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# Grassland bird response to enhanced vegetation diversity in restoration plantings in the Spring Run Complex of northwestern Iowa

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**Grassland bird response to enhanced vegetation diversity in restoration plantings in the  
Spring Run Complex of northwestern Iowa**

by

**Jennifer Amy Vogel**

A dissertation submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of

**DOCTOR OF PHILOSOPHY**

Major: Ecology and Evolutionary Biology

Program of Study Committee:  
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**ABSTRACT**

Loss of habitat is one of the primary factors affecting population declines of grassland birds, and recovery efforts have focused on increasing the amount of grassland habitat in the landscape. Assessing the value of habitat restorations for grassland birds is an essential component of grassland bird conservation. We compared grassland bird habitat use, reproductive success, nestling growth rates, nestling baseline corticosterone, and blood glucose levels among restored grasslands planted with seed mixes of varying plant species diversity. In addition, we tested the ability of a conspecific song playback system to attract Henslow's sparrows to previously unoccupied restored habitat. We selected 4 grassland planting types, cool-season, warm-season (newly planted and mature), and high diversity for our study to encompass the range of planting mixtures typically available to land managers. The most common bird species we encountered were Bobolink (*Dolichonyx oryzivorus*), Common Yellowthroat (*Geothlypis trichas*), Red-winged Blackbird (*Agelaius phoeniceus*), and Sedge Wren (*Cistothorus platensis*). Bird densities overall were not consistently higher or lower in any one of the 4 planting types. Bobolink densities, however, were higher in Cool-season fields than in any of the other field types. Models of the relationships between bird density/bird species richness and habitat characteristics revealed that both vegetation characteristics and food resources were important in explaining grassland bird densities. Our habitat models showed that different species, even those within a species category, were influenced by different habitat characteristics. Given the importance of cool-season, non-native grass plantings for Bobolinks in our study, consideration must be given to the impact that elimination of these plantings may have on the future of Bobolink populations. Red-winged Blackbird nest survival was influenced by year, visual obstruction, and variation in

the availability of invertebrate food resources throughout the nesting season. We found evidence for a difference in daily nest survival among planting types ( $P=0.06$ ). Specifically, estimated nest survival was more than twice as high in mature warm-season fields (36%) than in cool-season fields (14%). Red-winged Blackbird nestling size at fledging differed among grassland planting type. Male nestlings were larger than females at fledging with regard to mass, wing, and tarsus measurements. Nestling growth rates did not differ either among grassland restoration planting types or between sexes. Invertebrate food resource availability did not appear to affect growth rates of nestling Red-winged Blackbirds. Red-winged Blackbird nestling baseline corticosterone levels were lower in the warm-season planting type than in either cool-season or high diversity plantings. We found no evidence of differences in baseline corticosterone levels or blood glucose levels between males and females. We found no relationship between baseline corticosterone levels and body mass or between baseline corticosterone levels and blood glucose for male or female nestlings. Blood glucose levels in male nestlings had a significant positive relationship with nestling mass, but not in female nestlings. Management activity and brood size had positive relationships with baseline corticosterone, suggesting that more intensive management activity and larger brood sizes were related to increased stress levels. Nestling age and temperature during the nestling period were positively related to blood glucose levels. We successfully attracted Henslow's Sparrows to 3 of 7 treatment plots using conspecific song playbacks and we found no Henslow's Sparrows in control plots. The addition of social cues using playback systems in restored grassland habitats may aid conservation efforts of Henslow's Sparrows to available habitat. We conclude a variety of planting types and management strategies may be necessary to successfully conserve grassland birds.

## CHAPTER ONE: GENERAL INTRODUCTION

As a result of rapid settlement and conversion to agriculture, the tallgrass prairie ecosystem of North America is one of the most endangered ecosystems on earth (Smith 1981, Noss et al. 1995) and in Iowa, less than 0.01% of the original 12 million hectares of prairie remains (Sampson and Knopf 1994). Loss of habitat is one of the primary factors affecting population declines of grassland birds over the last several decades (Herkert 1995, Fletcher and Koford 2003, Herkert et al. 2003). Efforts to aid in the recovery of grassland bird populations have focused on increasing the amount of grassland habitat in the landscape. However, the extent to which the ecological function of these plantings has been restored is unknown.

Recent habitat restoration efforts focused on mitigating external environmental threats alone, such as habitat destruction, may not be enough to conserve imperiled songbird species (Ward and Schlossberg 2004, Ahlering and Faaborg 2006). Animal behavior has recently been recognized as playing an important role in species conservation (Ward and Schlossberg 2004, Ahlering and Faaborg 2006). Social information and conspecific attraction may be important for many species. In fact, a recent review found that in 20 out of 24 studies examining conspecific attraction in songbirds, birds were successfully attracted using social cue manipulation (Ahlering et al. 2010). In territorial songbirds, the presence of conspecific individuals may provide important cues about habitat use.

Grassland vegetation structure is a key habitat component for grassland songbirds (McCoy et al. 2001, Chapman et al. 2004). Seed mixtures used for grassland plantings in the Midwest vary widely. Thus, the resulting vegetation structure and composition vary significantly among different types of restored grassland plantings (McCoy et al. 2001). The

plant species diversity in seed mixes used for grassland restoration plantings may have lasting effects on the resulting grassland bird community.

Because grassland bird food resources in the form of arthropods vary with plant diversity (Jamison et al. 2002, Benson 2003, Leathers 2003, Harveson et al. 2004, Sutter and Ritchison 2005), the choice of planting mix for a grassland restoration may have important implications for the reproductive success of grassland birds. The availability of food resources near the nest location may contribute to the success or failure of grassland bird nests. Birds spend more time foraging and fly longer distances to forage when their nests are located in areas with reduced food availability (Adams et al. 1994). Sparling et al. (2007) found that, for Red-winged Blackbirds (*Agelaius phoeniceus*), habitat types with the lowest invertebrate diversity had the lowest levels of nest success. Birds that locate their nests in areas with high food availability may need to spend less time off the nest foraging for food. Less time spent away from the nest may translate into lower nest predation and brood parasitism. Food supplementation of Song Sparrows (*Melospiza melodia*) lowered nest predation rates through its influence on adult antipredator behavior (Rastogi et al. 2006). In addition, Dearborn et al. (1998) found that parental nest attendance is an important component of nest defense, and therefore, an important component of nest success.

Nestling growth rates in altricial birds are influenced by many external factors related to the availability of food resources (O'Connor 1984). Both the quality and quantity of food delivered to young in the nest are potentially important in determining growth and reproductive success (Boag 1987, Reynolds et al. 2003, Granbom and Smith 2006). Food resources may affect nestling growth rates differently in different systems (Granbom and Smith 2006). Differences in how food resources influence growth rates may be related to



food resource variability among habitat types and may depend on whether food resources are a limiting factor in those systems. Avian growth rates may also differ both seasonally and annually within habitats. Estimates of food resource availability for insectivorous birds have been measured as parameters of interest in the study of avian systems. Many of these studies have found that food availability is associated with bird abundance in both grassland and forested systems (Brush and Stiles 1986; Davros 2005, Benson et al. 2007).

Corticosterone is a steroid hormone that is released by the adrenal complex in response to stress in vertebrate animals (Siegel 1980). Baseline corticosterone levels in blood plasma are associated with food resource availability in birds (Kitaysky et al. 1999, Saino et al. 2003, Schoech et al. 2004, Pravosudov and Kittaysky 2006, Jenni-Eiermann et al. 2008). Increased baseline corticosterone levels have been associated with poor feeding conditions in adult and developing birds (Saino et al. 2003, Pravosudov and Kitaysky 2006, Kempster et al. 2007, Jenni-Eiermann et al. 2008). In addition, plentiful food resource availability has been linked to lower baseline corticosterone levels. Baseline corticosterone levels have also been used as indicators of habitat quality in birds. In a study of American Redstarts (*Setophaga ruticilla*), birds in lower quality habitat had higher baseline corticosterone levels than those in higher quality habitat (Marra and Holberton 1998).

Blood glucose levels in birds are higher and more variable than in other vertebrates (Braun and Sweazea 2008, Lobban et al. 2010). It is unknown how birds can tolerate these higher and more variable blood glucose levels without experiencing the negative effects, such as tissue damage and death that can occur in other animals (Beuchat and Chong 1998). Blood glucose levels are a reflection of the diet and the recent level of food ingestion by birds (Davey et al. 2002) and blood glucose levels in birds have been shown to be affected by

experimental food restrictions (Altan et al. 2005, Kempster 2007). Blood glucose levels may provide an additional indicator of an individual's physiological condition.

Red-winged Blackbirds are one of the most common bird species in North America (Beletsky 1996). They nest in a variety of habitat types including marshes and uplands (Beletsky 1996, Swain et al. 2003, Sparling et al. 2007). Because of their adaptability to different habitats, they provide an excellent opportunity to evaluate differences in nest survival, nestling growth, and physiological condition among different habitat types.

### **Dissertation Organization**

This dissertation is composed of seven chapters. Chapter one contains a general introduction to the dissertation. Chapter two is a paper written to be submitted to the *Journal of Wildlife Management*. Chapter two compares grassland bird habitat use among restored grasslands planted with seed mixes of varying plant species diversity. Chapter three is a paper written for submission to the journal *Restoration Ecology*. Chapter three examines the effects of grassland restoration planting type and invertebrate food resource availability on the reproductive success of a grassland specialist, the Red-winged Blackbird (*Agelaius phoeniceus*). Chapter four is a paper written to be submitted to the *Wildlife Society Bulletin*. Chapter four examines whether nestling growth rates and nestling size at fledging differed among grassland restoration plantings with varying plant species diversity and to examine how the availability of invertebrate food resources affected the growth rates of grassland songbirds. Chapter five is a paper written for submission to the *Journal of Wildlife Management*. Chapter five examines how restored grassland habitat type affects the physiological condition of nestling Red-winged Blackbirds in grasslands. Chapter six is a

paper that is published in *The Prairie Naturalist*. Chapter six tested the ability of a conspecific song playback system to attract Henslow's sparrows to previously unoccupied restored habitat. Chapter seven contains a general conclusion to the dissertation. All components of this dissertation including data collection, data analysis, and written text were completed by Jennifer A. Vogel under the guidance of Rolf R. Koford and David L. Otis.

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## CHAPTER TWO: BIRD RESPONSE TO ENHANCED VEGETATION DIVERSITY IN GRASSLAND RESTORATION PLANTINGS

A paper to be submitted to the *Journal of Wildlife Management*

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**ABSTRACT** Loss of habitat is one of the primary factors affecting population declines of grassland birds, and recovery efforts have focused on increasing the amount of grassland habitat in the landscape. We compared grassland bird habitat use among restored grasslands planted with seed mixes of varying plant species diversity. We selected 4 grassland planting types, cool-season, warm-season (newly planted and mature), and high diversity, for our study to encompass the range of planting mixtures typically available to land managers. The most common bird species we encountered were bobolink (*Dolichonyx oryzivorus*), common yellowthroat (*Geothlypis trichas*), red-winged blackbird (*Agelaius phoeniceus*), and sedge wren (*Cistothorus platensis*). Bird densities overall were not consistently higher or lower in any one of the 4 planting types. Bobolink densities, however, were higher in cool-season fields than in any of the other field types. Given the importance of cool-season, non-native grass plantings for bobolinks in our study, consideration must be given to the impact that elimination of these plantings may have on the future of bobolink populations. Our habitat models revealed that both vegetation characteristics and food resources were important in

explaining grassland bird densities and that different species, even those within a species category, were influenced by different habitat characteristics. Therefore, we conclude a variety of planting types and management strategies may be necessary to successfully conserve grassland birds.

**KEY WORDS** grassland birds, Conservation Reserve Program, habitat restoration

## **INTRODUCTION**

As a result of rapid settlement and conversion to agriculture, the tallgrass prairie ecosystem of North America is one of the most endangered ecosystems on earth (Smith 1981; Noss et al. 1995). Loss of habitat is one of the primary factors affecting population declines of grassland birds over the last several decades (Herkert 1995; Fletcher and Koford 2003; Herkert et al. 2003). Efforts to aid in the recovery of grassland bird populations have focused on increasing the amount of grassland habitat in the landscape. However, the extent to which the ecological function of these plantings has been restored is unknown.

In Iowa, less than 0.01% of the original 12 million hectares of prairie remains (Sampson and Knopf 1994). Historically, the region was characterized by a mix of mesic to dry tallgrass prairies. Current land use in Iowa is approximately 94% agricultural, with corn (*Zea mays*) and soybeans (*Glycine max*) as the primary crop types (Jackson et al. 1996). A major component in the conversion of agricultural lands back to grassland habitat has been the Conservation Reserve Program (CRP) of the United States Department of Agriculture (Johnson and Schwartz 1993). The goal of this program is to reduce soil erosion by removing erodible farm land from production. This goal is achieved by offering compensation to landowners who plant their marginal lands to perennial grasslands.

Planting mixtures used in CRP vary widely in composition from cool-season, non-native grass plantings to diverse mixtures of native forbs and grasses. The costs associated with planting and maintaining these different types of CRP plantings also vary. Seed costs range from approximately \$50/ha for cool-season, non-native plantings, to an intermediate cost of \$285/ha for native warm-season grasses, to \$2840/ha for a diverse mix of native grasses and forbs (Prairie Seed Farms 2008). In addition, the cost of maintaining a more diverse planting type is higher than the cost of maintaining a less diverse (grass only) planting type.

Choice of CRP planting type influences both the composition and structure of the resulting vegetation. Many studies have demonstrated relationships between bird abundance and vegetation structure and composition (King and Savidge 1995; Patterson and Best 1996; Delisle and Savidge 1997; Hughes et al. 1999; McCoy et al. 2001), however, individual species may respond differently. For example, dickcissel (*Spiza americana*) abundance has been associated with forb cover (Patterson and Best 1996) and tall, dense vegetation (Patterson and Best 1996; Delisle and Savidge 1997; Hughes et al. 1999) in grassland habitats. In contrast, bobolink (*Dolichonyx oryzivorus*) abundance has been negatively associated with both forb cover (Patterson and Best 1996) and vertical density (Delisle and Savidge 1997). Because of differences in how individual species respond to vegetation characteristics in grassland habitats, comparisons of overall bird abundance between different types of CRP plantings may show no differences based on planting type despite having significant differences in vegetation composition and structure (King and Savidge 1995; Delisle and Savidge 1997). Conservation of grassland birds as a group may require a diversity of grassland habitat types (Ribic et al. 2009).

Grassland bird food resources in the form of arthropods vary with plant diversity (Jamison et al. 2002; Benson 2003; Leathers 2003; Harveson et al. 2004; Sutter and Ritchison 2005). As a result, the choice of planting mix for a grassland restoration may affect use by grassland birds. Comparisons of short-grass CRP fields in Texas found that arthropods in CRP plantings can provide important prey resources for grassland birds (McIntyre and Thompson 2003). Among the different exotic and native short-grass CRP types studied in Texas, no differences in arthropod diversity or biomass were found (McIntyre and Thompson 2003). None of the short-grass CRP plantings studied, however, had a seeded forb component, and none had arthropod diversities that were comparable to native short-grass prairie (McIntyre and Thompson 2003). Adding a forb component to CRP plantings may increase invertebrate food resources for grassland birds (Doxon and Carrol 2007). For example, in Kansas CRP fields planted to native grasses, there was not a relationship between forb abundance and invertebrate abundance or biomass (Hull et al. 1996).

Given a strong relationship between vegetation type and arthropod food resource availability, it is difficult to separate the effects of each factor on bird use of grassland habitats. For example, evaluations of bobolink territory quality in Oregon found that territories of mated males had a higher percentage of forbs and higher caterpillar abundances than territories of un-mated males (Wittenberger 1980). Alternatively, in the pine barrens of New Jersey, arthropod biomass was a better predictor of bird habitat use than measures of vegetation and regardless of vegetation type; bird abundance was higher in areas with higher arthropod biomass (Brush and Stiles 1986).

The objective of our study was to compare bird use of restored grasslands planted with seed mixes of varying plant species diversity. We wanted to compare bird density/ bird species richness and vegetation composition/ structure in each of the planting types. We also wanted to examine the relationships between bird density/species richness and habitat characteristics such as vegetation structure/composition and food resource availability.

## **METHODS**

### **Study Area**

The Spring Run Wetland Complex is a mix of over 1600 ha of wetlands and reconstructed grasslands located in Dickinson County in northwest Iowa, USA. This area is managed by the Iowa Department of Natural Resources and is one of the largest examples of a prairie pothole landscape in the state.

We selected 4 restoration/planting types for our study to encompass the range of planting mixtures typically available to land managers. The planting types we selected were (1) cool-season - plantings of non-native, cool-season grasses (e.g.g. smooth brome (*Bromus inermis*), timothy (*Phleum pratense*), reed canary grass (*Phalaris arundinacea*), and Kentucky bluegrass (*Poa pratensis*)), (2) warm-season - a mix of native warm-season grasses divided into 2 groups by age of planting (e.g.g. switch grass (*Panicum virgatum*), Indian grass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and side-oats grama (*Bouteloua curtipendula*)), and (3) high diversity - a mixture of over 40 species of native grasses and forbs. Within the study area, reconstructed fields were selected or planted in a block design, with each of the planting types occurring in each block (Fig. 1). We surveyed a total of 6 complete blocks.

## **Vegetation Composition and Structure**

We surveyed upland vegetation at 25 m intervals along randomly located transects in each field during May and July each summer in 2007, 2008, and 2009. The 2 rounds of vegetation surveys coincided with peak height of cool season and warm-season grasses respectively. We established vegetation transects in the fields independently of the bird survey transects. The shape and size of the fields determined the number of transects and therefore the number of survey locations (ranged from 24-30 survey locations per field). At each survey point along the vegetation transect, we estimated the percent cover of warm-season native grasses, cool-season native grasses, warm-season exotic grasses, cool-season exotic grasses, native forbs, exotic forbs, standing dead vegetation, woody vegetation, bare ground, and litter in 0.5 m x 0.5 m Daubenmire frames (Daubenmire 1959). In addition, we recorded the number of species in each of the 6 grass/forb categories listed above to get a measure of species richness for each category within each field. We measured litter depth with a ruler and visual obstruction using a Robel pole in each cardinal direction at each survey location (Robel et al. 1970).

## **Density Estimates**

We surveyed grassland birds along 100 m transects in each field. We selected survey transect locations to maximize the number of transects in each field. The size and shape of each field determined the number of transects (ranged from 7 to 10 transects per field). We placed transects only in upland vegetation, and we did not locate transects near field edges or wetlands.

