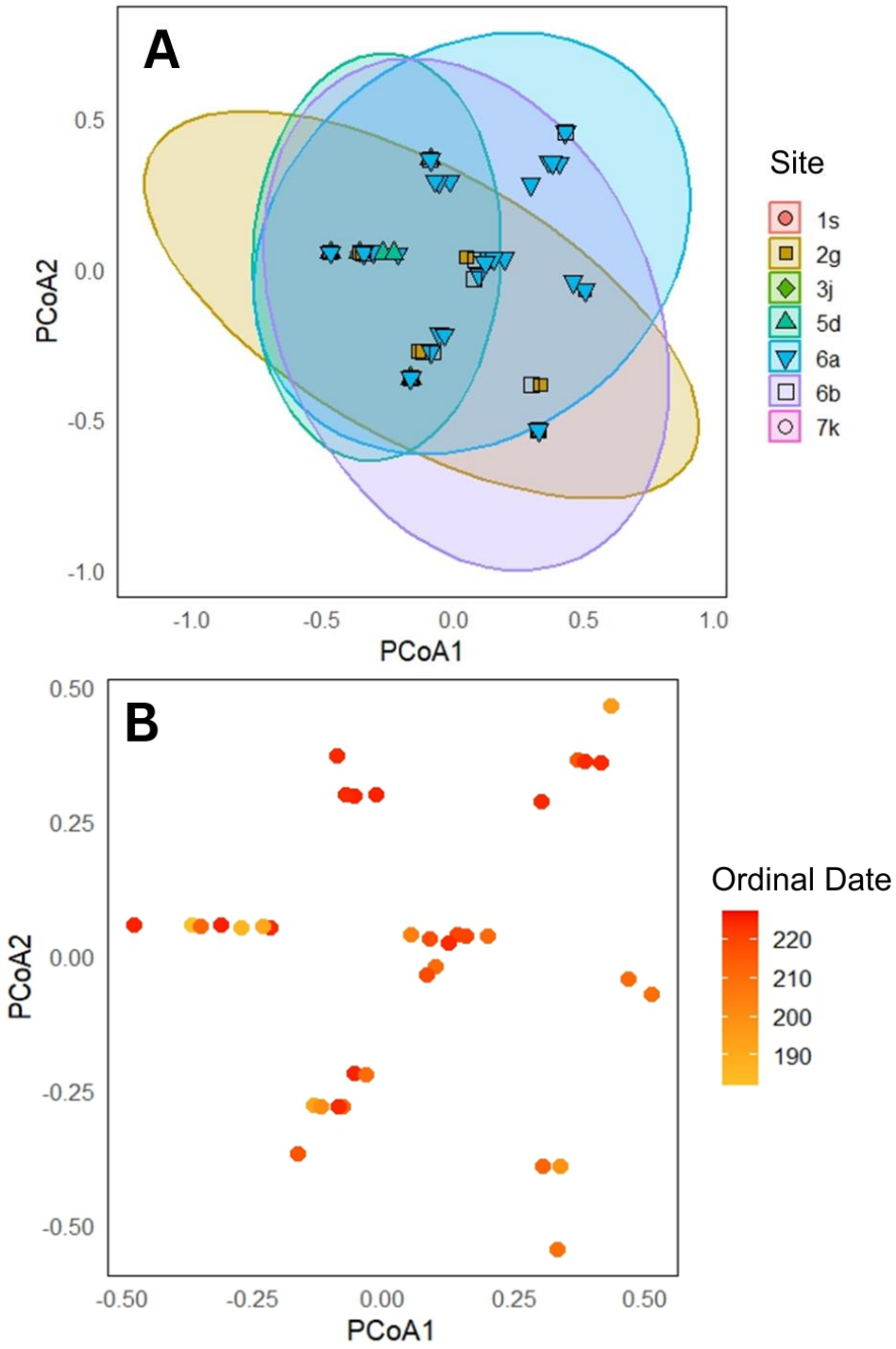


Figure 7. Principal coordinate analysis of the Jaccard dissimilarity matrix of insect genera detected in Bobolink fecal samples via DNA metabarcoding. Graph A presents samples by Site, with ellipses representing 95% confidence intervals, and no ellipses were drawn for sites with <5 samples. Graph B presents samples by Ordinal Date (continuous).

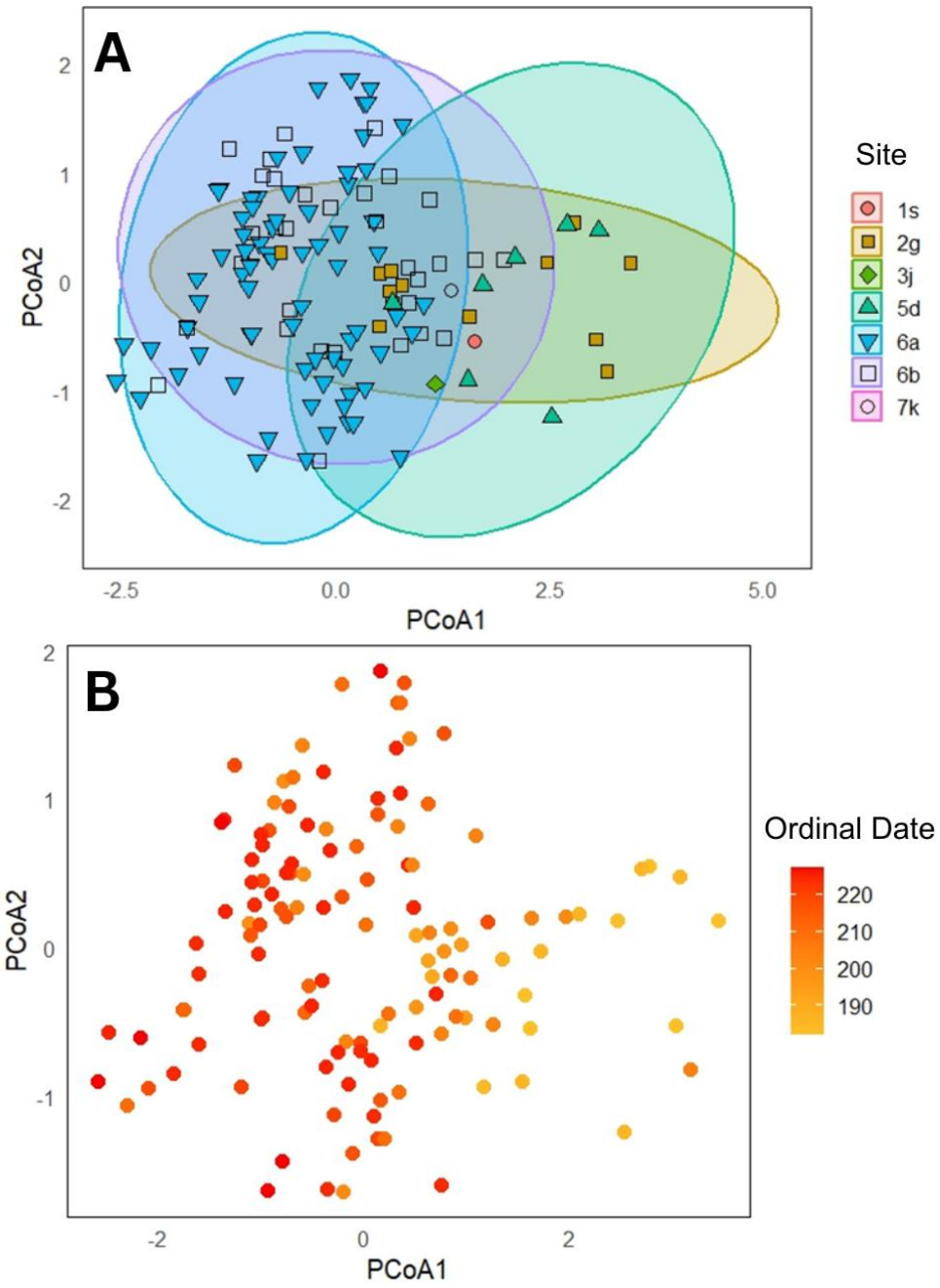


Figure 8. Principal coordinate analysis of the Jaccard dissimilarity matrix of plant genera detected in Bobolink fecal samples via DNA metabarcoding. Graph A presents samples by Site, with ellipses representing 95% confidence intervals, and no ellipses were drawn for sites with <5 samples. Graph B presents samples by Ordinal Date (continuous).

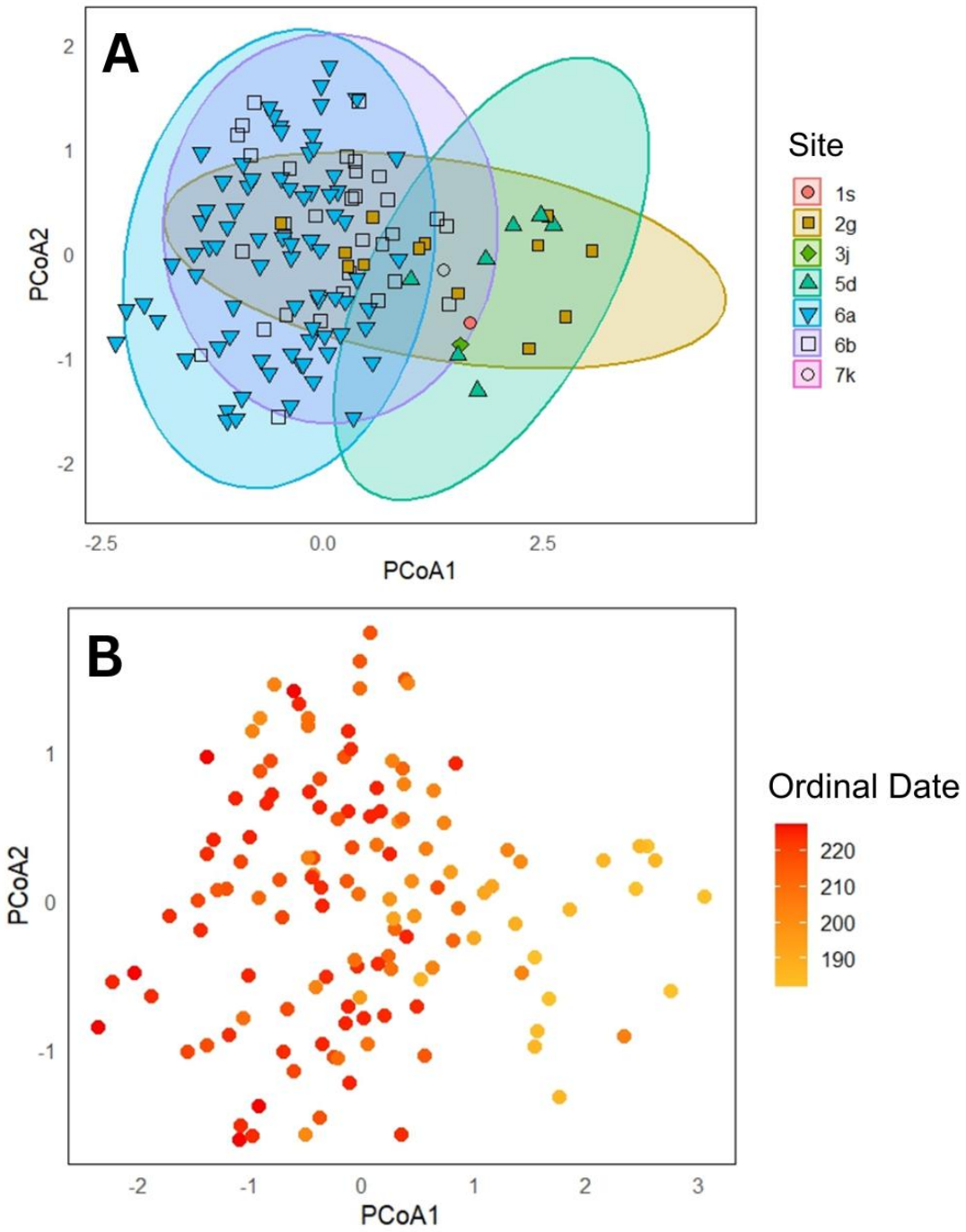


Figure 9. Principal coordinate analysis of the Jaccard dissimilarity matrix of both plant and insect genera detected in Bobolink fecal samples via DNA metabarcoding. Graph A presents samples by Site, with ellipses representing 95% confidence intervals, and no ellipses were drawn for sites with <5 samples. Graph B presents samples by Ordinal Date (continuous).