We conducted bird surveys in each field once per week for 6 weeks in June and July of 2007, 2008, and 2009 following the line-transect method of Buckland et al. (1993). We

conducted bird surveys between sunrise and 1000 hours and we did not conduct bird surveys on days where weather conditions could have impeded visibility or audibility (rain, fog, or wind in excess of 30 km/hr). Surveys consisted of an observer walking along the transect at a constant pace identifying birds by sight and sound within 35 m on either side of the transect. For each observation, we recorded the bird species and sex (if known). In addition, we used laser rangefinder binoculars to record the distance of the bird(s) from the observer and the compass bearing. Compass bearings, transect bearings, and observation distance were used to calculate the perpendicular distance of the birds from the transect line.

### **Invertebrate Abundance and Biomass**

We sampled invertebrates using 12-inch diameter sweep nets in each field on 6 randomly selected 25 m long sections of the vegetation transects described above. We completed 3 rounds of invertebrate surveys each year (2007-2009), in May, June, and July. Sweep net samples were taken only on warm, sunny days between 1000 and 1800 hours. To avoid trampling the vegetation on the transects, we off-set sweep net sampling 5 m to the left or right of the transect (randomly determined by a coin flip). Invertebrate surveys consisted of an observer walking at a pace of 1 sweep per meter, sweeping the vegetation within 1 m of the ground. We placed invertebrate samples in 3.8-liter sized zip-top bags at the completion of each survey. Immediately following sampling, we took the invertebrate samples to the lab and sorted them from vegetation debris using self-sorting tubes (Fig. 2). Invertebrate samples remained in the tubes for 24 hrs. During the 24 hr sorting time, invertebrates were drawn to the light end of the tubes and were carried down a funnel into labeled whirl-pak sample bags filled with 70% ethyl alcohol for preservation. At the end of the 24 hr sorting time, we removed vegetation and debris and inspected for remaining invertebrates. We

identified invertebrate samples to Order and then we counted, dried, and weighed each sample to obtain estimates of abundance and biomass.

### **Data Analysis**

To compare vegetation structure and composition among the 4 planting types, we used Analysis of Variance (ANOVA) in SAS Version 9.1 (SAS Institute, Cary, NC, USA) with PROC MIXED. We treated year as a repeated measure with a REPEATED statement in SAS. We averaged vegetation measurements taken within each field within each year. We conducted post-hoc pairwise comparisons among planting types using Tukey-Kramer adjustments for multiple testing. To evaluate vegetation diversity, we calculated and compared a Shannon Diversity Index value of the vegetation cover classes for each field. To calculate Shannon Diversity we used the formula:  $H' = -\sum (p_i * \ln p_i)$ , where  $p_i$  = the proportion of each vegetation cover class in each field. To provide a better visual representation of the above differences in vegetation characteristics among the planting types, we used Correspondence Analysis (CA). The differences in vegetation characteristics among the fields are represented as the physical distance in two dimensions (CA1 and CA2) of each field with respect to the others on the ordination plot. Fields that are near one another have more similar vegetation characteristics than those that are more distant on the plot.

We used program DISTANCE to estimate bird density (number of birds per ha) in each of the 4 planting types. We used the Multi-covariate Distance Sampling (MCDS) analysis engine in program DISTANCE to evaluate models of the detection functions for each species (Table 1). We considered detection function models that included a null model (no covariates) and combinations of 4 covariates in the set of candidate models: survey week (1, 2, 3, 4, 5, or 6 corresponding to the week each survey was conducted), planting type



(cool-season, warm-season (new), warm-season (old), or high diversity), planting age (in years – plantings  $\geq 10$  years were entered as 10 years), and management activity (0=none, 1=spot mow or spot herbicide treatment, 2=complete mow or hay, 3=prescribed fire). We tested half-normal and hazard-rate key functions with automatic sequential selection of adjustment terms. We selected the best model of the detection function from the set of candidate models by choosing the model with the lowest Akaike's Information Criterion (AIC) value and a chi-square goodness of fit test p-value  $\geq 0.10$  for each species. We post-stratified the data in program DISTANCE to obtain density estimates by field and year.

We categorized bird species into obligate grassland species and facultative grassland species based on habitat requirements (Vickery et al. 1999). Obligate species are those that will only use grassland habitats and do not use other habitat types (Vickery et al. 1999). Facultative species are those that commonly use grassland habitats, but also make use of other habitat types (Vickery et al. 1999).

We tested for differences in bird density among the 4 different planting types using ANOVA with PROC MIXED in SAS. Because each density estimate obtained from program DISTANCE has a sampling variance, we weighted bird density by the inverse of the variance using a WEIGHTED statement in SAS. We tested differences for each year (2007, 2008, and 2009) separately. We also combined the data from all 3 years (2007, 2008, and 2009) by including year as a repeated measure with a REPEATED statement in SAS. We conducted post-hoc pairwise comparisons among planting types using Tukey-Kramer adjustments for multiple testing.

We developed a set of a priori biological hypotheses of habitat covariates to compare models of breeding bird density (Table 2). For each bird species, we compared a set of 5

models using model selection and AIC to determine the best model from each set of candidate models (Burnham and Anderson 2002). The hypotheses evaluated for each bird response variable were represented by combinations of vegetation structure, vegetation diversity, food resources, native vegetation, grass cover, and management activity (see Table 2). We constructed the set of 5 candidate models individually for each bird response variable based on information about the species or set of species from the literature.

## **RESULTS**

### **Vegetation Composition and Structure**

Vegetation characteristics differed among the 4 planting types (Table 3, Fig. 3). Cool-season fields contained very little native warm-season grass in comparison to the other 3 planting types, but cool-season fields had 3 times more cover of exotic cool-season grasses than warm-season fields and 8 times more cover of exotic cool-season grasses than high diversity fields (Table 3).

High diversity fields had nearly 5 times higher percent cover of native forbs than warm-season fields and 27 times more cover of native forbs than the cool-season fields (Table 3). In addition, high diversity fields contained 5 times more native forb species than warm-season fields and nearly 20 times more native forb species than cool-season fields (Table 3). In contrast, cool-season fields contained far fewer exotic forbs than the other 3 planting types, all of which had about 5 times more cover of exotic forbs than cool-season fields (Table 3).

Cool-season fields had lower vegetation diversity and lower plant species richness than all 3 of the other planting types (Table 3). High diversity fields had 3 times higher plant

species richness than cool-season fields and about 1.5 times higher plant species richness than both of the warm-season planting types (Table 3).

Visual obstruction was lower in the newly planted warm-season fields than in 2 of the other planting types, but the high diversity fields and the newer warm-season fields both had more variation in visual obstruction among measurements within a field than the other 2 planting types (Table 3). The 2 mature planting types had nearly 6 times more litter depth than newly planted warm-season fields and about 2.5 times more litter depth than the high diversity fields (Table 3). The 2 mature planting types also had a much lower percent cover of bare ground than newly planted fields (Table 3).

### **Bird Density and Species Richness**

The most common bird species we encountered during our surveys were bobolink, common yellowthroat (*Geothlypis trichas*), red-winged blackbird (*Agelaius phoeniceus*), and sedge wren (*Cistothorus platensis*). We detected an average of 32 bird species during our annual surveys (38 species in 2007, 28 species in 2008, and 31 species in 2009). For all years combined, bird densities of some species and some groups of species differed among the planting types. Patterns of differences in bird densities in individual years were similar to those we found for all years (Appendix A). Therefore, we only present results of differences in bird densities for all years combined (Table 4).

Obligate species density was highest in the cool-season fields. However, this trend is primarily driven by one species – the bobolink. Bobolink density was 12 times greater in the cool-season fields than in older warm-season fields, 21 times greater than in newly planted warm-season fields, and more than 75 times greater than in high diversity fields (Table 4).

Sedge wren density was also higher in cool-season fields than in newly planted warm-season fields, but species densities were not different among the other planting types (Table 4).

Facultative species density was lower in cool-season and newly planted warm-season fields than high diversity fields, however there were no individual species where densities were different among the planting types (Table 4). One additional species that had differences among planting types was the song sparrow (*Melospiza melodia*). Although it had generally low densities in all of the planting types, song sparrow density was higher in high diversity fields than in cool-season fields (Table 4).

Although there were some differences in bird species richness in the first 2 years of the study, there was no evidence of a difference in bird species richness among the planting types during 2009 or when all years were combined (Table 5). In 2007, facultative species richness was higher in cool-season fields than in newly planted warm-season fields and, in addition, the combined species richness of facultative and obligate species was higher in cool-season fields than in newly planted warm-season fields (Table 5). In 2008, obligate species richness was lower in cool-season fields than in newly planted warm-season fields and the combined species richness of facultative and obligate species was also lower in cool-season fields than in newly planted warm-season fields (Table 5).

### **Habitat Models**

We evaluated habitat models for 15 different bird response variables. The vegetation structure model was included in the model set for all 15 bird response variables, and in 6 of the analyses, vegetation structure was included in at least one of the best supported models (Table 2). Vegetation structure was included in the best supported models for sedge wrens and common yellowthroats, and we found evidence that both visual obstruction ( $\beta_{\text{vis.obs.SEWR}}$ )

0.024, 95% CI 0.008, 0.04;  $\beta_{\text{vis.obs.COYE}} = 0.034$ , 95% CI 0.015, 0.053) and litter depth ( $\beta_{\text{L.d.SEWR}} = 0.01$ , 95% CI 0.006, 0.014;  $\beta_{\text{L.d.COYE}} = 0.007$ , 95% CI 0.002, 0.012) were positively associated with density. In addition, visual obstruction was positively associated with red-winged blackbird density ( $\beta_{\text{visual obstruction}} = 0.029$ , 95% CI 0.001, 0.057) but was negatively associated with the density of all species ( $\beta_{\text{visual obstruction}} = -0.381$ , 95% CI -0.754, -0.008).

Vegetation diversity was included in at least one of the best supported models in 5 out of 12 analyses (Table 2). In fact, we found evidence that plant species richness was positively associated with grasshopper sparrow density ( $\beta_{\text{plant sp.rich.}} = 0.073$ , 95% CI 0.032, 0.114), obligate bird species richness ( $\beta_{\text{plant sp.rich.}} = 0.551$ , 95% CI 0.222, 0.880) and total bird species richness ( $\beta_{\text{plant sp.rich.}} = 0.855$ , 95% CI 0.161, 1.548).

Two other hypotheses, food resources (9 out of 14) and grass cover (4 out of 6), were frequently included in at least one of the best models (Table 2). We found evidence that total grass cover was positively associated with bobolink density ( $\beta_{\text{total grass}} = 0.003$ , 95% CI 0.001, 0.006). Finally, we found evidence that native grasses ( $\beta_{\text{native grass.}} = 0.022$ , 95% CI 0.007, 0.036) and native forbs ( $\beta_{\text{native forb}} = 0.099$ , 95% CI 0.077, 0.122) were positively associated with the density of all facultative bird species.

## DISCUSSION

### Vegetation Composition and Structure

Many of the differences we found in vegetation structure and composition were expected based on the characteristics of the plant species used in each planting mix. Similar to our results, previous researchers have found that warm-season CRP plantings had higher species richness, more forb cover, and lower percent cover of grasses than cool-season

plantings (Henningsen and Best 2005). Few studies, however, have simultaneously examined high diversity fields and cool-season and warm-season fields.

Delisle and Savidge (1997) compared bird use and vegetation on cool-season and warm-season CRP plantings and found a few differences in vegetation characteristics between the 2 planting types during the breeding season, primarily with vegetation density and vegetation height. In contrast, we found many differences in vegetation characteristics between our cool-season and warm-season fields (Table 3). However, we did not find that warm-season fields (either planting age) had significantly taller vegetation than cool-season fields. The cover classes presented by Delisle and Savidge (1997) and others were broader than those we measured (e.g. their forb cover vs. our native or exotic forb cover) making specific comparisons difficult.

Native forb cover was 20 times greater in high diversity fields than in cool-season fields and 5 times greater in high diversity fields than in warm-season fields. Some grassland bird species, such as the dickcissel, have been associated with increased forb cover (Patterson and Best 1996), suggesting that increasing forb cover may provide better habitat for some species. Additionally, grassland invertebrate biomass and diversity have been associated with increased forb cover (Burger et al. 1993), suggesting that increased forb cover in CRP plantings may provide better food resources for grassland birds. However, we did not find that bird densities were higher in our fields with increased forb cover (high diversity). Similarly, neither bird abundance nor invertebrate biomass were correlated with forb abundance in Kansas CRP plantings (Hull et al. 1996).

### **Bird Density and Species Richness**

Bird densities were not consistently different among the 4 planting types. However, bobolink densities were consistently higher on cool-season fields than any of the other planting types (Table 4). Negus et al. (2010) found that although bobolinks were using and nesting in managed CRP fields, they were almost 3 times more abundant in undisturbed CRP fields that were comparable to the cool-season fields in our study. Similarly, other researchers have found that bobolink densities were higher in cool-season fields as compared to warm-season fields (Delisle and Savidge 1997). Many of the early non-native, cool-season CRP plantings are being converted to native warm-season or high diversity plantings, or are being managed to enhance vegetation diversity (Thompson et al. 2009, Negus et al. 2010). We expect that elimination of cool-season plantings may be detrimental to the future of bobolink populations in Iowa.

For most of the individual grassland bird species we examined, we found no differences in density among the 4 planting types (Table 4). King and Savidge (1995) found no differences in bird use (both richness and number of birds) between cool-season and warm-season fields during the breeding season in southeast Nebraska. Similarly, no differences in grassland bird abundance and/or species richness were found in comparisons between cool-season and warm-season CRP plantings in northern Missouri, eastern South Dakota, western Minnesota, or southeast Iowa (McCoy et al. 2001; Bakker et al. 2004; Henningsen and Best 2005; Bakker and Higgins 2009).

### **Habitat Models**

Models of the relationships between bird density/bird species richness and habitat characteristics showed that different species, even those within a species category, were

influenced by different habitat characteristics. Many previous researchers have found that vegetation characteristics are important predictors of bird use of grasslands and that responses tend to be species-specific. We found that the best models of dickcissel density included vegetation diversity (Table 2). In contrast, the best model for bobolink density included only grass cover (Table 2). Our findings confirm what others have found in the past; dickcissels are positively associated with forb cover and bobolinks are negatively associated with forb cover in grassland habitats (Patterson and Best 1996, Delisle and Savidge 1997).

Our habitat models revealed that both vegetation characteristics and food resources are important in explaining grassland bird densities. Researchers have recently advocated for the inclusion of information about food resources to better predict bird use of grassland habitats (Benson 2003; Davros 2005). In fact, we found that food resource availability was associated with density during the breeding season for 60% of the grassland bird species that we examined. Our findings are similar to those of Davros (2005) who found that habitat models for total bird abundance, total bird species richness, and common yellowthroat, sedge wren and song sparrow abundance were improved by adding information about food resources.

## **MANAGEMENT IMPLICATIONS**

Management of grassland habitat for grassland birds will not be successful under a “one size fits all” approach. Instead, we conclude that a variety of planting types and management strategies may be necessary to conserve grassland birds. Negus et al. (2010) advocated for management practices that would enable the inclusion of a mosaic of



vegetation characteristics to provide the variety of habitat types necessary for multiple grassland bird species. Additionally, Ribic et al. (2009) found that no single grassland habitat type would be adequate to conserve all grassland birds and that a variety of grassland habitat types would be required. Recommendations about grassland habitat plantings have advocated the use of native grass species over non-native grasses (Allen 1993) and avoiding single-species plantings of native or non-native grasses (McCoy et al. 2001). However, given the importance of cool-season grass plantings for bobolinks in our study, consideration must be given to the impact that elimination of these plantings may have on the future of bobolink populations. Future research is needed to assess whether the inclusion of additional native cool-season grass species into grassland plantings will provide the necessary vegetation structure for bobolinks.

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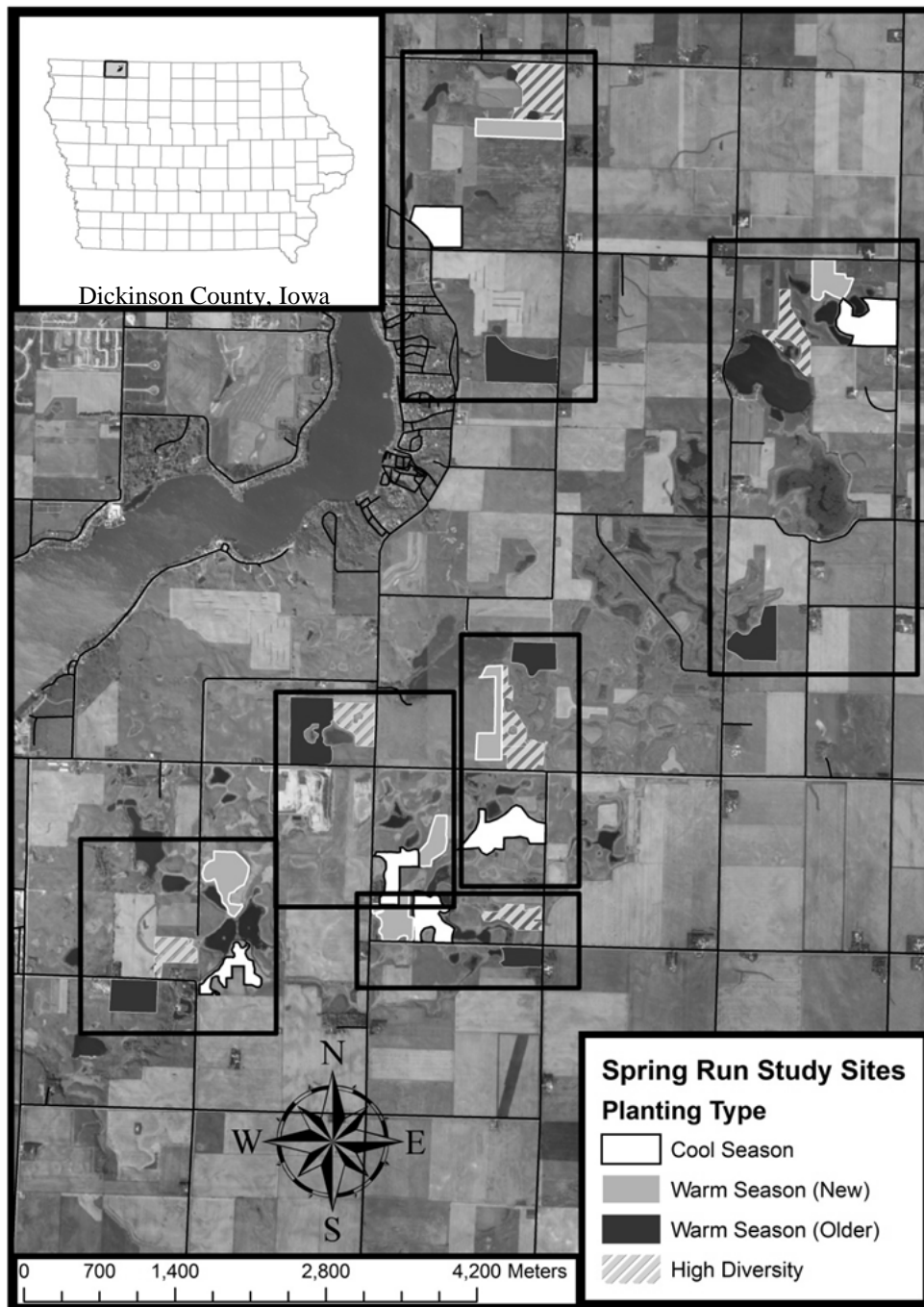


Figure 1. Study Sites were located in the Spring Run Complex in Dickinson County, Iowa, USA in 2007, 2008, and 2009. Solid white polygons indicate sites that were planted with cool-season grasses  $\geq 10$  years ago, solid light grey polygons indicate sites that were planted to warm-season grasses between 2005 and 2007, solid black polygons indicate sites that were planted to warm-season grasses  $\geq 10$  years ago, and hatched light grey polygons indicate sites that were planted with a high diversity seed mix between 2005 and 2007. Black rectangles indicate study site blocking.



Figure 2. Invertebrate self-sorting “tubes” used to sort invertebrates collected during sweep net sampling from vegetation debris. Invertebrate samples collected during sweep net sampling were placed in 1 gallon sized zip-top bags at the completion of each survey. Sample bags were cut open and placed into the back of each tube. Invertebrate samples remained in the tubes for 24 hours. During the 24 hour sorting time, invertebrates were drawn to the light end of the tubes and were carried down a funnel into labeled whirl-pak sample bags filled with 70% ethyl alcohol for preservation. At the end of the 24 hour sorting time, vegetation and debris were removed and inspected for remaining invertebrates.



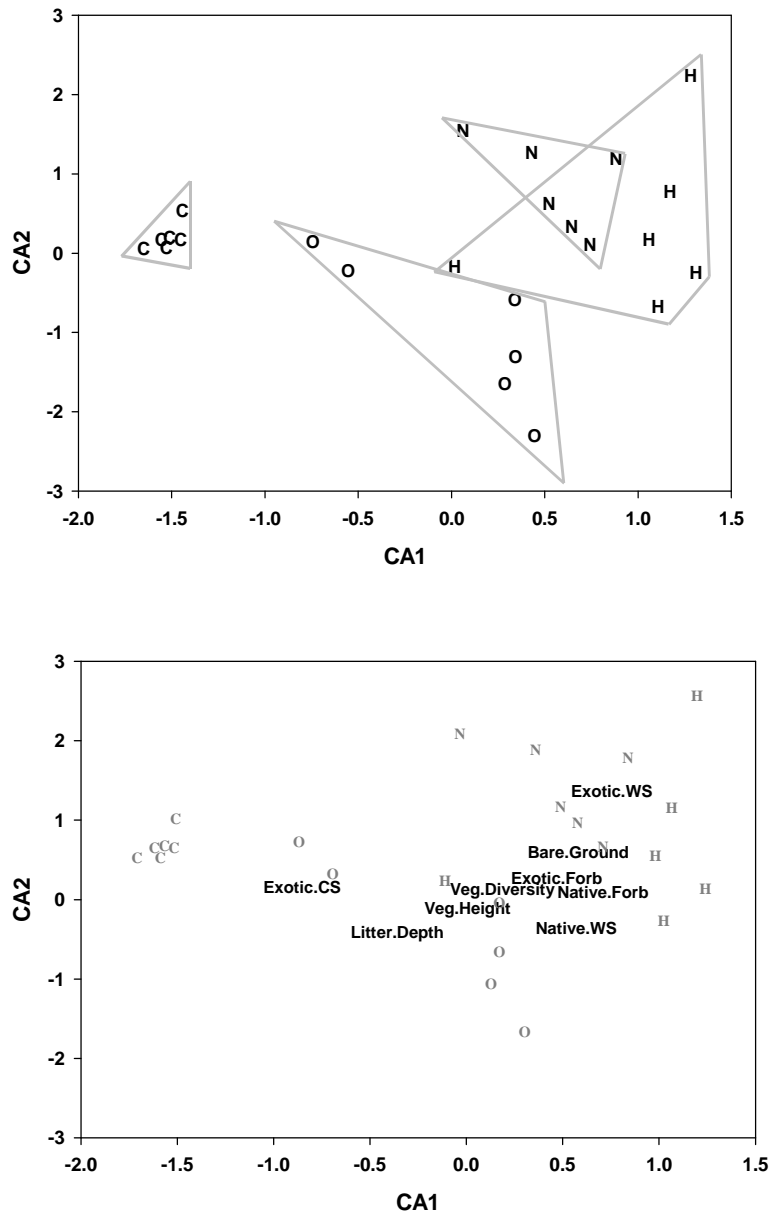


Figure 3. Correspondence Analysis ordination plots of vegetation characteristics measured in 2007, 2008, and 2009 in the Spring Run Complex, Dickinson County, Iowa, USA. Cool-season fields are indicated with C's, warm-season fields are indicated with N's for newly planted and O's for mature fields, and high diversity fields are indicated with H's. Vegetation measurements taken within each field were averaged for each year. Selected vegetation characteristic scores were plotted (bottom) for reference.

Table 1. Covariates included in models of detection functions for birds surveyed in 2007, 2008, and 2009 in the Spring Run Complex, Dickinson County, Iowa, USA. Models of detection functions were evaluated using the Multi-covariate Distance Sampling analysis engine of program DISTANCE. We tested half-normal and hazard-rate key functions with automatic sequential selection of adjustment terms. We included a null model (no covariates) and combinations of 4 covariates in the set of candidate models: Round (survey week), Planting Type, Planting Age, and Management Activity. The best model from the set of candidate models was selected using Akaike's Information Criterion and Chi-square Goodness of Fit Tests.

		Covariates	Curve Type	No. of Param.	$\Delta$ AIC	$\chi^2$ p-value	
<u>Obligate Grassland Species:</u>							
Bobolink <sup>♂</sup>	<i>Dolichonyx oryzivorus</i>	Planting Age	Half-normal	2	0.05	0.34	
Dickcissel <sup>♂</sup>	<i>Spiza americana</i>	Null Model	Half-normal	1	0.00	0.38	
Grasshopper Sparrow <sup>♂</sup>	<i>Ammodramus savannarum</i>	Management Activity	Hazard-rate	3	0.00	0.29	
Sedge Wren <sup>♂</sup>	<i>Cistothorus platensis</i>	Planting Type + Management Activity	Hazard-rate	4	1.99	0.18	
<u>Facultative Grassland Species:</u>							
Common Yellowthroat <sup>♂</sup>	<i>Geothlypis trichas</i>	Planting Type + Management Activity	Half-normal	3	2.72	0.14	
Ducks <sup>♀*</sup>		Management Activity	Half-normal	Cosine (1)	3	0.00	0.54
Killdeer <sup>♂♀</sup>	<i>Charadrius vociferus</i>	Planting Type + Management Activity	Half-normal	Cosine (2)	5	0.00	0.39
Red-winged Blackbird <sup>♂</sup>	<i>Agelaius phoeniceus</i>	Management Activity	Half-normal		2	4.40	0.16
Ring-necked Pheasant <sup>♀</sup>	<i>Phasianus colchicus</i>	Null Model	Hazard-rate	Cosine (3)	5	0.00	0.29
<u>Other Species:</u>							
American Goldfinch <sup>♂</sup>	<i>Spinus tristis</i>	Null Model	Hazard-rate	2	1.34	0.23	
Common Grackle <sup>♂</sup>	<i>Quiscalus quiscula</i>	Management Activity	Half-normal	2	0.00	0.69	
Song Sparrow <sup>♂</sup>	<i>Melospiza melodia</i>	Null Model	Hazard-rate	2	0.00	0.55	
Swamp Sparrow <sup>♂</sup>	<i>Melospiza georgiana</i>	Planting Type	Half-normal	2	0.00	0.30	
Yellow-headed Blackbird <sup>♂</sup>	<i>Xanthocephalus xanthocephalus</i>	Round	Half-normal	2	0.00	0.37	

♂ Males only included in analysis

♀ Females only included in analysis

♂♀ Males and females included in analysis

\* Includes Blue-winged Teal (*Anas discors*) and Mallard (*A. platyrhynchos*)

Table 2. Habitat models for bird density and species richness surveyed in 2007, 2008, and 2009 on the Spring Run Complex, Dickinson County, Iowa, USA. We developed a set of a priori biological hypotheses of habitat characteristics (see table footnote) to explain bird density and species richness. For each bird response, we developed a set of 5 models using these biological hypotheses (model set is indicated by the superscript numbers following each response variable) plus an intercept only model for each. Habitat models were evaluated using Akaike's Information Criterion for small sample sizes ( $AIC_c$ ). The number of parameters in the model (including the intercept) is indicated in column  $k$ ,  $\Delta AIC_c = AIC_c - \text{minimum } AIC_c$ , and  $W_i$  (model weight) =  $\exp[-\{\Delta AIC_c/2\}]/\sum \exp[-\{\Delta AIC_c/2\}]$ .

Species	Best Supported Model(s)	k	$\Delta AIC_c$	$W_i^*$
<u>Obligate Grassland Species Density</u>				
Bobolink <sup>1, 3, 1+3, 5, 1+3+5</sup>	Grass Cover	2	0.00	0.96
Dickcissel <sup>1, 3, 1+3, 2, 1+2+3</sup>	Food Resources	3	0.00	0.55
	Veg. Diversity	3	1.00	0.33
Grasshopper Sparrow <sup>1, 2, 3, 1+3, 6</sup>	Veg. Diversity	3	0.00	1.00
Sedge Wren <sup>1, 2, 3, 1+3, 5</sup>	Veg. Structure	4	0.00	0.71
	Veg. Structure + Food Resources	6	1.79	0.29
All Obligate Species <sup>1, 2, 3, 1+2+3, 4</sup>	Veg. Diversity	3	0.00	0.39
	Food Resources	3	0.30	0.34
<u>Facultative Grassland Species Density</u>				
Common Yellowthroat <sup>1, 2, 3, 1+3, 6</sup>	Veg. Structure + Food Resources	6	0.00	1.00
Ducks <sup>1, 2, 5, 6, 1+5</sup>	Grass Cover	2	0.00	0.53
Killdeer <sup>1, 3, 1+3, 5, 6</sup>	Grass Cover	2	0.00	0.59
Ring-necked Pheasant <sup>1, 3, 1+3, 5, 6</sup>	Grass Cover	2	0.00	0.48
	Food Resources	3	1.12	0.28
Red-winged Blackbird <sup>1, 2, 3, 1+3, 1+2+3</sup>	Veg. Structure + Food Resources +	8	0.00	0.78
	Veg. Diversity			
All Facultative Species <sup>1, 2, 3, 1+2+3, 4</sup>	Native Vegetation	3	0.00	1.00
All Species Density <sup>1, 2, 3, 1+3, 5</sup>	Veg. Structure + Food Resources	6	0.00	0.78
<u>Species Richness</u>				
Obligate Species <sup>1, 2, 3, 1+3, 1+2+3</sup>	Veg. Diversity	3	0.00	0.98
Facultative Species <sup>1, 2, 3, 1+3, 1+2+3</sup>	Food Resources	3	0.00	0.40
	Veg. Structure	4	0.54	0.31
	Veg. Diversity	3	1.10	0.23
All Species <sup>1, 2, 3, 1+3, 1+2+3</sup>	Veg. Structure + Food Resources +	8	0.00	0.37
	Veg. Diversity			
	Veg. Structure + Food Resources	6	0.31	0.31
	Veg. Structure	4	0.81	0.24

<sup>1</sup>Vegetation Structure Hypothesis (Visual Obstruction + Variation in Visual Obstruction + Litter Depth)

<sup>2</sup>Vegetation Diversity Hypothesis (Shannon Diversity Index of Cover Classes + Plant Species Richness)

<sup>3</sup>Food Resources Hypothesis (Biomass Preferred Invertebrate Orders + Biomass All Invertebrate Orders)

<sup>4</sup>Native Vegetation Hypothesis (% Cover of Native Grasses + % Cover of Native Forbs)

<sup>5</sup>Grass Cover Hypothesis (% Cover of Native Grasses + % Cover of Exotic Grasses)

<sup>6</sup>Management Hypothesis (Planting Age + Management Activity)

\*Akaike weights ( $W_i$ ) calculated with all 5 models for each response variable.

Table 3. Vegetation composition/structure variables measured in 2007, 2008, and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. *P*-values are given for ANOVA tests for differences among planting types (df = 3, 20). Different lettered subscripts following means indicate significant differences among treatment types for pairwise comparisons.

	Cool Season		Warm Season (new)		Warm Season (older)		High Diversity		<i>P</i>
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	
<u>Native Warm-season Grasses:</u>									
Number of Species	0.01 <sup>a</sup>	0.12	1.22 <sup>b</sup>	0.12	1.11 <sup>b</sup>	0.12	1.24 <sup>b</sup>	0.12	<0.001
% Cover	0.10 <sup>a</sup>	2.93	19.99 <sup>bc</sup>	2.93	28.90 <sup>b</sup>	2.93	17.83 <sup>c</sup>	2.93	<0.001
<u>Native Cool-season Grasses:</u>									
Number of Species	0.00 <sup>a</sup>	0.03	0.13 <sup>b</sup>	0.03	0.01 <sup>ab</sup>	0.03	0.33 <sup>c</sup>	0.03	<0.001
% Cover	0.04 <sup>a</sup>	0.44	1.43 <sup>a</sup>	0.44	0.29 <sup>a</sup>	0.44	4.20 <sup>b</sup>	0.44	<0.001
% Cover of All Native Grasses	0.15 <sup>a</sup>	3.05	21.42 <sup>b</sup>	3.05	29.19 <sup>b</sup>	3.05	22.03 <sup>b</sup>	3.05	<0.001
<u>Exotic Warm-season Grasses:</u>									
Number of Species	0.00 <sup>a</sup>	0.05	0.24 <sup>b</sup>	0.05	0.01 <sup>a</sup>	0.05	0.15 <sup>ab</sup>	0.05	0.002
% Cover	0.02 <sup>a</sup>	1.20	5.12 <sup>b</sup>	1.20	0.08 <sup>a</sup>	1.20	2.85 <sup>ab</sup>	1.20	0.009
<u>Exotic Cool-season Grasses:</u>									
Number of Species	1.65 <sup>a</sup>	0.09	0.86 <sup>b</sup>	0.09	0.76 <sup>b</sup>	0.09	0.35 <sup>c</sup>	0.09	<0.001
% Cover	60.15 <sup>a</sup>	2.44	16.24 <sup>bc</sup>	2.44	20.28 <sup>b</sup>	2.44	7.26 <sup>c</sup>	2.44	<0.001
% Cover of All Grasses	60.31 <sup>a</sup>	2.88	42.79 <sup>b</sup>	2.88	49.55 <sup>b</sup>	2.88	32.13 <sup>c</sup>	2.88	<0.001
<u>Native Forbs:</u>									
Number of Species	0.12 <sup>a</sup>	0.11	0.45 <sup>a</sup>	0.11	0.44 <sup>a</sup>	0.11	2.34 <sup>b</sup>	0.11	<0.001
% Cover	0.87 <sup>a</sup>	1.24	4.75 <sup>a</sup>	1.24	4.96 <sup>a</sup>	1.24	23.58 <sup>b</sup>	1.24	<0.001
<u>Exotic Forbs:</u>									
Number of Species	0.20 <sup>a</sup>	0.09	0.86 <sup>b</sup>	0.09	0.79 <sup>b</sup>	0.09	0.91 <sup>b</sup>	0.09	<0.001
% Cover	1.82 <sup>a</sup>	1.31	10.22 <sup>b</sup>	1.31	8.36 <sup>b</sup>	1.31	9.86 <sup>b</sup>	1.31	<0.001
Diversity of Cover Classes <sup>1</sup>	0.99 <sup>a</sup>	0.05	1.62 <sup>bc</sup>	0.05	1.48 <sup>b</sup>	0.05	1.71 <sup>c</sup>	0.05	<0.001
Plant Species Richness	1.98 <sup>a</sup>	0.18	3.77 <sup>b</sup>	0.18	3.12 <sup>b</sup>	0.18	5.32 <sup>c</sup>	0.18	<0.001
Visual Obstruction <sup>2</sup>	3.49 <sup>ab</sup>	0.37	2.20 <sup>a</sup>	0.37	4.43 <sup>b</sup>	0.37	4.27 <sup>b</sup>	0.37	<0.001
Variation Visual Obstruction <sup>3</sup>	1.18 <sup>a</sup>	0.13	1.14 <sup>a</sup>	0.13	1.85 <sup>b</sup>	0.13	1.96 <sup>b</sup>	0.13	<0.001
Litter Depth <sup>4</sup>	21.64 <sup>a</sup>	2.20	3.88 <sup>b</sup>	2.20	24.00 <sup>a</sup>	2.20	9.56 <sup>b</sup>	2.20	<0.001
% Cover Standing Dead	3.55	0.61	1.86	0.61	2.95	0.61	1.97	0.61	0.160
% Cover Woody Veg.	0.03	0.06	0.00	0.06	0.21	0.06	0.09	0.06	0.057
% Cover Bare Ground	2.73 <sup>a</sup>	2.71	24.56 <sup>b</sup>	2.71	5.49 <sup>a</sup>	2.71	18.53 <sup>b</sup>	2.71	<0.001
% Cover Litter	30.28 <sup>a</sup>	1.63	15.89 <sup>b</sup>	1.63	28.34 <sup>a</sup>	1.63	13.76 <sup>b</sup>	1.63	<0.001
% Cover Other	0.48	0.11	0.14	0.11	0.19	0.11	0.07	0.11	0.059

<sup>1</sup>Shannon Diversity Index of vegetation cover classes

<sup>2</sup>Measured in decimeters

<sup>3</sup>Standard deviation of visual obstruction

<sup>4</sup>Measured in millimeters

Table 4. Bird density (number of birds per hectare) measured in 2007, 2008, and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. *P*-values are given for ANOVA tests for differences among planting types (*df*=3, 20) for all 3 years combined. Different lettered subscripts following means indicate significant differences among treatment types for pairwise comparisons.