Table 3. Prey items present in Bobolink samples, identified by taxonomic group (Catalogue of Life 2021; Hobern et al. 2021). The table is organized alphabetically by genera, beginning with Kingdom Animalia and followed by Kingdom Plantae.

Phylum	Class	Order	Family	Genus
Arthropoda	Insecta	Lepidoptera	Hesperiidae	*Ancyloxypha*
Arthropoda	Insecta	Hemiptera	Cicadellidae	*Athysanus*
Arthropoda	Insecta	Lepidoptera	Erebidae	*Cisseps*
Arthropoda	Insecta	Lepidoptera	Coleophoridae	*Coleophora*
Arthropoda	Insecta	Coleoptera	Curculionidae	*Hypera*
Arthropoda	Insecta	Hemiptera	Miridae	*Lygus*
Arthropoda	Insecta	Lepidoptera	Noctuidae	*Mythimna*
Arthropoda	Insecta	Coleoptera	Phalacridae	*Olibrus*
Magnoliophyta	Liliopsida	Poales	Poaceae	*Agrostis*
Magnoliophyta	Liliopsida	Poales	Poaceae	*Alopecurus*
Magnoliophyta	Magnoliopsida	Asterales	Asteraceae	*Ambrosia*
Magnoliophyta	Liliopsida	Poales	Poaceae	*Anthoxanthum*
Magnoliophyta	Magnoliopsida	Fagales	Betulaceae	*Betula*
Magnoliophyta	Magnoliopsida	Asterales	Asteraceae	*Centaurea*
Magnoliophyta	Liliopsida	Poales	Poaceae	*Dactylis*
Magnoliophyta	Magnoliopsida	Apiales	Apiaceae	*Daucus*
Magnoliophyta	Liliopsida	Poales	Poaceae	*Hierochloe*
Magnoliophyta	Liliopsida	Poales	Poaceae	*Lolium*
Magnoliophyta	Liliopsida	Poales	Poaceae	*Phalaroides*
Magnoliophyta	Liliopsida	Poales	Poaceae	*Phleum*
Magnoliophyta	Liliopsida	Poales	Poaceae	*Poa*
Magnoliophyta	Magnoliopsida	Asterales	Asteraceae	*Solidago*
Magnoliophyta	Magnoliopsida	Caryophyllales	Caryophyllaceae	*Stellaria*
Magnoliophyta	Magnoliopsida	Asterales	Asteraceae	*Symphyotrichum*
Magnoliophyta	Magnoliopsida	Fabales	Fabaceae	*Trifolium*

2.4 Discussion

My study supports the hypotheses that Bobolinks change diet while staging for fall migration, and that the insect component of diet differs between adult and juveniles. My results demonstrate that Bobolink do not rely on a single plant or insect genus. No singular genus was found in every Bobolink's diet (or even 75% of diets). Plants appear to have a higher relative importance than insects to Bobolink diet given the high overall FOO (99.2%) and that the top five genera with the highest FOO were all plants (Figure 3, Table 1). The 17 common (>5% of samples) plant genera present made up six unique

plant families and six unique plant orders. However, insects still have importance to Bobolink diet, as insect genera were present in 71.2% of samples. The eight common (>5% of samples) insect genera identified were composed of eight unique insect families and three unique insect orders. These results emphasize the importance of a diverse, heterogenous prey community for the Bobolink's diet during fall staging.

Dietary differences over time

My study found that dietary community composition of Bobolink diet changed significantly over time. Early in the sampling period, Bobolinks consumed a higher FOO of certain genera, including *Mythimna* (moths) and *Anthoxanthum* (vernal grasses), later consuming other genera frequently such as *Cisseps* (moths), *Alopecurus* (foxtail grass), and *Phalaroides* (canary grass). Late in the staging period, genus *Olibrus* (shining flower beetles) was consumed in high frequency. Many migrant avian species, including songbirds, shift their diet selection seasonally and during pre-migratory periods as they prepare physiologically, morphologically, and behaviourally for migration (Bairlein 1998). Shifting demands in most frequently consumed prey resources may meet the costly energetic requirements of pre-migratory fuelling (Tsuru & Tonra 2026). This includes building fat reserves through pre-migratory hyperphagia (Fusani et al. 2009), as efficient fuel storage is crucial to the survival and success of migratory birds (Sillett & Holmes 2002; Warnock 2010). Therefore, the dietary shift I detected across the staging period may be intentional, to meet the energetic demands of fuelling for migration. These changes over time were visualized in Figure 5; however, this should be interpreted cautiously as Date was analyzed as a continuous variable and has only been binned into

weeks for ease of visual interpretation. The sample size differs between bins and therefore Figure 5 should be viewed prudently.

Additionally, the Bobolink may require different resources over time, not just to support staging but also to supply adequate resources for molt. The Bobolink is one of few bird species that undergoes two complete annual molts (Renfrew et al. 2020). This includes a full pre-basic molt in North America and a full pre-alternate molt in South America (Renfrew et al. 2011). Molting is energetically costly, as is pre-migratory staging, and this overlap of energetically intensive processes may result in a physiological trade-off (Rubolini et al. 2002). Rubolini et al. (2002) found that molting was associated with reduced fat stores during the pre-migratory period in another long-distance migrant, the Barn Swallow (*Hirundo rustica*), and that this energetic cost was greater for inexperienced juvenile birds, which could also explain differences I detected between age groups. Other migrant species molt and deposit fuel simultaneously where food is abundant and feeding conditions are favourable (Remisiewicz et al. 2017). Dietary shifts likely correspond to the energetic demands of multiple competing bodily processes that occur during this post-breeding period (Tsuru & Tonra 2026).

Temporal variation in diet is also partly explained by seasonal variation in prey availability. Insect dietary community composition in agricultural ecosystems can be highly variable; these differences can be explained by spatial variation, weather conditions, and seasonal dynamics (Scrosati et al. 2011; Burgess et al. 2024). Plant dietary community composition and structure changes seasonally as well, which can also affect insect dietary community composition (Stinson & Brown 1983). Plant and insect dietary community composition are also subject to phenological change as a response to

climate change, which could result in asynchrony between avian resource requirements and resource availability (Youngflesh et al. 2023; Belitz et al. 2025). This could indicate that Bobolinks may be changing their diet temporally, not because they are selecting the best resources for pre-migratory fuelling, but because these are the resources most abundant at the time.

Dietary differences between adults and juveniles

Insect consumption differed significantly between Bobolink age groups (although explained only a small proportion of total variance), which could reflect the different resource needs and foraging capabilities of adults versus juveniles. It is possible that the nutritional requirements of the juveniles differ from the adults, and that they may require a higher quantity or quality of foods to meet the energetic demands of maturation as bodily systems develop (McCabe & Guglielmo 2019). McCabe and Guglielmo (2019) found that juvenile birds had higher metabolic rates and energy expenditure.

Furthermore, while the adult birds are staging for migration, the young are still growing to adult size (Chandler et al. 2012). During staging before fall migration, juvenile Bobolink have only recently fledged and are transitioning from being fed by parents to learning how to forage independently (Wittenberger 1980; Skipper and Kim 2013; Renfrew et al. 2020). Additionally, immature birds have weaker beak musculature and delayed wing maturation, which could negatively affect manoeuvrability, energy expenditure in flight, ability to catch prey, and opening hard prey items (Marchetti & Price 1989). The insect community consumed may differ between age groups because juveniles may not yet be adept at catching live animal prey and are forced to rely on prey items that are easier to catch. This would explain why I found no significant difference

between the plant dietary community composition of adult and juvenile Bobolink. This may also explain why juvenile samples appeared to be more dispersed than adult samples when plotted using ordination, given a potentially more opportunistic feeding strategy of the juvenile Bobolink. Alternatively, there are potential advantages to weaker beak musculature, such as larger gape size and mandibular flexibility, which could allow juveniles to feed upon larger prey items than adults (Marchetti & Price 1989), further explaining differences in dietary community composition.

Comparisons with other dietary studies of Bobolink

This is the first study that quantifies Bobolink diet using the DNA metabarcoding of fecal samples. Although DNA in fecal samples is degraded during the process of digestion, the amplification of DNA using barcodes allows fecal metabarcoding to be a specific and less invasive tool, especially compared to ligatures, forced regurgitation techniques, and dissection (Garfinkel et al. 2021). I was able to examine diet at a finer taxonomic level than previous studies, which focused largely on order and family. For comparison, higher taxonomic levels of the genera identified have been summarized in Table 3 (Catalogue of Life 2021; Hobern et al. 2021).

Beal (1900) identified Bobolink dietary items from May to September, which is the only Bobolink study I could find that examined dietary items during the fall staging period of July and August. Beal's (1900) dietary results identified invertebrate orders Orthoptera and Lepidoptera (as did Wittenberger 1980; Skipper & Kim 2013 in nestlings), as well as Coleoptera (as did Lorenzón et al. 2019 in the wintering grounds) and Hymenoptera. Beal (1900) found Bobolink consumed plants mainly of family Poaceae, similar to Meanley and Neff (1953) and Lorenzón et al. (2019). Similar to

Beal's (1900) research, my study also identified invertebrate orders Coleoptera and Lepidoptera, as well as the plant family Poaceae, indicating these may be prey items consistently consumed during fall staging by the Bobolink. Beal (1900) also found that insect consumption was relatively high in May, June, and July (81.9%, 92.4%, and 84.5% of stomach contents respectively, the remaining percentage plant material) and that plant consumption was conversely high in August and September (80.6% and 92.9% respectively). Beal's (1900) findings indicate a dietary transition from July to August, and although my results cannot determine the volume of plant and insect matter consumed, I did find significant changes in dietary community over time, which also indicates a transition in the Bobolink's diet during staging.

Contrary to Beal's (1900) study, my results did not identify any insect prey items belonging to orders Orthoptera or Hymenoptera among the commonly consumed genera (>5% of samples; Table 3). However, I did identify Hymenoptera and Orthoptera as present across a small number of samples (≤5%), although these were excluded from my analyses. Wittenberger (1980) and Skipper and Kim (2013) also identified Orthoptera as common prey items, although these were being delivered to nestlings, which could explain why this order was not as common for post-fledging and adult consumption.