	Cool Season		Warm Season (new)		Warm Season (older)		High Diversity		<i>P</i>
	Density	S.E.	Density	S.E.	Density	S.E.	Density	S.E.	
<u>Obligate Species</u>									
Bobolink <sup>♂</sup>	1.280 <sup>a</sup>	0.09	0.060 <sup>b</sup>	0.07	0.104 <sup>b</sup>	0.07	0.017 <sup>b</sup>	0.07	<b>&lt;0.001</b>
Dickcissel <sup>♂</sup>	0.006	0.01	0.013	0.01	0.005	0.01	0.020	0.01	0.695
Grasshopper Sparrow <sup>♂</sup>	0.003	0.12	0.353	0.13	0.019	0.12	0.074	0.12	0.215
Sedge Wren <sup>♂</sup>	0.369 <sup>a</sup>	0.08	0.035 <sup>b</sup>	0.08	0.167 <sup>ab</sup>	0.08	0.269 <sup>ab</sup>	0.08	<b>0.046</b>
All Obligate Species	2.020 <sup>a</sup>	0.19	0.971 <sup>b</sup>	0.19	0.797 <sup>b</sup>	0.19	0.816 <sup>b</sup>	0.19	<b>0.001</b>
<u>Facultative Species</u>									
Common Yellowthroat <sup>♂</sup>	0.056	0.28	0.025	0.28	0.857	0.28	0.696	0.28	0.111
Ducks <sup>♀</sup>	0.075	0.02	0.035	0.02	0.031	0.02	0.024	0.02	0.450
Killdeer <sup>♂♀</sup>	0.020	0.01	0.027	0.02	0.000	0.01	0.017	0.01	0.597
Red-winged Blackbird <sup>♂</sup>	0.270	0.15	0.393	0.15	0.393	0.15	0.788	0.15	0.133
Ring-necked Pheasant <sup>♀</sup>	0.100	0.04	0.138	0.04	0.109	0.04	0.098	0.04	0.888
All Facultative Species	0.729 <sup>a</sup>	0.35	1.226 <sup>a</sup>	0.35	2.070 <sup>ab</sup>	0.35	2.904 <sup>b</sup>	0.35	<b>0.003</b>
<u>Other Species</u>									
American Goldfinch <sup>♂</sup>	0.003	0.01	0.020	0.01	0.034	0.01	0.034	0.02	0.371
Common Grackle <sup>♂</sup>	0.065	0.02	0.000	0.02	0.000	0.02	0.000	0.02	0.098
Song Sparrow <sup>♂</sup>	0.008 <sup>a</sup>	0.03	0.040 <sup>ab</sup>	0.03	0.053 <sup>ab</sup>	0.03	0.126 <sup>b</sup>	0.03	<b>0.051</b>
Swamp Sparrow <sup>♂</sup>	0.043	0.01	0.009	0.01	0.039	0.01	0.044	0.02	0.224
Yellow-headed Blackbird <sup>♂</sup>	0.016	0.01	0.027	0.01	0.018	0.01	0.014	0.01	0.901
Facultative and Obligate	2.749	0.45	2.120	0.45	2.867	0.45	3.721	0.45	0.169
Total Density	2.917	0.49	2.472	0.49	3.342	0.49	4.294	0.49	0.099

<sup>♂</sup> Males only included in analysis

<sup>♀</sup> Females only included in analysis

<sup>♂♀</sup> Males and females included in analysis

Table 5. Bird species richness (R.) measured in 2007, 2008, and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. *P*-values are given for ANOVA tests for differences among planting types (df=3, 20). Different lettered subscripts following means indicate significant differences among treatment types for pairwise comparisons.

	Year	Cool Season		Warm Season (new)		Warm Season (older)		High Diversity		<i>P</i>
		R.	S.E.	R.	S.E.	R.	S.E.	R.	S.E.	
Obligate	2007	2.50	0.42	1.50	0.42	2.00	0.42	2.17	0.42	0.420
Facultative	2007	5.17 <sup>a</sup>	0.34	3.17 <sup>b</sup>	0.34	4.00 <sup>ab</sup>	0.34	4.17 <sup>ab</sup>	0.34	<b>0.008</b>
Facultative & Obligate	2007	7.67 <sup>a</sup>	0.60	4.67 <sup>b</sup>	0.60	6.00 <sup>ab</sup>	0.60	6.33 <sup>ab</sup>	0.60	<b>0.232</b>
Total (All Species)	2007	13.67	1.18	10.33	1.18	12.17	1.18	12.00	1.18	0.299
Obligate Grassland	2008	2.33 <sup>a</sup>	0.31	3.67 <sup>b</sup>	0.31	2.50 <sup>ab</sup>	0.31	2.67 <sup>ab</sup>	0.31	<b>0.037</b>
Facultative Grassland	2008	3.83	0.44	5.00	0.44	3.50	0.44	3.50	0.44	0.086
Facultative & Obligate	2008	6.17 <sup>a</sup>	0.53	8.67 <sup>b</sup>	0.53	6.00 <sup>a</sup>	0.53	6.17 <sup>a</sup>	0.53	<b>0.008</b>
Total (All Species)	2008	8.33	0.88	11.50	0.88	8.83	0.88	10.50	0.88	0.080
Obligate	2009	2.67	0.51	4.00	0.51	3.17	0.51	3.17	0.51	0.345
Facultative	2009	3.67	0.44	3.83	0.44	4.33	0.44	4.83	0.44	0.274
Facultative & Obligate	2009	6.33	0.59	7.83	0.59	7.50	0.59	8.00	0.59	0.232
Total (All Species)	2009	9.67	1.07	9.00	1.07	10.83	1.07	12.17	1.07	0.214
Obligate	All	2.50	0.27	3.06	0.27	2.56	0.27	2.67	0.27	0.481
Facultative	All	4.22	0.28	4.00	0.28	3.94	0.28	4.17	0.28	0.878
Facultative & Obligate	All	6.72	0.41	7.06	0.41	6.50	0.41	6.83	0.41	0.813
Total (All Species)	All	10.56	0.66	10.28	0.66	10.61	0.66	11.56	0.66	0.558

Appendix A. Bird density (D) measured in 2007, 2008, and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. *P*-values are for ANOVA tests for differences among planting types. Different lettered subscripts following means indicate significant differences among treatment types for pairwise comparisons.

	Year	Cool Season		Warm Season (new)		Warm Season (older)		High Diversity		<i>P</i>
		D	S.E.	D	S.E.	D	S.E.	D	S.E.	
<u>Obligate Species</u>										
Bobolink	2007	1.27 <sup>a</sup>	0.12	0.04 <sup>b</sup>	0.02	0.03 <sup>b</sup>	0.02	0.00 <sup>b</sup>	0.02	<0.001
Dickcissel	2007	0.05	0.02	0.06	0.02	0.06	0.02	0.13	0.03	0.151
Grasshopper Sparrow	2007	0.00	0.18	0.38	0.18	0.00	0.18	0.03	0.18	0.385
Sedge Wren	2007	0.55	0.16	0.00	0.15	0.56	0.16	0.32	0.15	0.066
All Obligate Species	2007	2.08 <sup>a</sup>	0.24	0.67 <sup>b</sup>	0.24	0.75 <sup>b</sup>	0.24	0.93 <sup>b</sup>	0.24	<b>0.003</b>
<u>Facultative Species</u>										
Common Yellowthroat	2007	0.09 <sup>a</sup>	0.35	0.00 <sup>a</sup>	0.35	1.95 <sup>b</sup>	0.37	1.55 <sup>b</sup>	0.36	<b>0.002</b>
Ducks	2007	0.10	0.05	0.02	0.04	0.03	0.04	0.02	0.04	0.638
Killdeer	2007	0.03	0.02	0.03	0.03	0.00	0.02	0.05	0.03	0.515
Red-winged Blackbird	2007	0.32	0.23	0.40	0.24	0.42	0.23	1.22	0.25	0.065
Ring-necked Pheasant	2007	0.13	0.07	0.17	0.08	0.15	0.07	0.07	0.06	0.697
All Facultative Species	2007	0.80 <sup>a</sup>	0.38	1.26 <sup>ab</sup>	0.38	2.67 <sup>bc</sup>	0.38	3.43 <sup>c</sup>	0.38	<0.001
<u>Other Species</u>										
American Goldfinch	2007	0.01	0.02	0.03	0.02	0.06	0.03	0.04	0.03	0.503
Common Grackle	2007	0.20	0.04	0.12	0.04	0.16	0.04	0.09	0.03	0.167
Song Sparrow	2007	0.00	0.02	0.04	0.02	0.04	0.02	0.05	0.02	0.212
Swamp Sparrow	2007	0.07	0.12	0.02	0.12	0.34	0.13	0.13	0.13	0.361
Yellow-headed Blackbird	2007	0.06	0.02	0.09	0.03	0.07	0.03	0.04	0.02	0.510
Facultative and Obligate	2007	2.88 <sup>a</sup>	0.52	1.93 <sup>ab</sup>	0.52	3.43 <sup>ab</sup>	0.52	4.36 <sup>b</sup>	0.52	<b>0.031</b>
Total Density	2007	3.23 <sup>a</sup>	0.60	2.43 <sup>ab</sup>	0.60	4.37 <sup>ab</sup>	0.60	4.98 <sup>b</sup>	0.60	<b>0.037</b>
<u>Obligate Species</u>										
Bobolink	2008	1.38 <sup>a</sup>	0.13	0.21 <sup>b</sup>	0.08	0.24 <sup>b</sup>	0.08	0.03 <sup>b</sup>	0.06	<0.001
Dickcissel	2008	0.00	0.02	0.01	0.02	0.00	0.02	0.01	0.02	0.930
Grasshopper Sparrow	2008	0.00 <sup>a</sup>	0.13	0.55 <sup>b</sup>	0.15	0.01 <sup>a</sup>	0.14	0.15 <sup>ab</sup>	0.14	<b>0.049</b>
Sedge Wren	2008	0.35	0.21	0.06	0.21	0.73	0.22	0.50	0.21	0.195
All Obligate Species	2008	1.94 <sup>a</sup>	0.28	1.03 <sup>ab</sup>	0.28	1.02 <sup>ab</sup>	0.28	0.81 <sup>b</sup>	0.28	<b>0.054</b>
<u>Facultative Species</u>										
Common Yellowthroat	2008	0.08	0.52	0.28	0.52	1.49	0.53	2.03	0.54	0.054
Ducks	2008	0.07	0.04	0.06	0.05	0.03	0.04	0.01	0.03	0.675
Killdeer	2008	0.00	0.07	0.11	0.07	0.00	0.07	0.00	0.07	0.638
Red-winged Blackbird	2008	0.29	0.05	0.45	0.16	0.37	0.15	0.66	0.16	0.379
Ring-necked Pheasant	2008	0.01	0.06	0.18	0.07	0.09	0.06	0.25	0.08	0.395
All Facultative Species	2008	0.61 <sup>a</sup>	0.56	1.41 <sup>ab</sup>	0.56	2.05 <sup>ab</sup>	0.56	3.26 <sup>b</sup>	0.56	<b>0.027</b>
<u>Other Species</u>										
American Goldfinch	2008	0.00	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.938
Common Grackle	2008	0.01	0.03	0.01	0.04	0.00	0.04	0.00	0.04	0.990
Song Sparrow	2008	0.02 <sup>a</sup>	0.04	0.07 <sup>ab</sup>	0.04	0.14 <sup>ab</sup>	0.04	0.19 <sup>b</sup>	0.05	<b>0.046</b>
Swamp Sparrow	2008	0.03	0.02	0.01	0.02	0.04	0.03	0.08	0.03	0.328
Yellow-headed Blackbird	2008	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.980
Facultative and Obligate	2008	2.55	0.68	2.45	0.68	3.07	0.68	4.07	0.68	0.338
Total Density	2008	2.61	0.77	2.58	0.77	3.39	0.77	4.78	0.77	0.189

## Appendix A. Continued.

	Year	Cool Season		Warm Season (new)		Warm Season (older)		High Diversity		P
		D	S.E.	D	S.E.	D	S.E.	D	S.E.	
<u>Obligate Species</u>										
Bobolink	2009	1.26 <sup>a</sup>	0.14	0.28 <sup>b</sup>	0.10	0.26 <sup>b</sup>	0.10	0.02 <sup>b</sup>	0.10	< <b>0.001</b>
Dickcissel	2009	0.00	0.02	0.01	0.02	0.00	0.02	0.01	0.02	0.919
Grasshopper Sparrow	2009	0.01 <sup>a</sup>	0.08	0.45 <sup>b</sup>	0.10	0.12 <sup>b</sup>	0.09	0.27 <sup>b</sup>	0.09	<b>0.022</b>
Sedge Wren	2009	0.62	0.12	0.28	0.11	0.18	0.11	0.29	0.11	0.087
All Obligate Species	2009	2.03 <sup>a</sup>	0.26	1.21 <sup>ab</sup>	0.26	0.62 <sup>b</sup>	0.26	0.70 <sup>b</sup>	0.26	<b>0.005</b>
<u>Facultative Species</u>										
Common Yellowthroat	2009	0.13	0.18	0.08	0.18	0.38	0.19	0.62	0.19	0.185
Ducks	2009	0.07	0.04	0.03	0.04	0.05	0.04	0.05	0.04	0.908
Killdeer	2009	0.02	0.02	0.03	0.02	0.00	0.02	0.01	0.02	0.811
Red-winged Blackbird	2009	0.38	0.16	0.51	0.17	0.59	0.17	1.02	0.18	0.095
Ring-necked Pheasant	2009	0.09	0.07	0.11	0.08	0.16	0.09	0.09	0.07	0.934
All Facultative Species	2009	0.79	0.42	1.00	0.42	1.49	0.42	2.02	0.42	0.207
<u>Other Species</u>										
American Goldfinch	2009	0.00	0.03	0.01	0.03	0.03	0.03	0.08	0.03	0.309
Common Grackle	2009	0.07	0.08	0.00	0.08	0.00	0.07	0.00	0.07	0.901
Song Sparrow	2009	0.00 <sup>a</sup>	0.01	0.02 <sup>ab</sup>	0.02	0.02 <sup>ab</sup>	0.02	0.09 <sup>b</sup>	0.02	<b>0.053</b>
Swamp Sparrow	2009	0.05	0.02	0.01	0.02	0.03	0.02	0.03	0.02	0.628
Yellow-headed Blackbird	2009	0.00	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.993
<u>Facultative and Obligate</u>	2009	2.82	0.53	2.22	0.53	2.11	0.53	2.73	0.53	0.718
<u>Total Density</u>	2009	2.92	0.57	2.41	0.57	2.28	0.57	3.12	0.57	0.686



### CHAPTER THREE: REPRODUCTIVE SUCCESS OF RED-WINGED BLACKBIRDS IN RESTORED GRASSLANDS

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**ABSTRACT** Measuring reproductive success is a key element in assessing the value of habitat restoration to grassland birds. Our objective was to examine the effects of grassland restoration planting type and invertebrate food resource availability on the reproductive success of a grassland specialist, the Red-winged Blackbird (*Agelaius phoeniceus*). We modeled daily nest survival using the nest survival model in program MARK. Red-winged Blackbird nest survival was influenced by year, visual obstruction, and variation in the availability of invertebrate food resources throughout the nesting season. We found some evidence for a difference in daily nest survival among planting types ( $p=0.06$ ). Specifically, estimated nest survival was more than twice as high in mature warm-season fields (36%) than in cool-season fields (14%).

**KEY WORDS:** food resources, grassland birds, grassland invertebrates, nest survival, vegetation diversity, vegetation structure

## INTRODUCTION

Population declines of North American grassland birds over the last several decades have been attributed to the widespread conversion of native grasslands to agricultural uses (Herkert 1995; Fletcher & Koford 2003; Herkert et al. 2003). Restoration efforts have focused on increasing the amount of grassland habitat in the landscape, but, it is unknown to what extent the ecological functions of these plantings have been restored.

Although comparative studies of grassland bird use of restored vs. remnant grasslands have assessed the potential for restored grassland plantings to provide suitable habitat for breeding songbirds (Fletcher & Koford 2003; Chapman et al. 2004), little attention has been given to assessing the relative value of the different types of grassland restoration plantings in maintaining bird populations.

Grassland vegetation structure is a key habitat component for grassland songbirds (McCoy et al. 2001; Chapman et al. 2004). Seed mixtures used for grassland plantings in the Midwest vary widely. Thus, the resulting vegetation structure and composition vary significantly among different types of restored grassland plantings (McCoy et al. 2001). The costs associated with planting and maintaining these different types of plantings also varies. Seed costs range from approximately \$50/ha for cool-season, non-native plantings, to an intermediate cost of \$285/ha for native warm-season grasses, to \$2840/ha for a diverse mix of native grasses and forbs (Prairie Seed Farms 2008). In addition, the cost of maintaining a more diverse planting type is higher than the cost of maintaining a less diverse (grass only) planting type. The plant species diversity in seed mixes used for grassland restoration plantings may have lasting effects on the resulting grassland bird community.

Although many studies have reported bird use of grassland habitats in terms of abundance, species richness, or density, these metrics may not provide an accurate assessment of habitat quality (Van Horne 1983). Reproductive success may be a more meaningful index of habitat quality for grassland birds. For avian populations, reproductive success is often measured in terms of nest survival.

Reproductive success in grassland birds is affected by factors that may be related to the structure and composition of the surrounding vegetation. Nest predation has been cited as the primary reason for nest failure in grassland birds in many studies (Camp & Best 1994; Martin 1995; Winter 1999; Hall 2005; Galligan et al. 2006; Perkins & Vickery 2007; Utnup & Davis 2007; Giocomo et al. 2008). The ability of potential predators to locate and access nests may be affected by the vegetation in which the nest is situated. A nest located in tall, dense vegetation may be better hidden from or less accessible to potential predators, leading to higher nest success (Johnson & Temple 1990; Howard et al. 2001; Frey et al. 2008). For example, successful Dickcissel (*Spiza americana*) nests are associated with taller and more dense vegetation than unsuccessful nests (Winter 1999; Conover et al. 2011). In addition, more diverse vegetation may promote higher nest success for some species (Dion et al. 2000; Lloyd & Martin 2005). Other studies have found no relationship between nest site vegetation characteristics and nest success (Vickery et al. 1992; Newton & Heske 2001). The effects of vegetation characteristics on nest success may differ depending on the type of nest predator and species of bird (Dion et al. 2000).