Toner (2025), conducted in the neighboring province of New Brunswick, identified that $\delta^{13}C$ values of Bobolink samples decreased significantly from the beginning of July until mid-August, indicating increased reliance on food resources derived from C_3 plant communities as the season advances. Those findings align with this study's results, as photosynthetic pathway classification by Sage & Monson (1998) indicated that temperate grass genera and temperate dicot genera use the C_3-

photosynthetic pathway. Of all commonly (>5% of samples) consumed plant genera present in the samples, *Agrostis, Alopecurus, Anthoxanthum, Dactylis, Hierochloe, Lolium, Phalaroides, Phluem,* and *Poa* are temperate grasses; the remaining genera are temperate dicots (Sage & Monson 1998; Catalogue of Life 2021; Hobern et al. 2021).

Although my results show the dietary community composition of the Bobolink's diet changes over time, comparisons with other Bobolink dietary studies identify several commonly consumed foods of this species: the plant family Poaceae and insect orders Lepidoptera, Coleoptera, and Hemiptera (Beal 1900; Meanley & Neff 1953; Wittenberger 1980; Skipper & Kim 2013; Lorenzón et al. 2019). While all insect genera present in my results belonged to insect orders that have been identified in other Bobolink diet studies, this was not the case for identified plant prey. Plant families Apiaceae, Asteraceae, Betulaceae, Caryophyllaceae, and Fabaceae were present in >5% of the samples yet were not identified in published Bobolink diet studies. These differences could be explained by a shift in dietary preference over time, or parsimoniously, by variation in plant dietary community composition and resource availability over spatial scales, as this study is the only Bobolink dietary study to have taken place in Nova Scotia, Canada.

Potential limitations

An important caveat of my study is that inferring diet from DNA metabarcoding techniques and occurrence data can overestimate the importance of prey items consumed, especially those consumed in small quantities (Deagle et al. 2019; Ruvck & Koper 2024). When taxa consumed in small quantities is overestimated by occurrence data, this comes at the expense of the estimated importance of taxa eaten in large quantities (Deagle et al. 2019; Ruvck & Koper 2024). DNA metabarcoding does not allow for estimating

quantities of prey items consumed, as such my study identifies only occurrence in the diet of the Bobolink. Another potential limitation is that the PCR primers used for the DNA metabarcoding analysis have been documented to under-detect certain orders compared to others due to "primer bias", meaning I likely underestimated the frequency of some invertebrate orders (Zeale et al. 2011; Clarke et al. 2014). Therefore, my study puts emphasis on the presence of taxa, rather than the quantity or importance.

Fecal samples, although less invasive for sampling wild animals, can result in DNA degradation during the digestion and sample collection process (Ando et al. 2020). Although this is mitigated somewhat by freezing samples following defecation, it may make prey detection difficult during the DNA metabarcoding analysis (Ando et al. 2020). However, because the species accumulation curves reached a well-defined asymptote, I believe this did not affect the community diversity of my study, although it may still have influence FOO (De Ruyck & Koper 2024).

Implications regarding species-at-risk management

Prey availability directly affects an individual's energy for reproduction and survival, resulting in food resources being a principal determinant of populations (Newton 1998). In terms of staging for migration, prey availability has also been shown to account for pre-migratory movements (Bounas et al. 2018). Since World War II, agricultural intensification including industrialization, habitat conversion and loss, and use of pesticides and fertilizers has caused insect population and diversity decline at an accelerating rate within agricultural ecosystems (Raven & Wagner 2021; Abudulai et al. 2022). Benton et al. (2002) found that invertebrates and bird numbers are negatively associated with agricultural intensity, and that bird numbers may be linked to declines in

agricultural invertebrates (although connections can be difficult to demonstrate, see review in Stanton et al. (2018)).

Grassland bird populations in North America, including the Bobolink's, have been declining in recent decades. Among guilds, grassland bird populations are in the steepest decline in Canada (Birds Canada and Environment and Climate Change Canada 2024) and this has been linked strongly to agriculture and possibly prey decline (Stanton et al. 2018). This reflects the importance of holistic conservation decisions that account for not just the species-at-risk, but also the population declines of their prey items.

The federal Recovery Strategy for Bobolinks in Canada (Environment and Climate Change Canada 2022) identifies that there is inadequate information available on the Bobolink's staging and migration habitat. While my study is not focused on habitat, my results demonstrate that Bobolink consume a diverse array of prey, including both plants and insects, during the fall staging period. Therefore, habitat that supports the diversity of these plant and insect communities would be of great importance as Bobolink staging habitat and should be prioritized for conservation. This could be actualized by maintaining access to heterogenous habitats through leaving uncut refugia or delayed cutting, which would enhance food availability and support Bobolink while preparing physiologically for fall migration.

Furthermore, the differences I identified between adult and juvenile diets present the opportunity for demographic-focused conservation techniques. Current conservation efforts concerning hatch year birds are primarily focused on the nesting requirements, but recently more studies have focused on the understudied period post-fledging, while the young prepare for their first migration (Rivera et al. 1998; Vitz & Rodewald 2006;

Chandler et al. 2012; Cox et al. 2014). A quantitative summary of fledgling survival across 35 passerine species by Cox et al. (2014) found that most mortality occurred during the first three weeks post-fledging. Cox et al. (2014) suggested that management focused on post-fledging habitat can improve survival, as survival is often influenced by patch-level factors, and that conservation of a species is more effective when their complete life-history is known (Chandler et al. 2012). The findings of their study and others (Rivera et al. 1998; Vitz & Rodewald 2006; Chandler et al. 2012; Cox et al. 2014) highlight the importance of conservation actions focused on the post-fledging stage, quantified here as the post-breeding, pre-migratory staging period.

2.5 Conclusion

My study detailed the taxonomic composition of the Bobolink's diet during the post-breeding, pre-migratory period in Nova Scotia, Canada, and highlighted significant differences in dietary community composition over time and between age groups. My results provide the first description of prey items consumed by Bobolink during fall staging in over 100 years (Beal 1900), as well as the first Bobolink dietary study that uses DNA metabarcoding analysis. My study expands the collective knowledge of the Bobolink's annual diet and the genera consumed during a critical life stage, emphasizing the importance of a diverse, heterogenous prey dietary community composition. If interpreted with appropriate caution, this study has implications for conservation of this species-at-risk, by maintaining access to prey sources during this critical period.

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Chapter 3: Bobolinks pay their rent: Bobolinks may provide ecosystem services in breeding range

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Abstract

Agricultural ecosystems present complex trade-offs between human needs and the conservation of biodiversity. Birds can provide valuable ecosystem services, such as pest and weed control, yet these roles are not always quantified. The Bobolink (*Dolichonyx oryzivorus*), a grassland bird with a complex history within agroecosystems, is an example of this conflict, as it is a species-at-risk threatened by early hay harvest with an unclear dietary role in agricultural habitat. My study, which took place across eight agricultural sites in the Annapolis Valley of Nova Scotia, Canada, examined the dietary composition of the Bobolink in terms of contributions from native and non-native plants and pest and non-pest insects using DNA metabarcoding of fecal samples. Bobolink did not show preferential consumption between groups, suggesting an opportunistic feeding strategy. Notably, the Bobolink consumed not just intentionally cultivated hayfield plants, but also persistent, dominant, and economically costly weeds and pests. These results, combined with the fact that no natural enemies of pests were identified in the Bobolink's diet, indicate that the Bobolink may be providing ecosystem services in the agroecosystems they inhabit. Additionally, no aquatic insects were identified in the Bobolink's diet, indicating nutritional requirements are met through terrestrial food sources. By clarifying the trophic role of Bobolinks in these ecosystems, this study improves the understanding of ecosystem services provided by this species-at-risk, which may incentivize farmers to delay hay harvesting and promote ecologically focused, bird-friendly management.

3.1 Introduction

Agricultural land is considered one of the most significant human alterations to the world's natural environment (Matson et al. 1997), spans more than one-third of global land area (FAO 2025), and is the world's dominant form of land management (Power 2010). Agriculture is necessary to support global food demand but can invoke negative consequences for global biodiversity (Matson et al. 1997) through land use conversion, air and water pollution, disease transmission, and pesticide use (Garcia 2020). For example, agriculture has been identified as the main threat to declining grassland bird populations (Douglas et al. 2023). In Canada, grassland birds decreased by 67% from 1970-2022, the most extreme population change of any avian group in the country (Birds Canada and Environment and Climate Change Canada 2024).

Alternatively, agriculture can be multifunctional when management practices that promote biodiversity conservation enhance productivity and ecosystem health within agricultural systems and increase agroecosystem resilience (Perfecto & Vandermeer 2010; Liere et al. 2017). Wildlife can provide crucial ecosystem services (Garcia et al. 2020), defined as the benefits that humans obtain from ecosystems (Constanza et al. 1997; Zhang et al. 2007; Bommarco et al. 2013) such as pollination, nutrient cycling, aesthetic value, consumption of weed seeds, pest control, and seed dispersal. These services can lead to increased crop yield and economic benefits for agricultural landowners (Jones et al. 2005; Peisley et al. 2015; Pejchar et al. 2018; Gonthier et al. 2019; Garcia et al. 2020; Díaz-Siefer et al. 2022).

Conversely, birds can sometimes be seen as pests to agricultural systems (Pejchar et al. 2018). Birds may damage crops through defecation and crop contamination,

granivory and frugivory, transmission of food-borne pathogens, mechanical crop damage, and consuming natural enemies of pests (also known as intraguild predation) (Zhang et al. 2007; Martin et al. 2013; Pejchar et al. 2018; Garcia et al. 2020). This pest behaviour, known as disservices, can degrade agricultural crops and cause economic losses for farmers (Pejchar et al. 2018; Garcia et al. 2020). However, disservices can be often offset by services. Gonthier et al.'s (2019) study on strawberry-eating birds found that disservices of birds (3.2% of berries damaged) were counteracted by the services of the birds (reduced the number of berries damaged by insect pests by 3.8%). Similarly, Garfinkel et al. (2022) found that birds provided considerable services in corn (*Zea mays*), but disservices in soybean crops (*Glycine max*). Mols & Visser (2002) found that Great Tits (*Parus major*) significantly reduced caterpillar damage in apple orchards. A global meta-analysis of bird effects on crop productivity by Díaz-Siefer et al. (2022) found that crop yield significantly decreased while pest abundance and crop damage significantly increased when wild birds were absent.

Pest control is an ecosystem service that can be provided by birds in agro-ecosystems, benefitting the farmer with reduced pest control costs and benefitting the bird with an abundant food source. The mutual benefits are further apparent for species-at-risk (Lindell et al. 2018). The threatened New Zealand Falcon (*Falco novaeseelandiae*) was introduced to vineyards as pest control for frugivorous passerines (Kross et al. 2012). This resulted in potential savings of US$234/ha to US$326/ha for vineyard owners when the falcon was present and additionally provided strong incentive for the conservation of that species (Kross et al. 2012).

An effective study system for evaluating ecosystem services within agro-ecosystems should involve clear ecological and economic trade-offs, in particular, species that simultaneously provide potential benefits to agricultural landscapes while also causing management conflicts between human needs and conservation. Knowledge of ecosystem services are especially relevant for species-at-risk, as the identification of ecosystem services can enhance the perceived value of a species and generate enhanced support for conservation. Additionally, species without clearly recognized trophic roles present an important opportunity to quantify ecosystem services and inform management decisions.