Brood parasitism by Brown-headed Cowbirds (*Molothrus ater*) may also affect the reproductive success of grassland birds (Johnson & Temple 1990; Dearborn et al. 1998; Winter 1999). Parasitized nests tend to fledge fewer host young (Johnson & Temple 1990)

and host young that successfully fledge may be in worse body condition as a result of nest parasitism (Dearborn et al. 1998). In spite of the negative consequences to host species, recent evidence has shown that once a nest has been parasitized, a host's best option may be to raise the brood even with the added burden of the parasite chick (Hoover & Robinson 2007). Densely vegetated nest locations may help camouflage grassland bird nests (Johnson & Temple 1990). However, Winter (1999) found no differences in vegetation characteristics between parasitized and un-parasitized Dickcissel nests.

Because grassland bird food resources in the form of arthropods vary with plant diversity (Jamison et al. 2002; Benson 2003; Leathers 2003; Harveson et al. 2004; Sutter & Ritchison 2005), the choice of planting mix for a grassland restoration may have important implications for the reproductive success of grassland birds. The availability of food resources near the nest location may contribute to the success or failure of grassland bird nests. Birds spend more time foraging and fly longer distances to forage when their nests are located in areas with reduced food availability (Adams et al. 1994). Sparling et al. (2007) found that, for Red-winged Blackbirds (*Agelaius phoeniceus*), habitat types with the lowest invertebrate diversity had the lowest levels of nest success. Birds that locate their nests in areas with high food availability may need to spend less time off the nest foraging for food. Less time spent away from the nest may translate into lower nest predation and brood parasitism. Food supplementation of Song Sparrows (*Melospiza melodia*) lowered nest predation rates through its influence on adult antipredator behavior (Rastogi et al. 2006). In addition, Dearborn et al. (1998) found that parental nest attendance is an important component of nest defense, and therefore, an important component of nest success.

Red-winged Blackbirds are one of the most common bird species in North America (Beletsky 1996). They nest in a variety of habitat types including marshes and uplands (Beletsky 1996; Swain et al. 2003; Sparling et al. 2007). Because of their adaptability to different habitats, they provide an excellent opportunity to evaluate differences in nest survival among different habitat types. The objective of our study was to examine the factors affecting nest survival of Red-winged Blackbirds in restored grasslands including vegetation characteristics, food resource availability, management activity, field area, planting age, brood parasitism, and restoration planting type.

## **METHODS**

### *Study Area*

The Spring Run Wetland Complex is a mix of more than 1,600 ha of wetlands and reconstructed grasslands located in Dickinson County in northwest Iowa, USA. This area is managed by the Iowa Department of Natural Resources and is one of the largest prairie pothole remnants in the state.

We selected four restoration/planting types for our study to encompass the range of planting mixtures typically available to land managers. The habitat types we selected were (1) cool-season - plantings of non-native, cool-season grasses (e.g. smooth brome (*Bromus inermis*), timothy (*Phleum pratense*), reed-canary grass (*Phalaris arundinacea*)) planted > 10 years ago, (2) warm-season - a five species mix of native warm-season grasses (e.g. switch grass (*Panicum virgatum*), Indian grass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and side-oats grama (*Bouteloua curtipendula*)) divided into two groups by age of planting (mature fields planted > 10 years

ago and new fields planted between 2005 and 2007), and (3) high diversity - a mixture of over 40 species of native grasses and forbs planted between 2005 and 2007. Within the study area, restored fields were selected and/or planted in a block design, with each of the field types occurring in each block (Fig. 1). We surveyed a total of six complete blocks.

### *Nest Survival*

Red-winged Blackbird nests in upland vegetation within each field were located in 2007-2009 using systematic searches and behavioral observations. Nest searches consisted of observers walking systematically through the fields watching for birds flushed from nests. Once a bird flushed, the observer carefully searched through the vegetation near the flush location to find the nest. The Universal Transverse Mercator coordinates of nests were recorded using a Global Positioning System handheld unit. In addition, we tied flagging tape to a piece of vegetation at least 5m directly north and south of the nest location. At the time of nest location, we recorded information on the status of the nest, including nest contents (number of eggs and/or nestlings), nest condition, and ultimately the nest fate. Nests were visited approximately every three days.

We evaluated all nests in the nestling stage for evidence of nestling starvation and brood parasitism by Brown-headed Cowbirds. We defined nestling starvation as cases where an individual nestling disappeared from the nest or was found dead in the nest while the rest of the nest contents remained normal, resulting in partial brood loss (Robertson 1973; Caccamise 1976; Caccamise 1978). Brood parasitism occurred when either an egg or nestling (for nests found in the nestling stage) cowbird was present in the nest.

### *Vegetation Composition and Structure*

We surveyed upland vegetation in 2007-2009 at 25m intervals along randomly located transects in each field during twice each summer. The two vegetation surveys coincided with peak height of cool-season and warm-season grasses. Vegetation transects were established in the fields independently of the bird survey transects. The shape and size of the fields determined the number of transects and therefore the number of vegetation survey locations ranged from 24-30 survey locations. At each survey point along the vegetation transect, we estimated the percent cover of warm-season native grasses, cool-season native grasses, warm-season exotic grasses, cool-season exotic grasses, native forbs, exotic forbs, standing dead vegetation, woody vegetation, bare ground, and litter in 0.5 m x 0.5 m Daubenmire frames (Daubenmire 1959). We measured visual obstruction using a Robel pole in each cardinal direction at each survey location (Robel et al. 1970).

### *Invertebrate Abundance and Biomass*

We sampled invertebrates using 30.5 cm diameter sweep nets in each field on 6 randomly selected 25m long sections of the vegetation transects described above. We completed three rounds of invertebrate surveys each summer in 2007-2009, one in mid-May, one in mid-June and one in mid-July. These sampling periods coincided with important times for food resource availability for grassland birds. Sweep net samples were taken only on warm, sunny days between 10:00 and 18:00 hours. To avoid trampling the vegetation on the transects, sweep net sampling was offset 5m to the left or right of the transect. One observer walked at a pace of one sweep per meter, sweeping the vegetation within one meter of the ground. Invertebrate samples were placed in 3.8 L sized zip-top bags at the completion of each survey. Immediately following sampling, invertebrate samples were

taken to the lab and sorted from vegetation debris using an apparatus made from polyvinyl chloride tubes. The zip-top bags containing the invertebrate samples were opened with scissors and placed into the back portion of the sorting tubes. Invertebrate samples remained in the tubes for 24 hours. During the 24 hour sorting time, invertebrates were drawn to the light end of the tubes and were carried down a funnel into labeled whirl-pak sample bags filled with 70% ethyl alcohol for preservation. Invertebrate samples were identified to Order, counted, dried, and weighed to obtain estimates of biomass.

### *Data Analysis*

To evaluate nest success of Red-winged Blackbirds, we modeled daily nest survival using the nest survival model in program MARK (White & Burnham 1999; Dinsmore et al. 2002). We used Akaike's information criterion (AIC) to select the best model based on a set of a priori candidate models (Dinsmore & Dinsmore 2007; Cooch & White 2005). We developed models to represent different hypotheses about factors (year, nest stage, vegetation structure/composition, invertebrate abundance/biomass, field type, clutch size, brood parasitism) that accounted for variation in Red-winged Blackbird nest survival (Dinsmore & Dinsmore 2007).

We adopted a hierarchical approach to model selection. We first examined time trends in nest survival by comparing models of constant survival, a linear trend, and a quadratic trend during the nesting season, as well as annual variation. Next, we looked for stage-specific temporal trends in nest survival, by dividing the nesting period into incubation and nestling stages and we compared the same temporal trends (constant, linear, and quadratic) within each nest stage. We modeled observer effects on nest survival with day-specific covariates (Dinsmore & Dinsmore 2007).



We used the best temporal trend models ( $\Delta AIC < 2$ ) from the analyses above to then investigate the effects of habitat and nest covariates on Red-winged Blackbird nest survival. We added each of the individual covariates separately. First, we wanted to see if vegetation characteristics had an effect on nest survival. We included visual obstruction (in dm, averaged from all measurements taken in each field for each year) and vegetation diversity (Shannon Diversity Index of vegetation cover classes for each field for each year). Second, we included invertebrate food resource availability as a possible factor affecting nest survival. To represent food resource availability, we included invertebrate biomass (average biomass of all invertebrate samples in each field for each year) and the variation in invertebrate biomass (standard deviation of all invertebrate samples in each field for each year). Third, we investigated the effects of management activity (0=none, 1=spot mow or spot herbicide treatment, 2=complete mow or hay, 3=prescribed fire), planting age (in years – plantings  $\geq 10$  years were entered as 10 years), and field area on nest survival. Lastly, we looked to see if brood parasitism (the presence of Brown-headed Cowbird eggs or nestlings) had an effect on nest survival. We report model averaged beta values from the set of best supported models ( $\Delta AIC < 2$ ).

To further investigate potential differences in nest survival among the planting types, we tested for differences in daily nest survival among the four different field types incorporating our block study design using ANOVA with PROC MIXED in SAS. We used program MARK to obtain estimates of average daily nest survival for each field, combining the data from all three years (2007, 2008, and 2009). Because of low numbers of nests in the newly planted warm-season fields, we excluded this field type from the analysis. In addition, two mature warm-season fields and one cool-season field were excluded from the data set

due to inadequate numbers of nests. We conducted pairwise comparisons among planting types using Tukey-Kramer adjustments for multiple testing. We calculated overall nest survival for each field type by raising daily survival rates to the exponent of the number of days in the nesting cycle (incubation time of 11 days and nestling period of 10 days).

## RESULTS

We monitored a total of 185 Red-winged Blackbird nests from 25 May through 5 August over the 3-year study period (2007-2009) encompassing 1995 total exposure days. Clutch size ranged from 1 to 5 with an average clutch size of  $3.4 \pm 0.86$ . Nests fledged an average of  $2.4 \pm 0.98$  young. We found evidence of nestling starvation in 48% of successful nests and in 36% of all nests containing nestlings. Brood parasitism by Brown-headed Cowbirds occurred in 11% of all nests and in 5% of successful nests (Table 1). Thirty percent of all nests we monitored fledged young and predation was the most common cause of nest failure (Fig. 2).

Our ANOVA results provided some evidence for a difference in daily nest survival among planting types ( $p=0.06$ ). Specifically, daily nest survival was higher in mature warm-season fields than in cool-season fields, but did not differ among the other planting types (Table 2). Overall nest survival was lowest in cool-season fields (14%), highest in mature warm-season fields (36%) and intermediate in high diversity fields (22%).

We found evidence of year effects in daily nest survival, with 2007 having higher nest survival compared to 2009 ( $\beta_{2007}=0.59$  on logit scale, 95% CI 0.02, 1.15; Table 3). Visual obstruction had a positive effect on nest survival ( $\beta= 0.15$  on logit scale, 95% CI - 0.03, 0.28; Fig. 3). We also found weak evidence that food resources had an effect on nest

survival through variation in invertebrate biomass ( $\beta_{\text{var.invert.bio.}} = -1.73$  on logit scale 95% CI - 3.58, 0.11) suggesting that fields with more variation in invertebrate biomass over the nesting season may have lower nest survival (Fig. 4). We found no evidence for observer effects on nest survival.

## **DISCUSSION**

Our daily nest survival estimates are similar to previously reported values. For example, in roadside ditches in a North Dakota study, daily nest survival for Red-winged Blackbirds was 0.931 (Clark et al. 2004). Our overall nest survival estimates are also similar to what others have found regionally. For example, the average seasonal estimate of nest success in a study in the Rainwater Basin region of Nebraska was 37% (Post van der Burg et al. 2010). We found overall nest survival in mature warm-season fields was more than twice as high than in cool-season fields, suggesting that there may be factors affecting nest survival that are a result of grassland planting type. While overall nest survival in our high diversity planting type was not significantly different from the other two planting types, this may be in part because of the recent planting age of these fields ( $\leq 4$  years). Although our results suggested that daily nest survival differed among planting types, a concurrent study of grassland bird density found that Red-winged Blackbird density was not different across the same four planting types (Vogel 2011).

We found within our 3-year study, Red-winged Blackbird nest survival varied by year. In fact, others have reported that Red-wing Blackbird reproductive success can be highly variable among years (Orians 1980; Beletsky 1996; Dinsmore & Dinsmore 2007).

Year to year variation in reproductive success may be a result of factors such as food availability and precipitation (Fletcher & Koford 2004).

We found evidence of a relationship between Red-winged Blackbird nest survival and visual obstruction, suggesting that nests had higher survival in fields with taller vegetation. Nests in taller vegetation may be less susceptible to predation by mammals (Pribil 1998). In contrast, Pribil (1998) discovered that although Red-winged Blackbirds preferred taller vegetation, preferences for vegetation features did not translate into higher reproductive success. In addition, Caccamise (1977) found that vegetation height at the nest was negatively correlated with hatching success of Red-winged Blackbirds in New Jersey marshes, but that there was no relationship between nest height and fledging success. One possible explanation for these differences may be that our vegetation measurements represent the vegetation of the entire field in which the nests were located, whereas others have measured vegetation specific to each nest.

Our results suggest that Red-winged Blackbird nests located in fields with more variation in invertebrate biomass over the nesting season may have lower nest survival than those with less variation in invertebrate food availability over the nesting season. Red-winged Blackbirds feed their nestlings almost an entirely invertebrate diet during the nestling period (Voigts 1973; Sparling et al. 2007). Researchers have reported that the most common food items collected by Red-winged Blackbirds were from the Orders Lepidoptera, Orthoptera, and Odonata (Robertson 1973; Sparling et al. 2007). Red-winged Blackbirds nesting in uplands tend to forage in terrestrial areas near their nesting location (Orians 1980). Our data suggest that terrestrial invertebrate food availability, and particularly consistency in invertebrate food availability over the nestling period, is potentially important in nest

survival. In a concurrent study of grassland bird habitat use and density on the Spring Run Complex, visual obstruction and food resources were also important predictors of adult Red-winged Blackbird density (Vogel 2011).

We found evidence of nestling starvation in both successful and unsuccessful nests in our study area. Nestling starvation is a major factor in partial brood loss in Red-winged Blackbirds (Beletsky 1996). Starvation was the second most common source of mortality in a study of Red-winged Blackbirds in New Jersey marshes and approximately 24% of nestling deaths were attributed to starvation over 2 years (Caccamise 1976; Caccamise 1978). In addition, nestling starvation was more common in areas that had higher nest density (Caccamise 1977). Red-winged Blackbirds tend to start out with clutch sizes of three to five eggs, but only tend to fledge an average of 2.7 young; much of this difference has been attributed to nestling starvation (Beletsky 1996). Similarly, we found an average clutch size of 3.4, but only an average of 2.4 young fledged. Due to competition for nest sites and food resources, starvation of nestlings may be more common when Red-winged Blackbirds co-occur with Yellow-headed Blackbirds (Robertson 1973) as was the case on our study sites.

Predation is commonly cited as the most important source of nest failure in Red-winged Blackbirds (Beletsky 1996). For example, in a study of Red-winged Blackbirds in New Jersey marshes, predation was the cause of mortality in 42% of nest failures (Caccamise 1976). Predation rates tend to be greater in uplands than in marsh habitats (Robertson 1973; Picman et al. 1993). Consistent with what others have reported, we found that predation was our most common cause of nest failure. Additionally, Red-winged Blackbird nests located in uplands are exposed to a greater variety of predators than those in marshes (Picman et al.

1993). Raccoons (*Procyon lotor*) are a common predator of Red-winged Blackbird nests and of nesting females (Blakley 1976; Picman et al. 1993).

Brown-headed Cowbirds are common brood parasites of Red-winged Blackbirds and parasitism rates vary from 0% to over 50% (Beletsky 1996). Brood parasitism rates are highly variable even among years at the same location (Beletsky 1996). Although 11% of our nests were parasitized, we did not find brood parasitism by Brown-headed Cowbirds to have an effect on Red-winged Blackbird daily nest survival. Red-winged Blackbird nestlings are larger than Brown-headed Cowbird nestlings and therefore can potentially out-compete them for food (Beletsky 1996). The main effect that parasitism by Brown-headed Cowbirds may have on Red-winged Blackbird nest success is the loss of one of the host eggs (Beletsky 1996; Clotfelter & Yasukawa 1999).

## **IMPLICATIONS FOR PRACTICE**

- Measuring reproductive success is a key element in assessing the value of habitat restoration to grassland birds. Although adult Red-winged Blackbird densities were not different among the different planting types, nest survival was twice as high in warm-season grass plantings than in cool-season grass plantings, suggesting that adult density may not be a good indicator of reproductive success.