My study species, the Bobolink (*Dolichonyx oryzivorus*), represents a challenging conflict of interest between the human benefits of agriculture and avian conservation. The Bobolink is listed as Threatened under the Species at Risk Act (SARA) (Government of Canada 2017) but was re-assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as Special Concern in 2022 (COSEWIC 2022). Some of the steepest population declines of Bobolinks in Canada are seen in Nova Scotia at -3.0% annually (Birds Canada and Environment and Climate Change Canada 2024). As grassland ground-nesters in agricultural hayfields, Bobolink reproductive success can be negatively affected by hay harvest during the breeding period as nests and nestlings are destroyed by harvesting machinery (Diemer & Nocera 2016). Delaying hay harvest until Bobolink have finished breeding has clear benefits to Bobolink population recruitment (Bollinger et al. 1990; Herkert 1997). However, delaying hay harvest comes at a cost to the farmers, as the nutritional quality in hay necessary for feeding livestock declines throughout the season (Nocera et al. 2005).

The complex interactions between agricultural management and Bobolink conservation are not limited to the species' breeding grounds and continue on the nonbreeding grounds. As Bobolinks forage primarily on rice (*Oryza sativa*) during the nonbreeding season, they are often viewed as a pest of rice fields by farmers (Renfrew et al. 2017; Lorenzón et al. 2019), although Renfrew and Saavedra (2007) suggest that perceived damaged may be inflated. This has resulted in the shooting and trapping of Bobolinks, with some being sold into the pet trade in both domestic and international markets (Renfrew et al. 2020).

Given this multifaceted relationship between Bobolinks and agriculture that can lead to various viewpoints of the species, I seek to determine if the Bobolink provides ecosystem services in its breeding range during the post-breeding, pre-migratory period. There has been some mention of Bobolink as pest control on insect populations in their nonbreeding range (Renfrew & Saavedra 2007; Lorenzón et al. 2019), however, this has not been extensively studied in their breeding range (Beal 1900; Renfrew et al. 2020). In the Bobolink's breeding range, dietary studies have largely been focused on the breeding and nesting period and have not typically examined prey items at a finer taxonomic scale than order or family (Beal 1900; Wittenberger 1980; Skipper & Kim 2013; Nocera et al. 2007). A finer identification of potential ecosystem services of the Bobolink in its breeding range could have conservation implications, as it may incentivize farmers to value the species for pest control and be more accepting of delayed hay harvest to better ensure its breeding success.

I also aim to determine if Bobolinks are supplementing their diet with aquatic insects in place of, or in addition to, terrestrial insects, given the fatty acid benefits

obtained from aquatic insects (Rideout et al. 2025). Birds need to consume large amounts of fatty acids to fuel flight (Guglielmo 2010); fatty acid composition of fat stores changes seasonally in migratory birds, and that this composition impacts migratory flight performance (Pierce & McWilliams 2005; Price 2010). Emerging aquatic insects provide up to ten times the essential fatty acids of terrestrial insects, which are important dietary components for birds (Twining et al. 2016). Emerging aquatic insects can be found in agricultural landscapes, contributing to the ecosystem from nearby drainage ditches, creeks, ponds, and rivers (Fehlinger et al. 2023). Given these benefits for migratory flight and the long-distance travelled by the Bobolink, I look to identify whether its prey items are aquatic or terrestrial to further understand the nutritional quality of its diet.

I seek to qualitatively describe prey items consumed by Bobolink during July and August in its breeding range by using DNA metabarcoding techniques. My goal is to better understand how the Bobolink interacts with its ecosystem through diet, including the degree to which they consume different plant and insect taxa. From a functional ecology lens, dietary composition in birds can be driven by foraging availability, opportunistic generalist feeding, and energetic optimization (such as fatty acid composition) (McWilliams et al. 2004; Razeng & Watson 2015). Bobolinks may consume prey that maximizes energy during a time of high energetic demand, or they may feed in proportion to the relative availability of prey items. To evaluate this, I test whether diet composition deviates from availability-neutral expectations. Specifically, I test the hypotheses that plant genera in the diets of Bobolink differ based on origin (native and non-native), and that insect genera in the diets of Bobolink differ based on pest status (pest or non-pest). I define pest as an insect that causes harm or damage to cultivated

hayfields, often resulting in economic loss (Hill 1997). The testable prediction arising from these hypotheses is that Bobolinks consume similar frequencies of native vs. non-native plants and pest vs. non-pest insects. I expect this based on Beal's (1900) study which identified several orders and families of both plants and insects in the diet of the Bobolink during a similar period, implying potential generalist dietary behaviour.

3.2 Methods

Study Area

I selected 8 study sites within the surrounding area of Belleisle, Nova Scotia (44.8018° N, 65.4024° W). Belleisle is at the southern end of the Annapolis Valley and has an abundance of hayfields that support Bobolink. Upon arrival to the breeding grounds, Bobolinks exhibit a preference for grassland with higher density and height of vegetation (Bollinger 1988; Nocera et al. 2007) and hayfields with high litter cover and little bare soil (Diemer & Nocera 2014; Renfrew et al. 2020). These characteristics of vegetation, such as vegetation height and density, likely influence the presence and abundance of invertebrate populations (Diemer & Nocera 2014). Higher densities of Bobolinks have been observed in older hayfields where ≥8 years have passed since their last plowing and reseeding, and Bobolink tend to avoid habitat near edges (Fletcher & Koford 2003; Diemer & Nocera 2014; Renfrew et al. 2020). I strived to obtain access to larger sites as Bobolinks are area-sensitive and generally prefer a larger patch size (Johnson & Igl 2001; Diemer & Nocera 2014). I selected larger (>6.0, the largest being 12.1 hectares (ha)) patch sizes with minimal edges, forbaceous vegetation, and a higher density and height of vegetation. Prior to sampling, I confirmed the presence of Bobolinks at each of the selected sites using visual and audible recognition.

Capture and fecal collection

During July and August 2025, I collected Bobolink fecal samples for later analysis of dietary composition. Collecting fecal samples is a non-invasive method in which large quantities can be collected easily (Lorenzón et al. 2019). My bird capture methods followed the guidelines of Fair et al. (2010) and were in accordance with UNB animal care protocol # 202500111. Banding was conducted under Master permit #10801 and Canadian Wildlife Service-issued aluminum bird leg-bands were used. I banded when Bobolinks were most active: from sunrise until approximately 1000h (Robbins 1981) using mist nets (mesh size 36mm; length of 6m or 12m) on 2.5m tall poles in areas of frequent use by Bobolinks. I followed the fecal collection protocol of Knutie & Gotanda (2018)), which uses a flat-bottomed paper bag for collection. Following removal from the net, individual birds were placed in the paper bag for five minutes or until defecation and the bag was labelled with the band number of the individual and the date and put in a resealable bag. Captured birds were released away from the nets following data collection and the collected fecal samples were frozen until they could be analyzed.

DNA metabarcoding analysis

The frozen fecal samples were placed in individually labelled centrifuge tubes and covered with 95% ethanol for preservation, then sent to the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph to undergo DNA metabarcoding analysis. Metabarcoding analysis allows for prey identification using the sequencing of prey DNA from fecal or gut samples of predators (Rytkönen et al. 2019). Once the samples were prepared through thawing, homogenization, and desiccation, the DNA was extracted and

the CCDB determined the prey species composition of each fecal sample to the finest possible taxonomic unit (Rideout et al. 2025).

Samples were first centrifuged at 10,000 rotations per minute for one minute, then ethanol was removed and a sterilized steel bead was added to each tube. Samples were lysed in 500 microlitres (µL) of invertebrate lysis buffer containing 2mg/mL Proteinase K. Homogenization was then performed using a Bead Ruptor Elite and DNA was extracted from 50 µL of each homogenate using a validated glass fiber, plate-based method (Ivanova et al. 2006). DNA then was amplified using the arthropod-specific primer ZBJ ArtF1c_M13/ZBJ-ArtR2_M13 (ZBJ) which targets a 157 base-pair (bp) fragment of the cytochrome c oxidase subunit I (COI) gene (Zeale et al. 2011), and the plant-specific primer ITS-3p62plF1_t1/ITS-p4R2_t1 (ITS2), which targets a 350 bp fragment of the second internal transcribed spacer from nuclear ribosomal DNA (Kolter & Gemeinholzer 2021). Three polymerase chain reaction (PCR) replicates were performed for each sample, and sequencing was performed on an Illumina NextSeq 1000/2000 platform. The ITS2 library was run on a P1 flow cell using 600 cycles with 2 × 319 bp paired-end chemistry, while the ZBJ library was run on a P1 flow cell using 300 cycles with 2 × 166 bp paired end chemistry.

Resulting sequence reads were processed using an in-house bioinformatics pipeline (MAP) and a comprehensive Barcode of Life Data System (BOLD) reference library (Ratnasingham et al. 2024). Processed reads were clustered into ASVs (amplicon sequence variants) using vsearch --cluster_fast, and only ASVs meeting a minimum read depth threshold (≥2) and a minimum identity of 99% for ZBJ and 95% for ITS2 were retained. To distinguish contamination from genuine low-level detections and denoise the

data, the CCDB calculated a background noise threshold by examining ASV distributions in negative control wells relative to sample wells (N. Nguyen, personal communication, 25 February 2026). DNA metabarcoding cannot represent abundance, only presence, and therefore can inadvertently minimize the importance of certain prey items (Deagle et al. 2019; De Ruyck & Koper 2024). I considered a detection confident if the genera had >100 reads in each of at least two out of three PCR replicates and considered a taxon rare if it was present in ≤5% of our samples. They were assumed to be less important to the diet of the Bobolink and excluded from our statistical analyses and interpretation.

Identifying groups

The CCDB recommends analyzing dietary items at the genus-level due to limited sequence divergence among closely related plant species and the short length of the molecular marker used for identification of arthropod species (N. Nguyen, personal communication, 25 February 2026). I kept statistical analysis to the genus-level, however, I did use species-level identifications solely for the purpose of determining whether a plant genus was native or non-native and if an insect genus was a pest or non-pest and aquatic or terrestrial. When necessary, I observed the species-level for species of common (>5% of samples) genera with >1000 read counts in three out of three PCR replicates. These conservative parameters allowed me to have high confidence in our native and non-native and pest and non-pest categorizations. I used the Canadian Endangered Species Conservation Council's (2022) Wild Species Report to classify plant taxa as native or non-native, as well as thorough literature review for each species. For insect taxa, I only classified an insect as a pest if it was identified in the literature as a pest to grasses or forage, to maintain relevance to the breeding habitat of the Bobolink (Nocera

et al. 2007; Renfrew et al. 2020). If an insect taxon was considered a pest in another agricultural crop but not hayfields, I considered it a non-pest to ensure cautious interpretation. I classified insects as aquatic or terrestrial using a thorough literature review. There was one case where multiple species of a plant genus were present with >1000 read counts in three out of three PCR replicates, and some of those species were considered native and some were considered non-native. This genus was categorized as undetermined and omitted from the statistical analysis.

Statistical analysis

I conducted statistical analyses using the R statistical software (v4.5.2; R Core Team 2025). I used the *dplyr* (Wickham et al. 2023) and *tidyr* (Wickham et al. 2025) packages for cleaning and filtering data. I used the function *glmer* in the package *lme4* (Bates et al. 2015) to fit binomial generalized linear mixed models. To establish a baseline for taxonomic comparisons, I examined genus-level presence/absence data structured so that each observation represented the occurrence or non-occurrence of a given genus in within an individual sample. The presence or absence of confident, non-rare taxa was used as the dependent variable, compared to site and origin (for plants) or pest status (for insects) as our fixed effects. Genus was included as a random effect to account for inherent differences in baseline occurrence among taxa; thus, fixed effects evaluate whether ecological groupings explain variation beyond taxon-specific prevalence. Initially, I included sample as a random effect to account for non-independence among genera within samples, but the variance was negligible and it was excluded from final models for parsimony. I evaluated model assumptions using base R and the package *DHARMa* (Hartig 2024) and selected models using Akaike's Information

Criterion (AIC; Akaike 1974). I calculated marginal and conditional R^2 values and model averaging for models with $\Delta AIC < 4$ using the package *MuMIn* (Bartoń 2025). I visualized the results using the package *ggplot2* (Wickham 2016).

To complement the model-based analyses, I determined the frequency of occurrence (FOO) for prey at the genus level using the DNA metabarcoding results. This was calculated using the number of consumer species samples where a specific taxon was present, then divided by the total number of samples from the consumer species (Bowser et al. 2013). A FOO of 1.0 indicates that a taxon was detected in the diet of all Bobolinks sampled, and a FOO of 0.5 indicates that a taxon was detected the diets of half of the Bobolinks sampled (Deagle et al. 2019). I chose to use FOO as it allows comparison with other dietary studies and quantifies diet effectively without using proportion (Bowser et al. 2013). My analysis identifies potential for ecosystem services based on diet composition but does not quantify functional impacts such as pest suppression or yield effects. I visualized FOO using *ggplot2* (Wickham 2016).

3.3 Results

I caught 155 Bobolinks and 136 of them provided fecal samples with sufficient matter to be analyzed by the CCDB. Each sample was replicated three times, for a total of 408 replicates analyzed. After quality filtering, primer/adaptor trimming, and size selection, the NextSeq run and sequencing produced 108,815,892 reads for taxonomic analysis from the ITS2 primers and 81,365,388 reads for taxonomic analysis from the ZBJ primers (N. Nguyen, personal communication, 25 February 2026).

Once correcting for a minimum of >100 reads in two of the three PCR replicates, all 136 samples had at least one insect or plant genera present. The sample size was

reduced to 135 by removing one mislabelled sample. I confidently detected 54 unique genera of plants across our samples, and 17 of those were present in >5% of our samples. Of those 17, six plant genera were identified as native, ten as non-native, and one genus was considered undetermined as it was comprised of both native and non-native plant species. I confidently detected 133 unique genera of insects across our samples, and eight of those were present in >5% of our samples. Of those eight, four genera were identified as non-pest and four were identified as pest.

Presence/absence of plant genera was best explained by the model including Site with Genus as the random effect. Based on my model results, there was no evidence of preferential occurrence between groups of genera. I found no evidence that native and non-native plant genera differed in their probability of occurrence ($\beta = 0.087$, $p = 0.807$, Table 4), suggesting that both groups are similarly consumed by Bobolink. Site effects were also not statistically significant. For the top plant model, the marginal R^2 was 0.4% and the conditional R^2, which included Genus as a random effect, was 20.7% (Table 5), indicating that most variance in plant occurrence can be attributed to differences among genera rather than site or native or non-native plant type.

Similarly, the presence/absence of insect genera was best explained by the null model and adding sample as an additional random effect did not improve model fit (Table 6). I found no evidence that pest and non-pest genera differed in their probability of occurrence ($\beta = -0.087$, $p = 0.824$, Table 4), indicating that both groups are being similarly consumed by Bobolink, and no site-level effects were detected; the conditional R^2 was 30.3% (Table 5). The same was true for the model comparing both native and

non-native plants and insect pests and non-pests (Table 5, Table 6), with the top model having a marginal R^2 of 4% and a conditional R^2 of 28.4% (Table 5).

Models met assumptions and diagnostics indicated no evidence of overdispersion (for plants: Pearson $X^2 = 2028.85$, df = 2090, dispersion ratio = 0.97, $p = 0.83$; for insects: Pearson $X^2 = 705.53$, df = 734, dispersion ratio = 0.96, $p = 0.77$; for both: Pearson $X^2 = 2606.81$, df = 2696, dispersion ratio = 0.97, $p = 0.89$). These results indicate that both native and non-native plants and pests and non-pests contribute to the diet of the Bobolink during this period, and that genera explain the most variation in presence and absence of insects and plants.

All identified insect genera in our samples were categorized as terrestrial insects and none as aquatic insects. The FOO of specific genera across all 135 Bobolink fecal samples is presented in Table 7 (plant genera by origin) and Table 8 (insect genera by pest status). The models show that these differences are driven by genus identity rather than their differences among groups, which is clear when visualized (Figure 10, 11).

Table 4. Binomial generalized mixed model results for presence and absence of plant and insect genera in Bobolink fecal samples detected via DNA metabarcoding. Model averaging was performed for models with ΔAIC < 3 and full averages are reported below.

Dependent Variable: Presence and Absence of Plant Genera				
Variable	**Estimate**	**Standard Error**	**z-value**	**p-value**
Intercept	-1.148	0.397	2.888	0.004
Site 5d	0.273	0.305	0.895	0.371
Site 6a	-0.122	0.175	0.698	0.485
Site 6b	-0.064	0.171	0.373	0.709
Non-native	0.087	0.356	0.244	0.807
Dependent Variable: Presence and Absence of Insect Genera				
Variable	**Estimate**	**Standard Error**	**z-value**	**p-value**
Intercept	-1.514	0.394	3.835	1.3e-04
Pest	-0.087	0.390	0.223	0.824
Dependent Variable: Presence and Absence of Plant and Insect Genera				
Variable	**Estimate**	**Standard Error**	**z-value**	**p-value**
Intercept	-1.235	0.270	4.579	4.7e-06
Site 5d	0.251	0.271	0.926	0.354
Site 6a	-0.085	0.145	0.585	0.558
Site 6b	-0.057	0.149	0.380	0.704

Table 5. The marginal and conditional R^2 from the top binomial generalized mixed models (ΔAIC = 0.0) for presence and absence of plant and insect genera in Bobolink fecal samples detected via DNA metabarcoding. The top dataset for insect genera was the null model, and therefore the marginal R^2 is zero.

Top Models from Each Dataset	**Marginal R^2**	**Conditional R^2**
Plant Genera	0.004	0.296
Insect Genera	0.000	0.207
Both Plant and Insect Genera	0.004	0.284

Table 6. Binomial generalized linear mixed model selection table for presence and absence of plant and insect genera in Bobolink fecal samples detected via DNA metabarcoding. Genus and Sample were used as random effects. Models were selected using Akaike's Information Criterion (Akaike 1974).

Dependent Variable: Presence and Absence of Plant Genera				
Model	**K**	**AIC**	**ΔAIC**	**logLik**
Site + (1 | Genus)	5	2070.5	0.0	-1030.2
1 + (1 | Genus)	2	2071.7	1.2	-1033.8
Origin + Site + (1 | Genus)	6	2072.3	1.8	-1030.1
Origin + (1 | Genus)	3	2073.4	2.9	-1033.7
Dependent Variable: Presence and Absence of Insect Genera				
Model	**K**	**AIC**	**ΔAIC**	**logLik**
1 + (1 | Genus)	2	682.9	0.0	-339.5
Pest + (1 | Genus)	3	684.7	1.8	-339.4
Site + (1 | Genus)	5	688.1	5.2	-339.1
Pest + Site + (1 | Genus)	6	689.9	7.0	-339.0
Dependent Variable: Presence and Absence of Plant and Insect Genera				
Model	**K**	**AIC**	**ΔAIC**	**logLik**
Site + (1 | Genus)	5	2632.1	0.0	-1311.0
1 + (1 | Genus)	2	2633.1	1.0	-1314.6
Group + Site + (1 | Genus)	8	2637.1	5.0	-1310.5
Group + (1 | Genus)	3	2638.2	6.1	-1314.1

Table 7. Frequency of occurrence (FOO) of plant genera detected across 135 Bobolink fecal samples via DNA metabarcoding. Native plants are compared to non-native plants. The origin of one genus could not be determined and is classified as undetermined. Genera are arranged from highest FOO to lowest. Each taxon was compared to the Canadian Endangered Species Conservation Council's (2022) Wild Species Report. The plant genus of undetermined origin was omitted from statistical analyses.

Genus	**Origin**	**Frequency of Occurrence**	**Citation for Classification**
Phalaroides	Non-native	0.713	Lavergne & Molofsky 2024; Lavergne & Molofsky 2006
Alopecurus	Non-native	0.636	Weintraub 1953
Symphyotrichum	Native	0.606	Djan-Chékar et al. 2004; Semple et al. 2024
Trifolium	Non-native	0.538	Vahdati et al. 2012; Mollashahi et al. 2023
Anthoxanthum	Non-native	0.447	Weintraub 1953
Solidago	Native	0.242	Baglyas et al. 2023
Agrostis	Native	0.227	Hitchcock 1905
Centaurea	Non-native	0.204	Lejeune & Seastedt 2001; Gudžinskas 2022
Phleum	Non-native	0.177	Weintraub 1953
Poa	Non-native	0.152	Weintraub 1953
Ambrosia	Native	0.144	Hodgins & Rieseberg 2011
Stellaria	Undetermined	0.121	Sharples 2019; Kučera et al. 2021
Hierochloe	Native	0.106	Cody et al. 2003
Betula	Native	0.098	Erdmann 1990
Lolium	Non-native	0.098	Weintraub 1953
Daucus	Non-native	0.075	Muturi et al. 2019
Dactylis	Non-native	0.053	Weintraub 1953

Table 8. Frequency of occurrence (FOO) of insect genera detected across 135 Bobolink fecal samples via DNA metabarcoding. Pest insects are compared to non-pest insects. Genera are arranged from highest FOO to lowest. Genera with asterisks have been identified as pests in other crops, but not hayfields.