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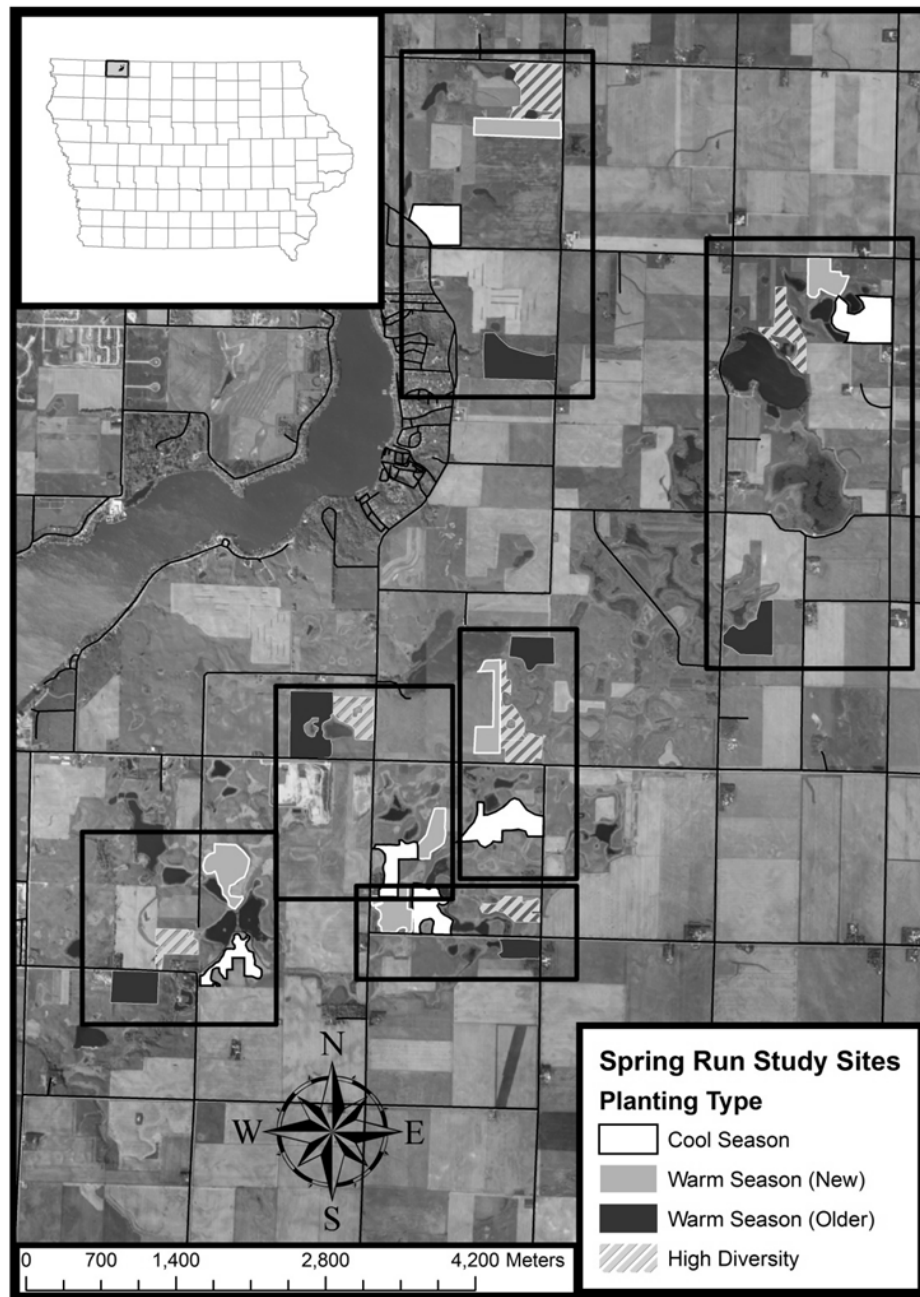


Figure 1. Study sites were located in the Spring Run Complex in Dickinson County, Iowa, USA in 2007, 2008, and 2009. Solid white polygons indicate sites that were planted with cool-season grasses  $\geq 10$  years ago, solid light grey polygons indicate sites that were planted to warm-season grasses between 2005 and 2007, solid black polygons indicate sites that were planted to warm-season grasses  $\geq 10$  years ago, and hatched light grey polygons indicate sites that were planted with a high diversity seed mix between 2005 and 2007. Black rectangles indicate study site blocking.

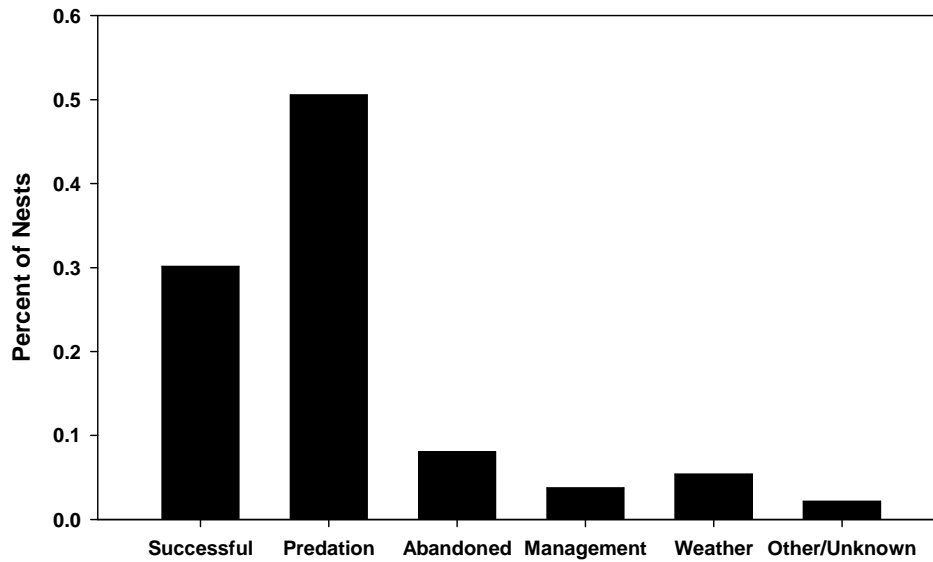


Figure 2. Fate of Red-winged Blackbirds nests monitored in 2007, 2008, and 2009 on the Spring Run Complex, Dickinson County, Iowa, USA. Successful nests fledged at least one young. Failed nests were categorized into losses from predation, abandonment, management activity (e.g. mowing), weather, or other/unknown causes.

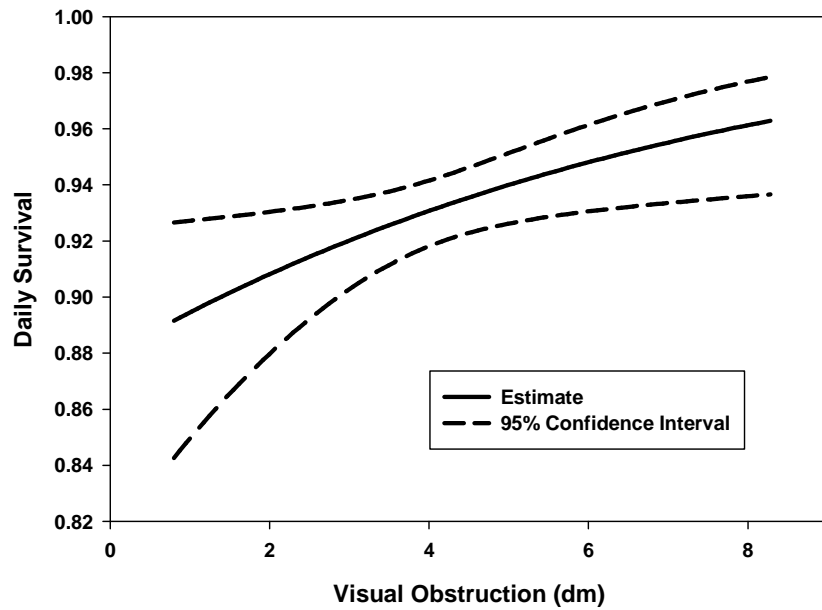


Figure 3. Predicted daily nest survival of Red-winged Blackbird nests monitored in 2007, 2008, and 2009 on the Spring Run Complex, Dickinson County, Iowa, USA. Daily nest survival was estimated for the range of visual obstruction readings we recorded in the study area. Estimates of daily survival were produced with the assumption of constant survival over the nesting season.



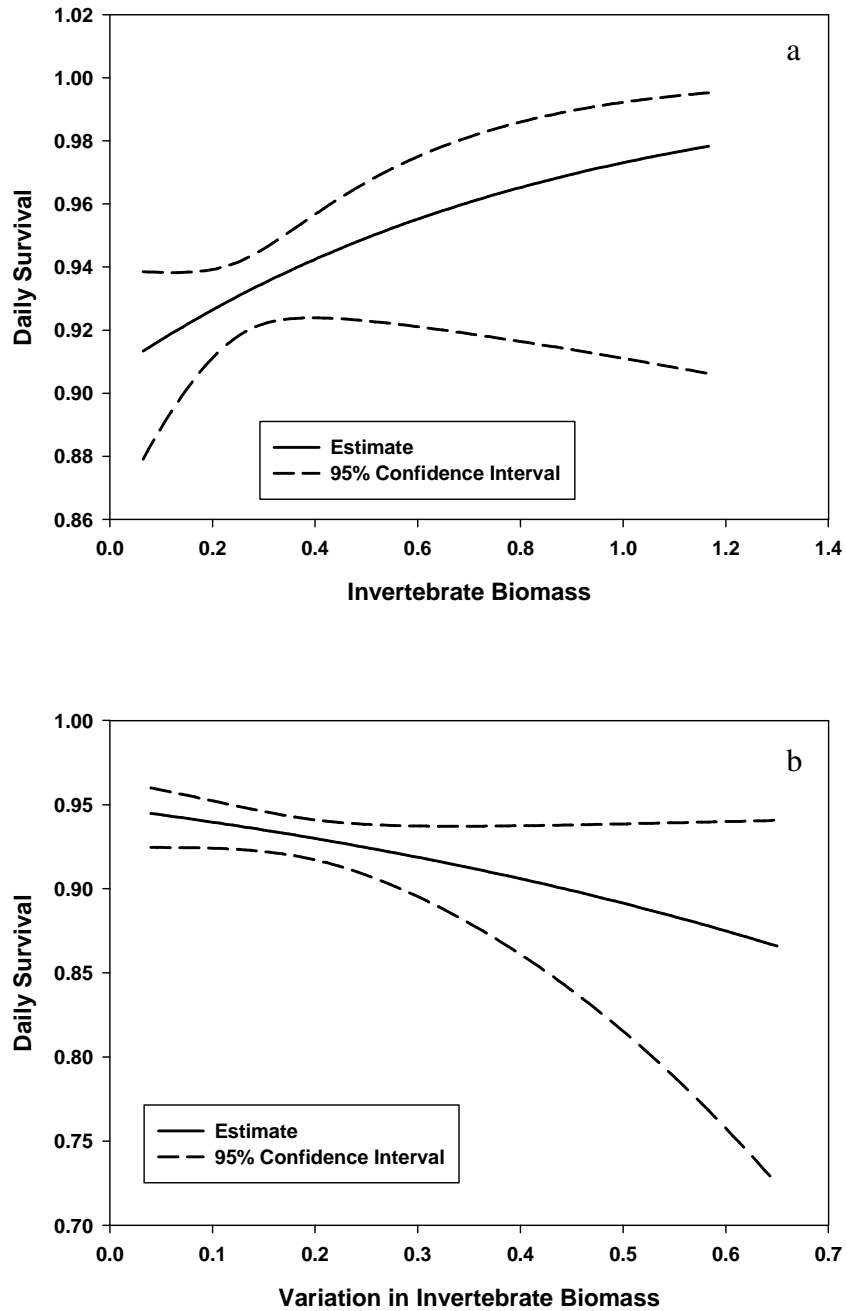


Figure 4. Predicted daily nest survival of Red-winged Blackbird nests monitored in 2007, 2008, and 2009 on the Spring Run Complex, Dickinson County, Iowa, USA. Daily nest survival was estimated for the range of values for invertebrate biomass (a) and variation in invertebrate biomass (b) we recorded in the study area. Estimates of daily survival were produced with the assumption of constant survival over the nesting season.

Table 1. Means and standard deviation of covariates by planting type included in models of daily nest survival for Red-winged Blackbird nests in 2007, 2008, and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. The covariates included were field area (in hectares), planting age (in years – plantings  $\geq 10$  years were entered as 10 years), management activity (0=none, 1=spot mow or spot herbicide treatment, 2=complete mow or hay, 3=prescribed fire), vegetation diversity (Shannon Diversity Index of vegetation cover classes for each field for each year), visual obstruction (in dm) invertebrate biomass (in grams), variation in invertebrate biomass (standard deviation of invertebrate biomass), and brood parasitism (percent of nests with Brown-headed Cowbird eggs or nestlings).

	Cool Season		Warm Season (new)		Warm Season (mature)		High Diversity	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Field Area	14.42	4.12	10.96	2.07	14.56	5.39	13.13	4.31
Planting Age	10.00	0.00	1.50	0.99	10.00	0.00	2.33	1.28
Management Activity	0.00	0.00	1.50	1.15	0.44	1.04	0.72	1.23
Vegetation Diversity	0.99	0.17	1.62	0.26	1.48	0.25	1.71	0.15
Visual Obstruction	3.49	0.97	2.20	1.42	4.43	1.49	4.27	2.29
Invertebrate Biomass	0.35	0.25	0.24	0.12	0.29	0.16	0.26	0.14
Var. Invert. Biomass	0.19	0.21	0.08	0.13	0.14	0.19	0.11	0.17
Brood Parasitism	13%	-	0	-	7%	-	22%	-

Table 2. Daily nest survival rates for Red-winged Blackbird nests in 2007, 2008, and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. Adjusted  $p$ -values are given for pair-wise comparison among planting types.

	<u>Cool Season</u>		<u>Warm Season (mature)</u>		<u>High Diversity</u>	
	Daily Survival	SE	Daily Survival	SE	Daily Survival	SE
	0.9110	0.010	0.9527	0.012	0.9305	0.009
<b>Pair-wise Comparisons</b>						
Cool Season	-		$p=0.057$		$p=0.370$	
Warm Season (mature)	$p=0.057$		-		$p=0.339$	
High Diversity	$p=0.370$		$p=0.339$		-	

Table 3. Nest survival model selection results for Red-winged Blackbirds in 2007, 2008, and 2009 on the Spring Run Complex, Dickinson County, Iowa, USA.

Model	AIC <sub>c</sub>	Δ AIC <sub>c</sub>	w <sub>i</sub>	K	Deviance
Year	672.54	0.00	0.26	3	666.52
Constant Survival (Intercept Only)	673.24	0.70	0.19	1	671.24
Linear by Stage	673.57	1.03	0.16	4	665.55
Nest Stage	673.85	1.31	0.14	2	669.84
Quadratic Survival	674.90	2.37	0.08	3	668.89
Linear Survival	674.93	2.39	0.08	2	670.92
Observer Effect	675.14	2.60	0.07	2	671.13
Quadratic by Stage	677.22	4.68	0.03	6	665.17
Constant Survival + Visual Obstruction	668.74	0.00	0.17	2	664.73
Nest Stage + Visual Obstruction	669.09	0.36	0.14	3	663.08
Linear by Stage + Visual Obstruction	669.45	0.71	0.12	5	659.41
Linear by Stage + Visual Obstruction + Food Resources	669.83	1.09	0.10	7	655.77
Constant Survival + Vis. Obstruction + Food Resources	669.90	1.16	0.09	4	661.88
Nest Stage + Visual Obstruction + Food Resources	669.91	1.17	0.09	5	659.87

**CHAPTER FOUR: THE EFFECTS OF VEGETATION SPECIES DIVERSITY AND  
INVERTEBRATE FOOD RESOURCE AVAILABILITY ON RED-WINGED  
BLACKBIRD (*AGELAIUS PHOENICEUS*) NESTLING GROWTH RATES IN  
RESTORED GRASSLANDS**

A paper to be submitted to the *Wildlife Society Bulletin*

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**ABSTRACT** Nestling growth rates in altricial birds are influenced by many external factors related to the availability of food resources. Grassland bird food resources in the form of arthropods vary with plant diversity. As a result, the choice of planting mix for a grassland restoration may affect food resource availability for grassland birds. Our objectives were to determine whether nestling growth rates and nestling size at fledging differed among grassland restoration plantings with varying plant species diversity and to examine how the availability of invertebrate food resources affected the growth rates of grassland songbirds. We selected three planting types to encompass the range of planting mixtures typically available to land managers. The planting types were (1) cool-season - plantings of non-native, cool-season grasses, (2) warm-season - a five species mix of native warm-season grasses, and (3) high diversity - a mixture of over 40 species of native grasses and forbs. We measured 79 red-winged blackbird (*Agelaius phoeniceus*) nestlings in 2008 and 2009.

Nestling size at fledging differed among grassland planting type with warm-season fields having smaller nestlings. Male nestlings were larger than females at fledging with regard to mass, wing, and tarsus measurements. Nestling growth rates did not differ either among grassland restoration planting types or between sexes.

**KEY WORDS** nestling growth, grassland birds, grassland invertebrates, food resources

## **INTRODUCTION**

Habitat loss is one of the primary factors affecting population declines of grassland birds over the last several decades (Herkert 1995; Fletcher and Koford 2003; Herkert et al. 2003). Recovery efforts for grassland bird populations have focused on increasing the amount of grassland habitat in the landscape. However, it is unknown to what extent the ecological functions of these grassland plantings have been restored. For example, although the densities of common bird species were similar in restored and remnant grasslands, Fletcher and Koford (2003) found that habitat structure differed between the two vegetation types.

Grassland bird food resources in the form of arthropods vary with plant diversity (Jamison et al. 2002; Benson 2003; Leathers 2003; Harveson et al. 2004; Sutter and Ritchison 2005). As a result, the choice of planting mix for a grassland restoration may affect food resource availability for grassland birds. Few studies have evaluated the response of invertebrate food resources to grassland habitat plantings. McIntyre and Thompson (2003) found that, in addition to differences in vegetation structure and composition between restored and remnant grasslands, arthropod abundance and diversity were higher on remnant

sites. In a study of planted exotic grasslands in Montana, chestnut-collared longspurs (*Calcarius ornatus*) had slower nestling growth and smaller mass at fledging than in native grasslands (Lloyd and Martin 2005). Although restored sites may not be functionally similar to remnant sites, restored grasslands can support invertebrate populations that are important food resources for grassland birds (McIntyre and Thompson 2003). Evaluating the availability of food resources in restored grasslands and how these resources relate to the growth rate of grassland songbirds warrants further investigation.

Nestling growth rates in altricial birds are influenced by many external factors related to the availability of food resources (O'Connor 1984). Both the quality and quantity of food delivered to young in the nest are potentially important in determining growth and reproductive success (Boag 1987; Reynolds et al. 2003; Granbom and Smith 2006). Laboratory studies have shown that zebra finch (*Poephila guttata*) nestling growth rates are significantly affected by diet quality (Boag 1987). However, studies on food supplementation and reduction in the field have not consistently demonstrated strong effects of food availability on avian growth rates. Increased parental foraging effort may compensate for limited food resources (Adams et al. 1994; Tremblay et al. 2005; Zalik and Strong 2008). For example, Adams et al. (1994) found that reducing invertebrate food resources did not produce adverse effects on growth and survival of vesper sparrow (*Pooecetes gramineus*) nestlings. Similarly, diet supplementation of Florida scrub-jays (*Aphelocoma coerulescens*) produced minimal improvement in nestling growth rates (Reynolds et al. 2003).

Estimates of food resource availability for insectivorous birds have been used as response variables in the study of avian systems. Many of these studies have found that food

availability is associated with bird abundance in both grassland and forested systems (Brush and Stiles 1986; Davros 2005; Benson et al. 2007; Vogel 2011). Food resources may affect nestling growth rates differently in different systems (Granbom and Smith 2006).