Genus	Pest Status	Insect Type	Frequency of Occurrence	Citation for Classification
Mythimna	Pest	Terrestrial	0.386	Philip et al. 2018; Falcon-Brindis & Villanueva 2025
Cisseps	Non-pest*	Terrestrial	0.242	Hudon & Perron 1970; Cantelo & Jacobson 1979
Olibrus	Non-pest	Terrestrial	0.227	Majka et al. 2008
Lygus	Pest	Terrestrial	0.083	Philip et al. 2018
Coleophora	Pest	Terrestrial	0.083	Landry 2003; Kaur et al. 2024
Athysanus	Non-pest*	Terrestrial	0.061	Saguez et al. 2014
Hypera	Pest	Terrestrial	0.061	Philip et al. 2018
Ancyloxypha	Non-pest*	Terrestrial	0.053	Smith & Robinson 1983; Smith 1986

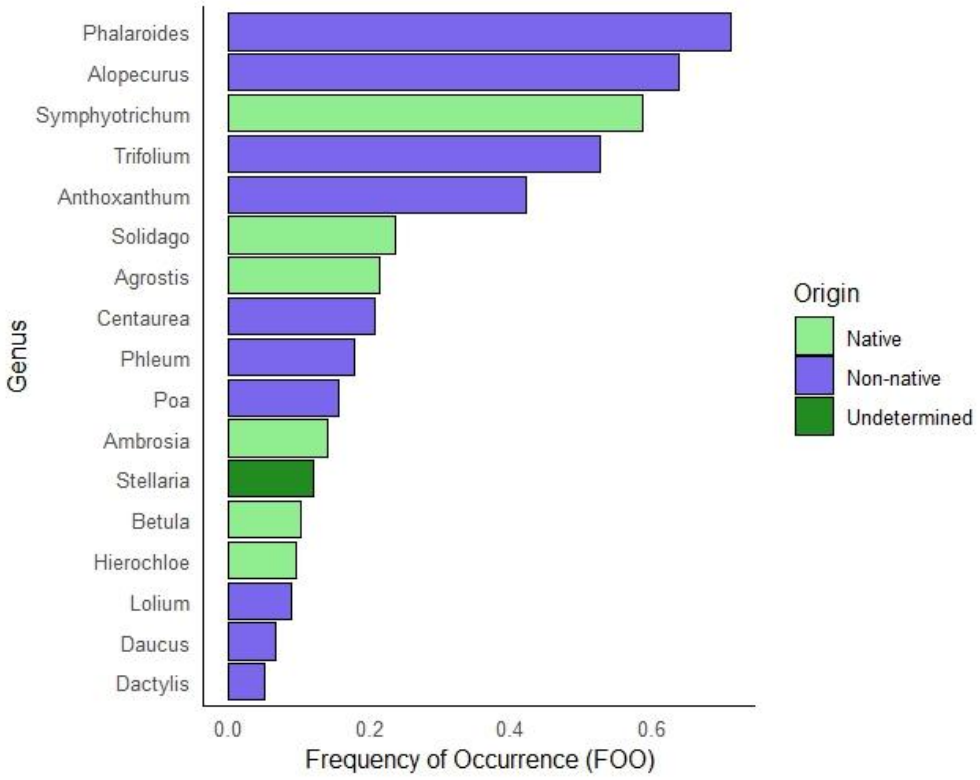


Figure 10. Frequency of occurrence (FOO) of plant genera detected across 135 Bobolink fecal samples via DNA metabarcoding. Plant genera are identified as native (light green), non-native (blue), and undetermined (dark green).

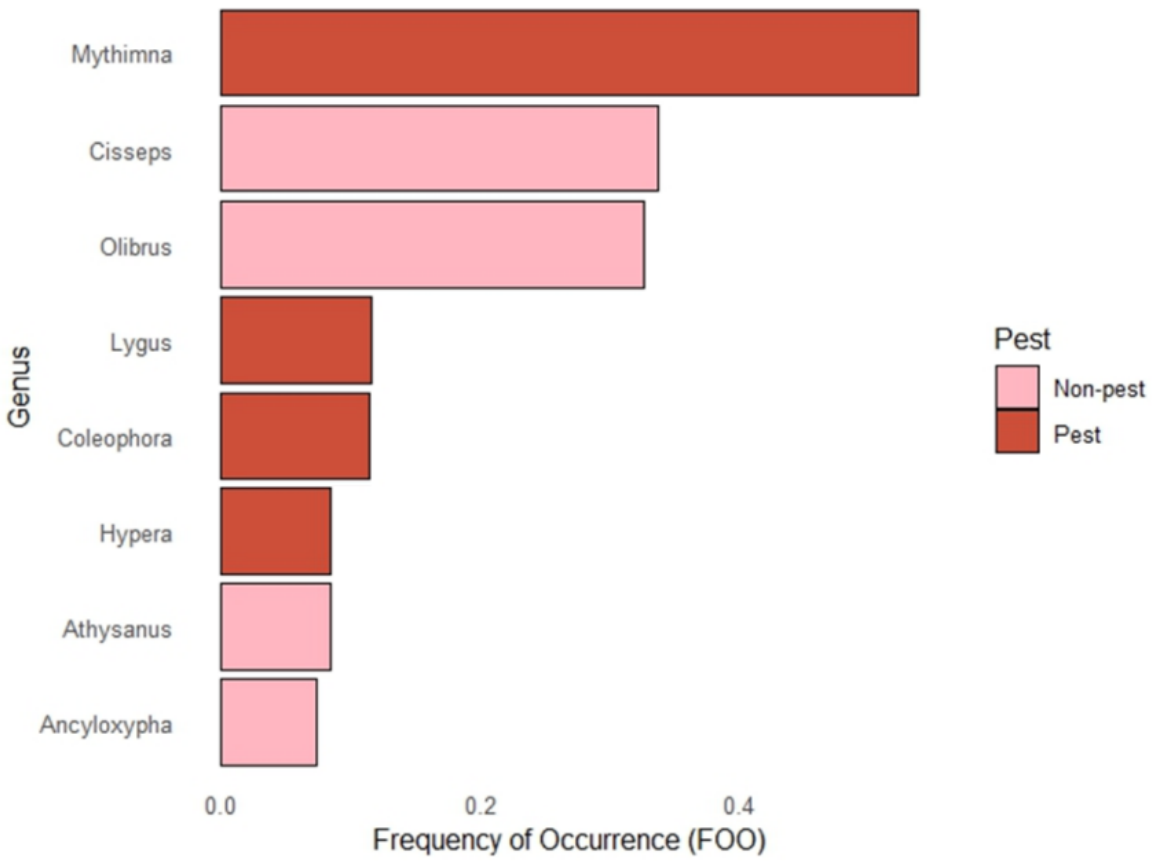


Figure 11. Frequency of occurrence (FOO) of insect genera detected across 135 Bobolink fecal samples via DNA metabarcoding. Insect genera are identified as pest (red) and non-pest (pink). The genus with the highest FOO, *Mythimna*, is considered a pest.

3.4 Discussion

I found that Bobolinks consume a diverse community of prey items composed of native and non-native plants and pest and non-pest insects. Bobolinks did not show different consumption of groups; rather, the presence of taxa appears to be driven by characteristics of individual genera. Site effects were insignificant and small, indicating that local conditions do not substantially influence taxonomic differences. These results establish a baseline for examining the consumption of native and non-native plants and

pest and non-pest insects by the Bobolink, including potential ecosystem services or disservices.

Native versus non-native plants

Our results show the Bobolink consumes a mixture of both intentionally cultivated hayfield plants as well as unwanted weeds and invasives. Cultivated plants include eight of the seventeen commonly consumed plant genera: *Agrostis, Dactylis, Hierochloe, Lolium, Phalaroides* (widely recognized synonym *Phalaris*; COL 2025)*, Phleum, Poa,* and *Trifolium* (Prigge et al. 1999; Barkworth et al. 2007). While some studies of seed-eating birds have found that birds have strong top-down effects on community structure through foraging (Guo et al. 1995), others indicate that even moderate to high seed predation does not limit the seed availability for germination and recruitment, which was true for even highly selective seed-eating birds (Marone et al. 2008). Given that the Bobolink consumes a wide range of plants and insects and does not appear to rely solely on a select number of species (none with a FOO > 0.713), any top-down effects would likely be less suppressive or community altering.

Despite consuming commonly cultivated grasses, I also found several weeds in the Bobolink's diet. *Ambrosia* (FOO = 0.144)*,* also known as common ragweed (*Ambrosia artemisiifolia*), can dominate hayfields due to its large seed count and rapid and efficient germination (Bazzazz 1974). Common ragweed has been identified as an agricultural weed that can be quite financially costly for farmers as it can reduce harvest yield (Rosenbaum et al. 2011). As Bobolinks consume ragweed seeds, if the viability of the seed is destroyed during digestion, they may be contributing to the suggested long-

term management of this weed, which is to reduce the seed bank (Milakovic & Karrer 2016).

Another plant genus identified in the diet of the Bobolink is *Daucus* (FOO = 0.075), also called Queen Anne's lace or wild carrot (*Daucus carota*), which has been categorized as an aggressive invasive (Muturi et al. 2019). As a noxious weed, it has been found to potentially cause tissue damage and other toxicity related diseases in livestock, especially in high quantities (Ayeni 2018). Knapweed (genus *Centaurea,* FOO = 0.204) was also found in the Bobolink's diet and is considered an invasive noxious weed as well, sometimes outcompeting desirable hayfield species (DiTomaso 2000). Knapweed can also reduce the value of hay and is unpalatable to livestock (Sheley et al. 1998). *Stellaria* (FOO = 0.121) has also been identified as a weed in hayfields (Batra 1979), although has not been studied extensively. While these plants are identified as weeds, interpretation around weed control must be made cautiously. It is unknown if digestion destroys the seed when consumed by the Bobolink; if it is viable after defecation this would not aid weed control.

Given that our analysis remains at the genus level, but the results of our DNA metabarcoding analysis allow us to cautiously interpret at the species level, some plant genera have complex results. For example, two *Anthoxanthum* (FOO = 0.447) species were identified in the metabarcoding analysis with >1000 reads in three out of three PCR replicates. *Anthoxanthum odoratum,* although not a dominant crop grass, does occur intentionally in agricultural hayfields (Smith et al. 2023). Meanwhile, *A. aristatum* has been identified as a weed in agroecosystems (Destefano 2013; Drapikowska et al. 2020). Similarly, *Alopecurus* (FOO = 0.636) has been identified as an intentionally cultivated

hay species (Robins & Jensen 2011) but also as a crop weed (Destefano 2013). Although the *Solidago* (FOO = 0.242) species identified in our DNA metabarcoding analysis (*Solidago rugosa*) has not been extensively categorized as a weedy plant, it is common in grasslands and is closely related and shares similar biological mechanisms to *S. canadensis*, which is considered a noxious weed that can spread rapidly and uncontrollably in hayfields (Havryliuk et al. 2023; Kelleher et al. 2025).

Plant genera *Betula* (FOO = 0.098) and *Symphyotrichum* (FOO = 0.606) were not identified as weeds nor as intentionally cultivated hayfield plants (Houle & Payette 1990; Djan-Chékar et al. 2004; Semple et al. 2024). Therefore, the Bobolink consuming these plant genera should not negatively affect the cultivated crops in which they spend their breeding season, or the crop yield of the farmers.

Pest versus non-pest insects

The insect genus that appeared across the most Bobolink samples was *Mythimna* (FOO = 0.386), commonly known as the true army worm (*Mythimna unipuncta*), which has been identified as a field crop pest in Canada (Philip et al. 2018). This genus causes severe damage to major crops and has been identified in field crops, grasses, and grains, including Timothy (*Phleum*) and bluegrass (*Poa*) (Falcon-Brindis & Villanueva 2025). Three other hayfield pests were found in the diet of the Bobolink, *Coleophora* (FOO = 0.083), *Lygus* (FOO = 0.083), and *Hypera* (FOO = 0.061). *Coleophora* is considered an invasive insect pest that causes significant economic damage to *Trifolium* species, which are cultivated in hayfields (Prigge et al. 1999). *Lygus* feeds on developing seeds and can cause yield losses and cause significant economic losses (Philip et al. 2018; George et al. 2021; Oeller et al. 2021). *Hypera* is considered a severe agricultural pest, particularly to

Trifolium species and alfalfa (*Medicago satvia*) (Chan et al. 1990; Bloem et al. 2002; Philip et al. 2018).

The second most frequently consumed genus by the Bobolink was *Cisseps* (FOO = 0.242). Although I did not consider this a pest as I found no literature indicating it as a pest in hayfields, *Cisseps* has been previously identified as an economic destructive pest in other *Poaceae* crops in Canada (Hudon & Perron 1970; Cantelo & Jacobson 1979). Similar to *Cisseps*, I did not find evidence that insect genera *Ancyloxypha* (FOO = 0.053) and *Athysanus* (FOO = 0.061) were pests in agricultural hayfields, however, they have been identified as pests in other crops. *Ancyloxypha* is a persistent pest that has been found to cause significant economic damage in cultivated rice fields (Smith & Robinson 1983; Smith 1986) and *Athysanus* has been previously identified as a pest in Canadian vineyards that can result in significant yield losses through feeding and disease transmission (Saguez et al. 2024).

I cautiously did not identify *Cisseps, Athysanus,* and *Ancyloxypha* as pests given the lack of literature indicating their pest status in hayfields. However, the fact that they are considered pests elsewhere may indicate they could be pests in hayfields but have not been identified as such due to lack of research. Furthermore, *Ancyloxypha* and *Cisseps* are pests of other Poaceae crops, such as rice and corn (Hudon & Perron 1970; Cantelo & Jacobson 1979; Smith & Robinson 1983; Smith 1986). Given that Bobolink hayfields in the breeding season are composed dominantly of plants belonging to Poaceae (Nocera et al. 2007; Renfrew et al. 2020), it seems likely that where rice and/or corn are absent, these insect genera may feed on similar and related plants (hayfield grasses).

The only insect genus that was not identified as a pest of any kind was *Olibrus*, which has only been identified in Nova Scotia in the last 20 years and has been found in the heads of plants belonging to *Asteraceae*, but not any cultivated crop species (Majka et al. 2008). In our literature review, I could not find evidence of *Olibrus* being a natural enemy, and in fact none of the common insect genera found in the diets of the Bobolink were identified as natural enemies. Natural enemies are the predators and parasites of pests and are seen as a beneficial pest control responsible for 50-90% of biological pest control in crop fields (Pimentel 2005). Often, bird species may consume natural enemies, reducing biological pest control in agroecosystems (Martin et al. 2013). I consider the fact that Bobolink are not consuming natural enemies as a very positive result for farmers or landowners with Bobolink present in their hayfields, as consuming natural enemies is considered an ecosystem disservice (Martin et al. 2013). The lack of natural enemy consumption amplifies the argument that Bobolinks may be benefitting the ecosystems in which they breed.

Terrestrial versus aquatic insects

Given that invertebrate populations in agricultural habitat are in decline (Mancini et al. 2023) and this may be a significant contributor to declines in grassland bird populations (Benton et al. 2002), I expected that Bobolinks may be supplementing their diet with emergent aquatic insects. Surprisingly, this was not the case, as all the commonly consumed insect genera I identified were terrestrial. This is interesting given that emerging aquatic insects provide up to ten times the essential fatty acids than that of terrestrial insects (Twining et al. 2016) and birds need a very high capacity of fatty acids to perform migratory flight (Guglielmo 2010). The fact that these are not consumed by

the Bobolink, especially during staging for a long-distance migration, is unexpected and indicates that terrestrial insects must be providing a beneficial trade-off.

Although terrestrial insects provide significantly less essential fatty acids compared to aquatic insects (Twining et al. 2016), it is possible that terrestrial insects consumed by the Bobolink exist in such high quantities in agroecosystems that Bobolinks can acquire sufficient fatty acids for migration with terrestrial insects alone. While insects in general are experiencing a global decline (Simmons et al. 2019), crop pests such as those identified in my study may still be abundant in agroecosystems due to the plentiful resources and the generalist, adaptable traits of pests (Tamburini et al. 2020). This speculation is supported by the fact that none of the insect genera I identified in fecal samples are imperiled in Canada (Canadian Endangered Species Conservation Council). In Nova Scotia, where my study takes place, *Mythimna* is considered apparently secure, *Ancyloxypha, Lygus,* and *Olibrus* are considered secure, *Cisseps* is considered unrankable (although considered generally secure in Canada), and *Athysanus, Coleophora,* and *Hypera* are considered exotic (Canadian Endangered Species Conservation Council 2022). Given the apparent abundance of these insects, the Bobolink may be able to sustain its fatty acid consumption from terrestrial rather than emergent aquatic insects.

Potential limitations

I analyzed dietary items at the genus level due to ensure statistical robustness of the data (N. Nguyen, personal communication, 25 February 2026). However, for interpretation of plant origin and insect pest status, I needed to examine the DNA metabarcoding results at the species-level, as species may differ within genera. I limited inference to species-level observations to those belonging only to common insect genera

in >5% of samples and those with >1000 read counts in all three PCR replicates. I believe this strengthens the interpretation of these results, although species-level evaluations should still be interpreted with caution. Another potential limitation is that DNA metabarcoding cannot quantify the abundance of prey items consumed and is only a measure of presence (Rytkönen et al. 2019). Therefore, I cannot infer the abundance of prey items, meaning the implications of ecosystem services and disservices of the Bobolink require further study to properly quantify. Furthermore, all my hayfield sites were biased towards low-intensity, small-scale landowners who were generally quite interested in conservation and supporting a project with potential management outcomes for the Bobolink. While I observed minimal site differences, these results may differ in large-scale hayfields with high agricultural intensity. Lastly, my study took place in Nova Scotia, Canada, and generalizations to the broader breeding range of the Bobolink should be made with caution.

Implications for conservation

Bobolink food consumption appears to potentially provide a complex array of ecosystem services and some minor disservices, although future studies would need to quantify consumption rates of prey items to confirm this. Intentionally cultivated hayfield plants are present in the Bobolink's diet, which could be considered a disservice to landowners are this may damage or reduce the yield of their crops. However, the Bobolink also consumes several plant genera that have been identified as highly financially costly, noxious, and dominant weeds. The consumption of weed seeds can provide ecosystem services for landowners, as this may suppress or slow the spread of undesirable plants. Additionally, I found four genera of hayfield pests and zero natural

enemies in the diet of the Bobolink. This indicates that insect consumption by Bobolink could act as biological pest control, contributing ecosystem services to hayfields and potentially even resulting in higher yield of crops and minimizing economic losses (as measured in other studies: Mols & Visser 2002; Gonthier et al. 2019; Díaz-Siefer et al. 2022; Garfinkel et al. 2022). Furthermore, as no natural enemies were identified in my results, this implies that the Bobolink may be contributing pest control without suppressing natural enemies of pests, which increases the benefits of their potential ecosystem services.

My results align with other Bobolink dietary studies in their nonbreeding range, which indicated they may contribute to the control of pests in rice fields. Lorenzón et al. (2019) found potential insect prey items in the diets of Bobolink belonging to orders with several species that damage rice crops, indicating a possible beneficial role of pest regulation in agroecosystems. Another study by Renfrew and Saavedra (2007) found that Bobolinks fed intensively on insects during the nonbreeding season, which may reduce caterpillar populations and benefit soybean production.

Results such as mine may provide the foundation for a "rebranding" of the Bobolink away from its status as a nuisance and better incentivize farmers to support the conservation of this species, with the caveat that these results cannot determine if the Bobolink is eating enough pests to alter crop productivity. By hosting Bobolinks on agricultural lands, landowners could see not only lower losses of yield to pests, but also economic benefits of "bird-friendly" crop management. A consumer survey on bird management methods by Herrnstadt et al. (2016) indicated that consumers were willing to pay more for fruit grown with natural practices. In recent years, the costs of pesticides

have become more apparent, both economically and ecologically, and both consumers and farmers are valuing natural alternatives (Herrnstadt et al. 2016; Lindell et al. 2018; Shave et al. 2018). If farmers used birds as biological control in lieu of pesticides, this could be a mutually beneficial relationship providing both a natural alternative and benefitting the birds, as grassland bird population decline can be in part attributed to pesticide use (Perfecto & Vandermeer 2008; Mineau & Whiteside 2013).

If farmers wanted to further support Bobolinks in their agricultural ecosystems for their conservation and/or for their potential ecosystem services, a strongly impactful and well-known management technique is to delay hay harvest until breeding has finished. Delaying hay harvest allows young Bobolinks to fledge the nests before they would be destroyed by hay machinery (Bollinger et al. 1990; Herkert 1997). However, delayed harvest can be a cost to farmers, as the nutritional quality in hay necessary for feeding livestock declines greatly throughout the season (Nocera et al. 2005). I posit that perhaps this cost could be offset by the Bobolink's possible ecosystem services of providing pest and weed control.

3.5 Conclusion

The Bobolink's history within agroecosystems is a complex one. While the Bobolink historically bred in the natural prairies and grasslands of North America, the conversion and destruction of this habitat has caused the Bobolink to adopt agriculturally associated grassland habitats including hayfields (Herkert 1997). This has resulted in trade-offs between declining populations of a grassland songbird and economically feasible hay harvesting (Diemer & Nocera 2016). However, my DNA metabarcoding results indicate that during the breeding season, the Bobolink consumes not just

intentionally cultivated hayfield plants, but also persistent, dominant, and economically costly weeds and pests. These results could imply that Bobolinks provide ecosystem services to the agricultural land they inhabit, potentially incentivizing farmers to delay hay harvesting and promote ecologically focused, bird-friendly management.

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Chapter 4: General Discussion

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I used DNA metabarcoding of fecal samples to describe the diet of the Bobolink (*Dolichonyx oryzivorus*) approaching fall migration. I compared differences in diet over time, between age groups, and between native vs non-native plants and pests vs non-pests. I sought to fill a knowledge gap surrounding this long-distance migratory species-at-risk, with implications for Bobolink conservation and management.

4.1 Hypotheses and Predictions

My first hypothesis was that Bobolinks prepare for migration through dietary change. If true, then I predicted I would see increasing degrees of change over time; i.e., the community composition of prey items, ascertained by their frequency of occurrence (FOO), would change significantly from July to August. My results supported this hypothesis and aligned with my prediction for both plant and insect genera consumed by the Bobolink.

My second hypothesis was that dietary composition will differ between adults and juveniles during the staging period, as juveniles have additional energy requirements as their bodily systems and foraging skills develop (Chandler et al. 2012). My prediction was that juvenile Bobolinks would have a different FOO of invertebrates in their diet when compared to adults. My results supported this hypothesis, as insect community composition differed significantly between adult and juvenile age groups. The plant community composition did not differ significantly between age groups; however, this was not unexpected given that foraging may be easier for plants compared to insects (Vanderhoff & Eason 2007).

My last test was to determine if plant genera in the diets of the Bobolink differ based on origin (native and non-native) and if insect genera in the diets of Bobolink differ

based on pest status (pest or non-pest). My prediction was that the Bobolink would consume a diverse community of prey items composed of both native and non-native plants and pest and non-pest insects. My results supported this hypothesis, as the differences between native and non-native plants and pest and non-pest insects were not statistically significant, indicating a diverse range of prey items in the diet of the Bobolink.

4.2 Limiting factors of the post-breeding period

I found that Bobolinks shifted their dietary patterns during the fall staging period, which could reflect differing resources needed to prepare for migration. The plant and insect community composition of Bobolink diet changed significantly over time, indicating possible changes in prey consumption or nutritional requirements from the start of July until mid-August. During this time, I also found significant differences between the insect community composition, but not the plant community composition, of the diets of adult versus juvenile Bobolink. These differences may highlight the population limiting factors that occur during the post-breeding, pre-migratory period, as an energetically costly time that has historically received less research attention than other life history stages (Tsuru & Tonra 2026). During this period, migrants must manage the competing nutritional and energetic demands associated with preparing for migration, molting, and juvenile development (Tsuru & Tonra 2026). The overlap of these processes may be evidence of the Energetic Allocation Hypothesis, where migrants juggle the increasing resource demands and energetic costs of these processes because the benefit is the reduction of temporal constraints or later more imposing energetic conflicts (Tsuru & Tonra 2026). If the Bobolink is engaging in these energetically demanding processes

simultaneously, this may also indicate that late-season breeding habitat must be rich in supporting resources.