Differences among habitats in the influence of food resources on growth rates may be related to food resource variability and may depend on whether food resources are a limiting factor in those systems.

A relationship between nestling weight and juvenile survival has been demonstrated for some bird species (Magrath 1991; Ragusa-Netto 1996). For other species, no such trend has been demonstrated; however, there is no evidence of a negative relationship between nestling mass and juvenile survival (see Table 5 in Magrath 1991). For blackbirds (*Turdus merula*), nestlings that were heavier at 8 days had higher survival than lighter nestlings and were more likely to return to breed than lighter nestlings (Magrath 1991). A similar relationship has been reported for black-capped donacobius (*Donacobius articapillus*) where nestling mass at 15 days was also correlated with juvenile survival (Ragusa-Netto 1996).

Red-winged blackbirds (*Agelaius phoeniceus*) are one of the most common bird species in North America (Beletsky 1996). They nest in a variety of habitat types including marshes and uplands (Beletsky 1996; Swain et al. 2003; Sparling et al. 2007). Red-winged blackbirds regularly forage for invertebrate food in upland habitats during the breeding season (Orians 1980). Because of their abundance and adaptability to different habitats, they provided an excellent opportunity to evaluate differences in nestling growth rates and size at fledging among different habitat types. The objectives of our study were to determine whether nestling growth rates and nestling size at fledging differs among grassland restoration plantings with varying plant species diversity. We also examined the relationship



between invertebrate food resources and growth rates of grassland songbirds and how vegetation characteristics and weather (temperature and precipitation) affected both invertebrate food resource availability and nestling growth rates in restored grasslands.

## **METHODS**

### **Study Area**

The Spring Run Wetland Complex is a mix of more than 1600 ha of wetlands and reconstructed grasslands located in Dickinson County in northwest Iowa, USA. The area is managed by the Iowa Department of Natural Resources and is one of the largest prairie pothole remnants in the state.

We selected three planting types for our study to encompass the range of planting mixtures typically available to land managers. The planting types we selected were (1) cool-season - plantings of non-native, cool-season grasses (e.g. smooth brome (*Bromus inermis*), timothy (*Phleum pratense*), reed-canary grass (*Phalaris arundinacea*)) planted > 10 years ago, (2) warm-season - a five species mix of native warm-season grasses (e.g. switch grass (*Panicum virgatum*), Indian grass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and side-oats grama (*Bouteloua curtipendula*)) and (3) high diversity - a mixture of over 40 species of native grasses and forbs. Within the study area, reconstructed fields were selected or planted in a block design, with each of the field types occurring in each block (Fig. 1). We surveyed a total of 6 complete blocks.

## **Nestling Growth**

We located nests in uplands from May through August in 2008-2009 using systematic searches and behavioral observations. Systematic nest searches consisted of observers walking in a systematic pattern through the fields watching for birds to flush from nests. After a bird flushed, an observer carefully searched through the vegetation near the flush location to find the nest. To aid in the relocation of nests, we recorded the Universal Transverse Mercator coordinates of nests using a Global Positioning System handheld unit. In addition, we tied flagging tape to a piece of vegetation 5m directly north and south of the nest location. At the time of nest location, we recorded the status of the nest including the nest contents (number of eggs and/or nestlings, presence of brood parasitism), nest condition, and ultimately the nest fate (whether a nest successfully fledged young or failed). Nests were monitored approximately every 3 days.

We assessed nestling growth rates. We individually marked nestlings by dyeing the down on the top of their heads with a non-toxic felt tipped pen. Once the nestlings were large enough (approximately 4 days old), we banded them with a unique combination of color and numbered aluminum bands. At each visit, we determined the mass of nestlings to the nearest 0.01g using a portable electronic balance, and we measured tarsus length and the length of the outermost primaries to the nearest 0.1mm using dial calipers.

## **DNA Sexing**

We determined the sex of each nestling that reached fledging age with DNA sexing. We collected blood from nestlings just prior to fledging (9-10 days). Blood samples were collected in accordance with the Ornithological Council's Guidelines to the Use of Wild Birds in Research (Gaunt et al. 1999) and Iowa State University's Institutional Animal Care

and Use Committee (permit # 8-06-6203-A). We sent blood samples on Permacode sample cards to Animal Genetics, Inc., Tallahassee, FL for processing. Samples were assayed using a Polymerase chain reaction (PCR) to amplify the DNA in each sample which contains the different sized W and Z bird sex chromosomes. This PCR-based method has been used to sex many different species of birds in many different life stages (Santamaria 2010).

### **Vegetation Composition and Structure**

We surveyed upland vegetation at 25 m intervals along randomly located transects in each field during May and July each summer in 2008 and 2009. The 2 rounds of vegetation surveys coincided with peak height of cool-season and warm-season grasses. The shape and size of the fields determined the number of vegetation transects and therefore the number of survey locations (ranged from 24-30 survey locations per field). At each survey point along the vegetation transect, we estimated the percent cover of warm-season native grasses, cool-season native grasses, warm-season exotic grasses, cool-season exotic grasses, native forbs, exotic forbs, standing dead vegetation, woody vegetation, bare ground, and litter in 0.5 m x 0.5 m Daubenmire frames (Daubenmire 1959). We measured visual obstruction using a Robel pole in each cardinal direction at each survey location (Robel et al. 1970). We averaged vegetation measurements taken within each field within each year. To evaluate vegetation diversity, we calculated a Shannon Diversity Index value of the vegetation cover classes for each field. To calculate Shannon Diversity we used the formula:  $H' = -\sum (p_i * \ln p_i)$ , where  $p_i$  = the proportion of each vegetation cover class in each field.

We recorded the management activity that occurred in each field during each nesting season. Fields in our study either had no management or they were managed with prescribed

fire. We also recorded the planting age of each field at the start of each nesting season. Fields that were planted more than 10 years ago were listed as 10 years.

### **Invertebrate Biomass**

We sampled invertebrates using 30.5 cm diameter sweep nets in each field on 6 randomly selected 25 m long sections of the vegetation transects described above. We completed three rounds of invertebrate surveys, one in mid-May, one in mid-June and one in mid-July in 2008 and 2009. We sampled invertebrates only on warm, sunny days between 1000 and 1800 hours. In order to avoid trampling the vegetation on the transects, we off-set sweep net sampling 5m to the left or right of the transect. During the surveys, one observer walked at a pace of one sweep per meter, sweeping the vegetation within one meter of the ground. Invertebrate samples were placed in 3.8 L sized zip-top bags at the completion of each survey. Immediately following sampling, invertebrate samples were sorted from vegetation debris using insect self-sorting tubes. Invertebrate samples remained in the tubes for 24 hours. During the 24 hour sorting time, invertebrates were drawn to the end of the tubes and were carried down a funnel into labeled whirl-pak sample bags filled with 70% ethyl alcohol for preservation. We identified invertebrate samples to Order, counted, dried, and weighed them to obtain estimates of biomass. We calculated a Shannon Diversity Index of invertebrate Orders using biomass.

### **Weather Data**

We obtained daily weather data from the nearest National Oceanic and Atmospheric Administration's National Climatic Data Center weather station (number 14972). For each nestling in our dataset, we compiled the daily precipitation and temperature data for the 10

days corresponding to the days in the nest. We averaged daily temperature and total precipitation for each 10-day period.

### **Data Analysis**

We estimated nestling growth rates using PROC NLIN in SAS Version 9.1 (SAS Institute, Cary, NC, USA) with the following logistic growth curve equation for altricial birds (Ricklefs 1968; Ricklefs 1983; McCarty 2001) in the model statement:

$$M_{(x)} = \frac{M_{(\infty)}}{1 + \left[ \frac{M_{(\infty)} - M_{(0)}}{M_{(0)}} \right] e^{-Kx}}$$

$M_{(x)}$  is the nestling mass at age  $x$ ,  $M_{(\infty)}$  is the predicted asymptotic mass of the nestling,  $M_{(0)}$  is the mass of the nestling at age 0, and  $K$  is the growth rate (Ricklefs 1983, McCarty 2001). We entered initial values for each parameter based on information from Beletsky (1996). We produced three growth curves for each nestling (mass, wing, and tarsus). Because we were not able to measure all of the nestlings more than 4 times during the nestling period, we were unable to obtain variances for all growth rate estimates, and therefore were unable to include the variance of our estimates in our subsequent analyses.

We tested for differences in nestling growth rates ( $K$ ) for mass, wing, and tarsus and for differences in mass, wing, and tarsus measurements at fledging among the different planting types using Analysis of Variance (ANOVA) with PROC MIXED in SAS. We included Nest in a RANDOM statement to account for the non-independence of nestlings within a nest. We included Block, Year, Sex, and Planting Type in the model statement. We also tested for differences in invertebrate biomass and diversity among the different planting types using ANOVA with PROC MIXED in SAS. We included Year, Block, and Planting

Type in the model statement. We conducted post-hoc pairwise comparisons among planting types using Tukey-Kramer adjustments for multiple testing.

We examined the effects of habitat, weather, and nest characteristics on avian growth rates and size at fledging using a model selection procedure. We developed a set of habitat, weather, and nest covariates (Table 1) that we predicted would affect growth rates in nestling red-winged blackbirds. We used PROC MIXED in SAS and included Nest in a random statement to account for the non-independence of nestlings within a nest. We used Akaike's Information Criterion (AIC) to evaluate the support for each model and determine which models were best supported ( $\Delta AIC < 2$ ) by the data (Burnham and Anderson 2002). We report beta values where the 95% confidence interval does not include zero for covariates in the best supported models ( $\Delta AIC < 2$ ). We also examined the effects of vegetation characteristics (Table 2) on invertebrate diversity and biomass using the same model selection procedure described above with a separate set of vegetation covariates.

## RESULTS

We measured 79 nestlings from 30 red-winged blackbird nests. Of the 79 nestlings, we were able to determine the sex of 71 individuals. The sex ratio of the nestlings was 36 males and 35 females. Brood size ranged from 1 to 4, with an average brood size of 2.8 nestlings. Only 2 out of the 30 nests (7%) were parasitized by brown-headed cowbirds (*Molothrus ater*).

Male nestlings were larger than females at fledging with regard to mass, wing, and tarsus measurements (Table 3). Nestlings were smaller at fledging in the warm-season planting type than in either high diversity or cool-season plantings (Table 4). We found no

evidence of differences in nestling growth rates among the three planting types (Table 4). In addition, we found no evidence of differences in nestling growth rates between males and females (Fig. 2-4, Table 3). There was no evidence of differences among years for growth rates or nestling size at fledging.

We found evidence that vegetation structure (visual obstruction) had a negative effect on nestling mass at fledging ( $\beta_{\text{visual obstruction}} = -1.279$ , 95% CI -2.458, -0.010) suggesting that fields with taller, dense vegetation may produce smaller nestlings (Table 5, Fig.5).

Vegetation structure also had a negative effect on nestling tarsus growth rates ( $\beta_{\text{visual obstruction}} = -0.032$ , 95% CI -0.061, -0.002; Table 5, Fig.5). Nestling wing growth rate was negatively related to food resource availability ( $\beta_{\text{invert. biomass}} = -0.285$ , 95% CI -0.430, -0.139) and positively related to management activity ( $\beta_{\text{management}} = 0.039$ , 95% CI 0.018, 0.059; Table 5, Fig. 6).

Invertebrate diversity did not differ among the planting types (Table 6). Araneae biomass was highest in cool-season fields than in either warm-season fields or high diversity fields (Table 6). Four additional invertebrate Orders had higher biomass in cool-season fields, but these differences were not statistically significant (Table 6). Overall invertebrate biomass was also higher in cool-season fields, but this difference was not statistically significant (Table 6). We found that the amount of native vegetation in a grassland restoration was negatively related to Araneae biomass ( $\beta_{\text{native grasses}} = -0.0002$ , 95% CI -0.0004, -0.00003;  $\beta_{\text{native forbs}} = -0.0003$ , 95% CI -0.0005, -0.0001; Table 8). Additionally, we found that planting age had a negative effect on Diptera (true flies) biomass ( $\beta_{\text{planting age}} = -0.0034$ , 95% CI -0.0061, -0.0006; Table 7) while vegetation diversity had a positive effect on Diptera biomass ( $\beta_{\text{vegetation diversity}} = 0.0301$ , 95% CI 0.0011, 0.06; Table 8).

## DISCUSSION

Although male red-winged blackbird fledglings are larger than females, we found that growth rates did not differ between the sexes. This result was consistent with previous work that suggested growth rates for male and female red-winged blackbird nestlings were similar (Holcomb and Twiest 1970). Male and female red-winged blackbird nestlings appear to be growing at the same rate, but female nestlings stop growing sooner than male nestlings. Holcomb and Twiest (1970) found that female red-winged blackbird nestlings had earlier feather tract development, achieved their adult size earlier, and fledged earlier than male nestlings. The average secondary sex ratio for red-winged blackbirds is 53:47 in favor of females (Beletsky 1996). The sex ratio for nestlings in our study was slightly in favor of males at 49:51 females to males.

Red-winged blackbird nestlings were smaller at fledging in warm-season fields than in either of the other two planting types. In addition, we found that vegetation structure (visual obstruction) was negatively associated with nestling mass at fledging, yet warm-season fields in the Spring Run Complex did not have higher visual obstruction readings than either cool-season or high diversity fields (Vogel 2011). Although vegetation structure was not associated with invertebrate biomass; taller, dense vegetation may make invertebrate prey more difficult to find, resulting in conditions that may be less favorable for foraging.

We did not find that temperature had an influence on red-winged blackbird nestling growth rates. A negative relationship between maximum daily temperature and nestling growth has been reported for sage sparrows (*Amphispiza belli*) in Idaho and both skylarks (*Alauda arvensis*) and yellowhammers (*Emberiza citronella*) in the United Kingdom (Petersen et al. 1986; Bradbury et al. 2003). Although warmer temperatures may increase



invertebrate activity and therefore lead to increased food availability, warmer temperatures may also lead to increased costs to both nestlings and adults (Bradbury et al. 2003). Adult costs associated with higher temperatures may include spending more time shading nestlings on the nest, leaving less time for foraging, while nestlings may have increased costs in hotter conditions due to heat dissipation requirements (Bradbury et al. 2003).

We found no evidence in our models that brood size had a significant effect on red-winged blackbird nestling growth or nestling size at fledging. Many studies have investigated the effects of experimentally manipulating brood size on nestling growth and nestling condition and have generally concluded that larger brood sizes produce smaller, slower growing nestlings (Dijkstra et al. 1990; Robinson and Rotenberry 1991; DeKogel 1997). Studies of naturally occurring brood sizes on nestling growth and condition have not come to such consistent conclusions. For example, brood size had no effect on nestling growth rates of tree swallows (*Tachycineta bicolor*), but had significant negative effects on nestling mass (Parsons 2009) and for Bachman's sparrows (*Aimophila aestivalis*) in Arkansas, brood size did not have an effect on nestling mass or tarsus growth (Haggerty 1994).

Brown-headed Cowbirds commonly parasitize the nests of red-winged blackbirds. Parasitism rates vary from 0% to more than 50% and are highly variable even among years at the same location (Beletsky 1996). We found that brood parasitism by brown-headed cowbirds may have an effect on red-winged blackbird nestling primary length at fledging; however, only 7% of our nests were parasitized. Others have found that red-winged blackbird nestlings were of similar size (mass and tarsus) in nests parasitized by brown-headed cowbirds and in unparasitized nests (Clotfelter and Yasukawa 1999). Red-winged

blackbird nestlings are larger than brown-headed cowbird nestlings and therefore can potentially out-compete them for food (Beletsky 1996).

Food resource availability was negatively related to nestling wing growth rates. However, we found no evidence of differences in overall food resource availability (invertebrate biomass) among the planting types. Red-winged blackbirds regularly forage for invertebrates in upland areas during the breeding season (Orians 1980). Previous work has suggested deficits in food resource availability may be compensated for by increased foraging time and/or foraging distance in adults (Adams et al. 1994; Tremblay et al. 2005; Zalik and Strong 2008).

Interestingly, we found that Araneae biomass was highest in cool-season fields. Because our cool-season fields contained non-native, cool-season grasses, it was not surprising that we also found a negative relationship between Araneae biomass and the percent cover of native vegetation. It is unclear why spiders in the Order Araneae would prefer exotic cool-season fields to the other planting types. Cool-season fields in the Spring Run Complex have significantly lower variation in visual obstruction readings than warm-season or high diversity fields, resulting in more homogenous cover (Vogel 2011). Perhaps the more homogenous nature of cool-season fields results in better web construction locations, less web obstruction due to vegetation variation, and therefore increased prey capture.

## **MANAGEMENT IMPLICATIONS**

Although we selected a broad range of planting types, we did not find that planting type was an important factor affecting the growth rates of red-winged blackbird nestlings.

We selected red-winged blackbirds for this study because they were abundant in all of our planting types. Future research may need to focus on whether the results we found for red-winged blackbirds translate to more grassland-dependent species such as dickcissels or bobolinks. From a human perspective, the qualitative differences we observe between monotypic grassland plantings and diverse grass/forb plantings are significant. However, these qualitative differences do not appear to translate into large quantitative differences in invertebrate food resource availability (biomass).