Efficient pre-migratory fuelling is crucial for the survival and success of migratory birds given the elevated risk of mortality during migration as well as the benefits of a successful migration (Sillett & Holmes 2002; Warnock 2010). Many migrant songbirds shift their diet seasonally during pre-migratory periods to prepare morphologically, physiologically, and behaviourally for migration (Bairlein 1998). Given the impressive distance travelled by the Bobolink (20,000 kilometres annually round-trip; Renfrew et al. 2020), this preparation stage may be especially crucial. The statistically significant differences in dietary community composition I observed over time could indicate a dietary shift to meet the demands of pre-migratory preparation, such as the accumulation of fat stores (hyperphagia) (Warnock 2010).

For juveniles, the time between breeding and fall migration is even more critical to survival (Chandler et al. 2012). During the post-breeding period, juvenile birds are not only preparing for their first migration but also growing to adult size and developing foraging and mobility skills (Chandler et al. 2012). Because of the high energy expenditure during the post-breeding period, juveniles may require higher quantity and quality of foods to support these energetic costly processes, and these requirements should be explored further in future studies (Vanderhoff & Eason 2007; McCabe & Guglielmo 2019). Additionally, immature birds have less developed wings and weaker beak musculature, which may impact energy expenditure and the ability to catch and manipulate prey items (Marchetti & Price 1989). Recently, more studies have emphasized the importance of conservation efforts focused on the post-fledging stage during the

understudied post-breeding, pre-migratory period (Rivera et al. 1998; Vitz & Rodewald 2006; Chandler et al. 2012; Cox et al. 2014).

A study monitoring juvenile Dickcissels (*Spiza americana*) and juvenile Eastern Meadowlarks (*Sturnella magna*) found that an increase in body mass reduced the risk of mortality and emphasized the importance of body condition on post-fledging survival (Suedkamp Wells et al. 2007). Further studies on Dickcissels (Berkeley et al. 2007) and Eastern Meadowlarks (Kershner et al. 2004) highlighted the vulnerability of juveniles and the high rates of mortality during the post-fledging period. These studies and others spotlight the importance of this critical life history period and the lack of information on the post-fledging period especially in grassland birds (Adams et al. 2001; Hovick et al. 2011). Furthermore, Cox et al. (2014) found that fledgling age was a strong predictor of survival, with mortality being highest during the first three weeks post-fledgling. Their study strongly emphasized the post-breeding, pre-migratory period as a critical and limiting period affecting survivorship, and survivorship can be influenced by food availability and habitat quality (Cox et al. 2014). Additionally, juvenile birds get lost easier than adults (Newton 2008) and may be competitively excluded by adults from high quality nonbreeding habitats (Sherry and Holmes 1996). Given these challenges of the first migration for juvenile birds, efficient preparation may be even more critical.

Molting is an energetically demanding process that likely requires additional resources during fall staging. The Bobolink undergoes two complete annual molts, with the first prebasic molt occurring primarily from July until October (Renfrew et al. 2020). This molt begins on or near the breeding grounds but may be completed during migration at stopover sites or on the nonbreeding grounds (Renfrew et al. 2020). The costs of

molting include increased exposure to suboptimal temperatures, increased risk of predation, and high energy demands, but this can be outweighed by benefits such as increased flight efficiency, insulation, and optimal plumage (Contina et al. 2023). Molting and preparing for migration require different physiological processes; molt requires a high proportion of a bird's bodily proteins to be replaced, resulting in increased energy use and protein synthesis (Jenni-Eiermann & Jenni 1996), while staging for migration involves the rapid accumulation of fat stores through hyperphagia (Warnock 2010). The overlap of energetically costly processes, molting and staging, may result in a physiological trade-off that corresponds with a dietary shift to meet the demands of multiple competing bodily processes (Rubolini et al. 2002; Tsuru & Tonra 2026). Furthermore, molting during staging may be an evolved strategy, as neither are as energetically demanding as migration itself (Contina et al. 2023). Rubolini et at. (2002) found that this energetic cost was greater for inexperienced juvenile birds during the pre-migratory period of another long-distance migrating passerine, the Barn Swallow (*Hirundo rustica*), further highlighting this period as costly and limiting for juvenile birds.

To ensure the conservation of bird populations in decline, including the Bobolink and other grassland or long-distance migratory birds, it is important to understand their physiological demands across spatial and temporal scales as well as across demographics. My study contributed knowledge of the energetically demanding period post-breeding and prior to migration, when multiple bodily processes including staging, molting, and juvenile development are competing for resources. By furthering the understanding of the post-breeding period, I am contributing to a holistic understanding of the life history of

the Bobolink but also emphasizing the importance of this time for avian life history in general, which may have implications for other species-at-risk and can help inform management decisions.

4.3 Management implications for a population in decline

My thesis has emphasized the importance of diet in preparation for fall migration, especially for a long-distance migrant in population decline, the Bobolink. Food resources are a limiting factor to survival, especially during the energetically demanding staging period, and should be protected to support the Bobolink during this crucial period. The Bobolink's diet during staging was distinctively diverse and it is clear from my results that this species is not relying on one or two specific prey items. Despite being the most frequently present genus, *Phalaroides* only occurred in 71.3% of samples. Out of 135 Bobolinks, 40 (29.6%) only consumed plant genera (when examining commonly occurring genera in >5% of samples). Of those 135, 94 consumed both plants and insects (69.6%), and one individual consumed only insect genera (0.7%). The 17 common (>5% of samples) plant genera present made up six unique plant families and six unique plant orders; the eight common (>5% of samples) insect genera identified were composed of eight unique insect families and three unique insect orders. This included native and non-native plants, intentionally cultivated and weedy invasive plants, and both crop pests and non-pest insects. These results indicate large variation in diet among individual Bobolinks during the staging period and could indicate a similar variation in nutritional needs.

To reflect the diverse and complex diet of the Bobolink during the breeding season, management should focus on ensuring the presence of heterogenous habitat that

supports these prey items and resource supply for the Bobolink. The lack of reliance on one cultivated plant species implies that the Bobolink may prefer non-monoculture hayfields and grasslands. Hološková et al. (2025) found that farmland bird species richness was improved by enhancing diverse landscape elements and that heterogenous landscapes consistently outperformed homogenous, crop-dominated fields. The study also revealed that bird species richness was improved by invertebrate abundance caused by reducing pesticide use and that invertebrate abundance was particularly high in fallow fields (Hološková et al. 2025). These results highlight the importance of smaller scale, agroecosystems that are less intensive, more heterogenous, and do not rely on pesticides.

My results demonstrate that the Bobolink eats a mixture of grasses and forbs, which indicate that conservation-focused hayfield management could facilitate a less intensive approach that rotates patches of hayfield so that some remain/become fallow while others are actively harvested (Hološková et al. 2025). This idea is supported by Allen et al.'s (2019) study that found that Bobolink and Grasshopper Sparrows (*Ammodramus savannarum*) used uncut refugia more than cut areas, and that even just a small (0.25 hectares) inclusion of refugia reduced field abandonment following harvest. These results strengthen the argument to include refugia, which could include fallow patches, for Bobolink conservation management especially during the post-breeding season, as this period typically occurs after hay harvest.

My DNA metabarcoding results indicate that the Bobolink may perform ecosystem services in hayfields, as I identified invasive weeds and crop pests in their diet. This implies that the Bobolink could be suppressing, to some unknown degree, the spread of undesirable plants that compete with intentionally cultivated crops and acting as

biological pest control by consuming insect pests, although this should be interpreted cautiously as it is unknown if the seeds are viable once defecated by the Bobolink. Such consumption has been found to result in higher yield of crops and minimized economic losses in pest control studies of other bird species (Díaz-Siefer et al. 2022).

Additionally, farmers could support Bobolink by delaying hay harvesting until after breeding. This has strongly positive benefits for Bobolink nest success and population recruitment (Bollinger et al. 1990; Herkert 1997), however, it can come at a cost to the farmers as nutritional quality in hay declines throughout the season (Nocera et al. 2005). If the Bobolink provides ecosystem services to hayfields through pest and weed control, perhaps this trade-off would be worth it. Another benefit of potential Bobolink pest control in hayfields is the emphasis on natural practices that has become more popular in recent years especially as an alternative to pesticides, which are economically and ecologically costly (Lindell et al. 2018). Herrnstadt et al. (2016) conducted a consumer survey on bird management methods and found that consumers were willing to pay more for fruit grown with natural practices, such as bird pest control. If avian research and agricultural management put an emphasis on biological control such as birds instead of pesticides, this could have a mutually beneficial relationship for birds, who may be in population decline due to pesticide use, and farmers looking to market to consumers who prefer natural products (Mineau & Whiteside 2013).

Lastly, management of the Bobolink and other grassland long-distance migrants should account for juveniles and adults as separate groups, specifically during this period of development post-breeding and prior to migration. A quantitative review of fledgling survival across 35 songbird species by Cox et al. (2014) found that food availability and

habitat characteristics and quality were factors influencing survival, indicating that management focused on post-fledging habitat and prey items can improve survival rates of juveniles. Cox et al. (2014) also put emphasis on the benefits of heterogenous habitat for passerine fledgling survival, which reiterates the importance of managing for heterogenous agroecosystems. Furthermore, knowledge of demographics such as differences between juveniles and adults can be important parameter for modelling population growth (Suedkamp Wells et al. 2007) Both Suedkamp Wells et al. (2007) and Hovick et al. (2011) are studies of juvenile grassland passerines that put emphasis on incorporating demographics in models (Hovick et al. 2011).

4.4 Recommendations for future research

I believe that my results provide a hypothesis-generating basis for much additional research. For the Bobolink specifically, more knowledge is needed on the ideal habitat to support this staging behaviour. Future studies could focus on comparisons between complex, heterogeneous habitat and monoculture crops for supporting successful populations. Research should also quantify specific habitat use during staging, to further identify habitat for conservation priority. Other Bobolink dietary studies should utilize DNA metabarcoding techniques to examine prey at finer taxonomic levels than studies have historically, especially across temporal and spatial scales. These studies could compare adults and juveniles as I have done, to see if these observed dietary differences continue onto the nonbreeding grounds.

Furthermore, to determine if Bobolinks are providing ecosystem services in hayfields, specific studies should be done comparing Bobolink presence and absence versus pest and weed abundance, as well as how much damage the Bobolink is inflicting

on the crops versus how much damage they are preventing. Studies by Renfrew and Saavedra (2007) and Lorenzón et al. (2019) suggest that Bobolinks may contribute to pest control in their nonbreeding range. Research in the Bobolink's nonbreeding range could utilize DNA metabarcoding techniques to identify pests to the genus- or species-level to further support these suggestions.

My results also demonstrate the potential importance of the post-breeding, pre-migratory season as a potentially limiting period across avian life history stages. Other grassland or long-distance passerines may also change their dietary composition as fall migration approaches to adequately prepare to migration. Future research should explore this further, especially given the dramatic declines in grassland bird populations (Birds Canada and Environment and Climate Change Canada 2024). My results emphasize the need for conservation management of the Bobolink and potentially other grassland birds that focuses on maintaining heterogenous habitat that supports a diverse range of prey items, which in turn may provide the necessary resources for pre-migratory preparation.

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Curriculum Vitae

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