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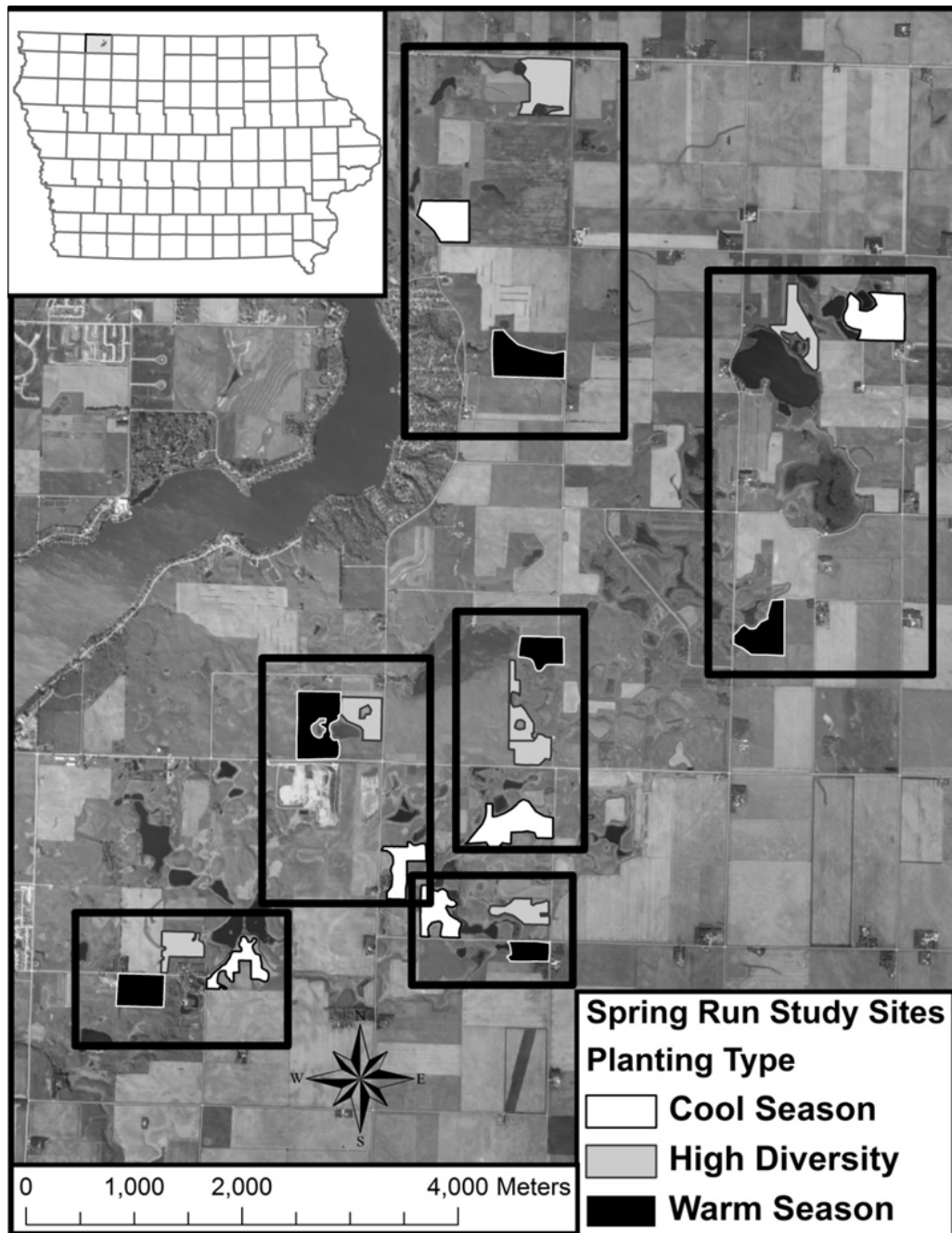


Figure 1. Study sites were located in the Spring Run Complex in Dickinson County, Iowa, USA in 2008, and 2009. Solid white polygons indicate sites that were planted with cool-season grasses  $\geq 10$  years ago, solid black polygons indicate sites that were planted to warm-season grasses  $\geq 10$  years ago, and solid light grey polygons indicate sites that were planted with a high diversity seed mix between 2005 and 2007. Black rectangles indicate study site blocking.

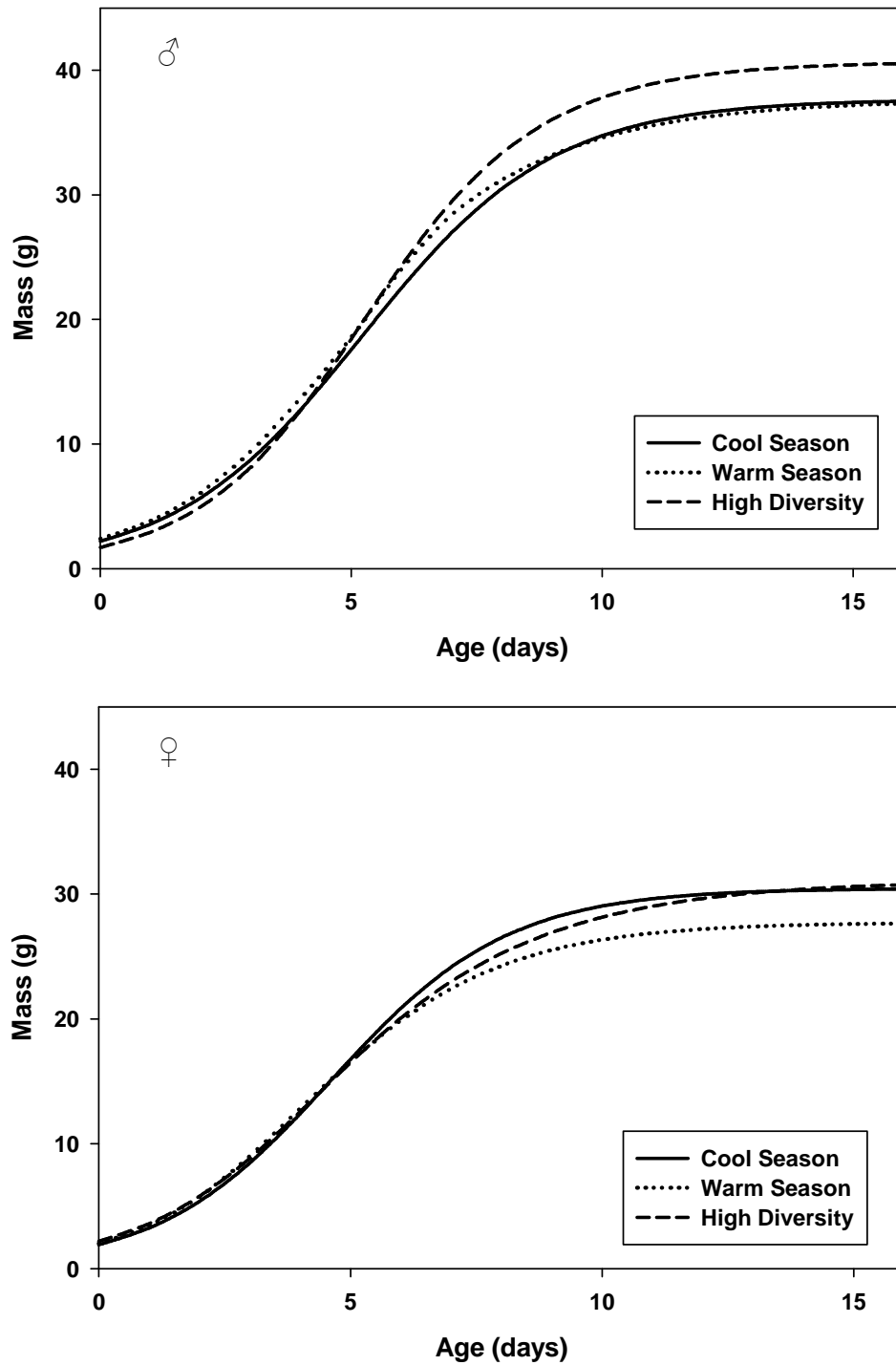


Figure 2. Predicted growth curves for male (top) and female (bottom) red-winged blackbird nestling mass measured in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

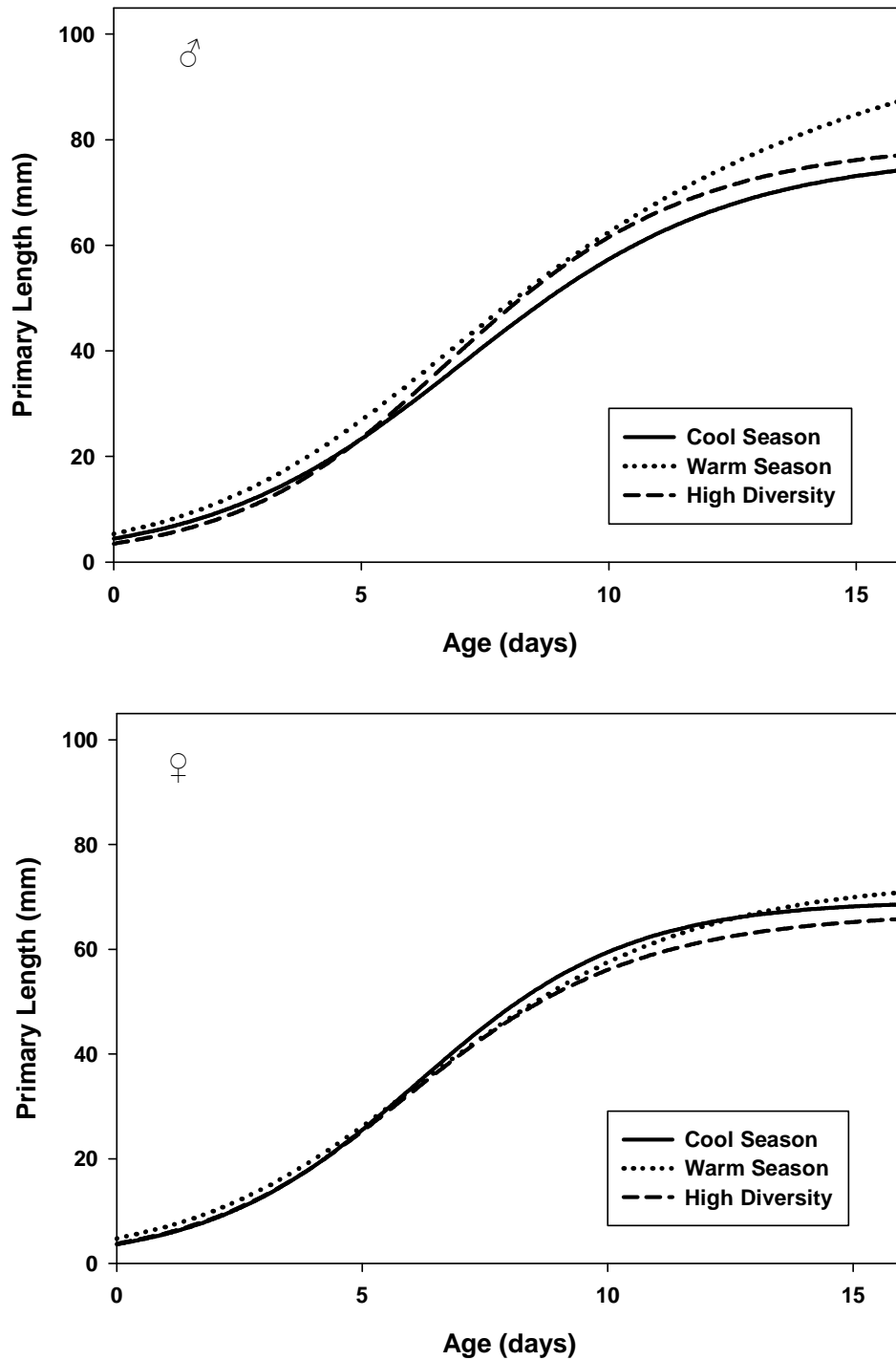


Figure 3. Predicted growth curves for male (top) and female (bottom) red-winged blackbird nestling primary length measured in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

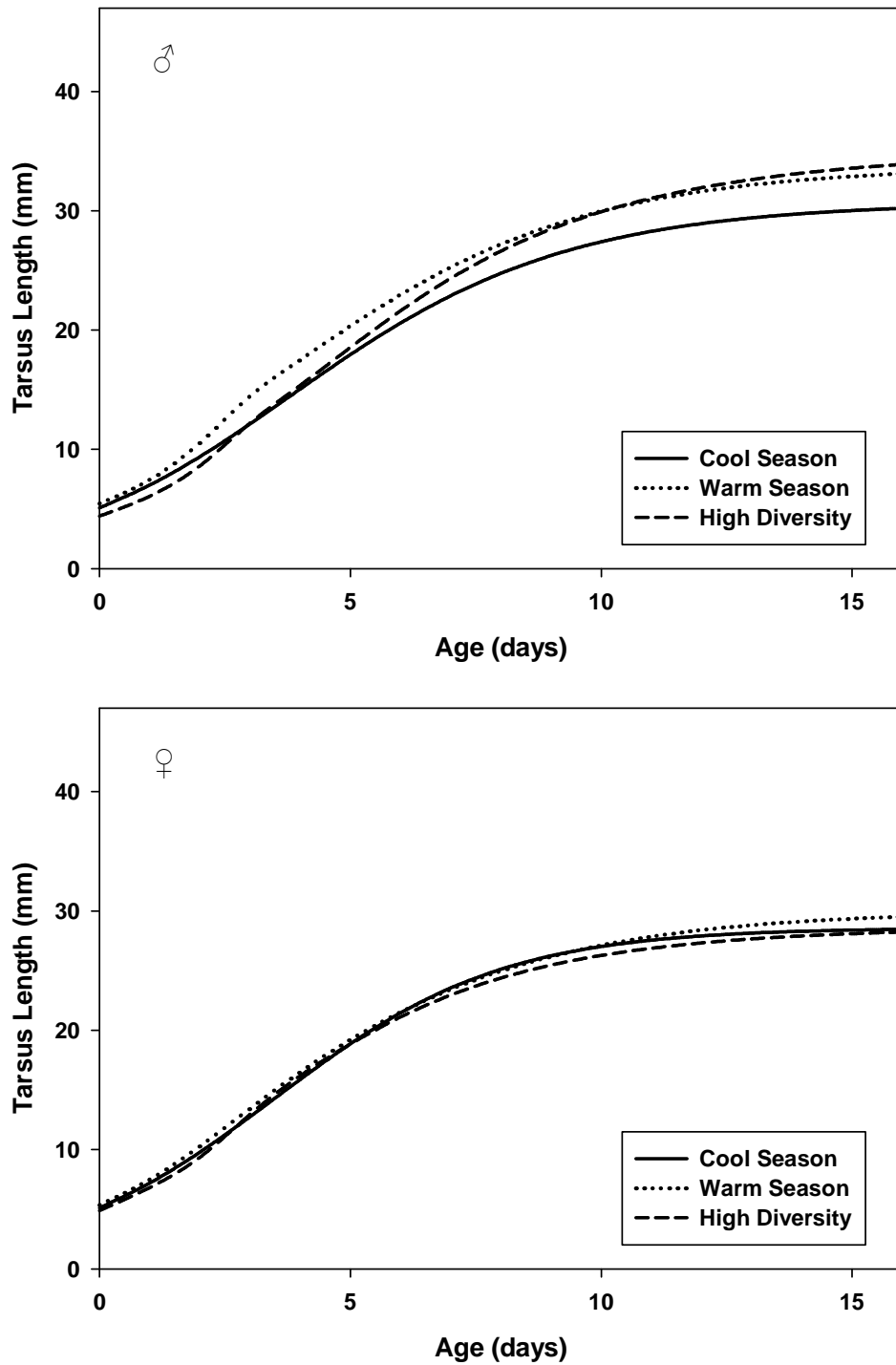


Figure 4. Predicted growth curves for male (top) and female (bottom) red-winged blackbird nestling tarsus length measured in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

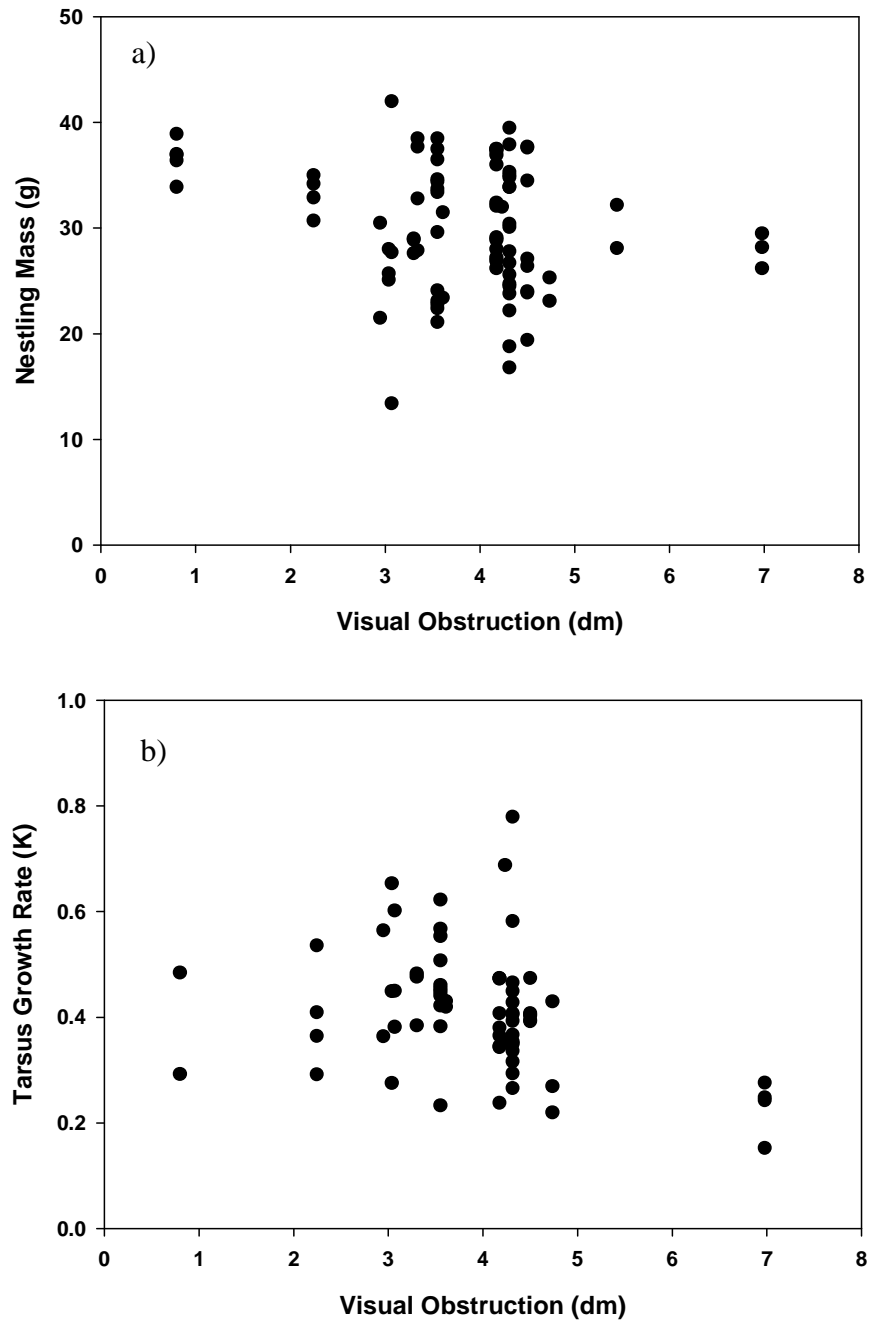


Figure 5. Relationship between vegetation structure (visual obstruction) versus a) red-winged blackbird mass at fledging and b) red-winged blackbird tarsus growth rate measured in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

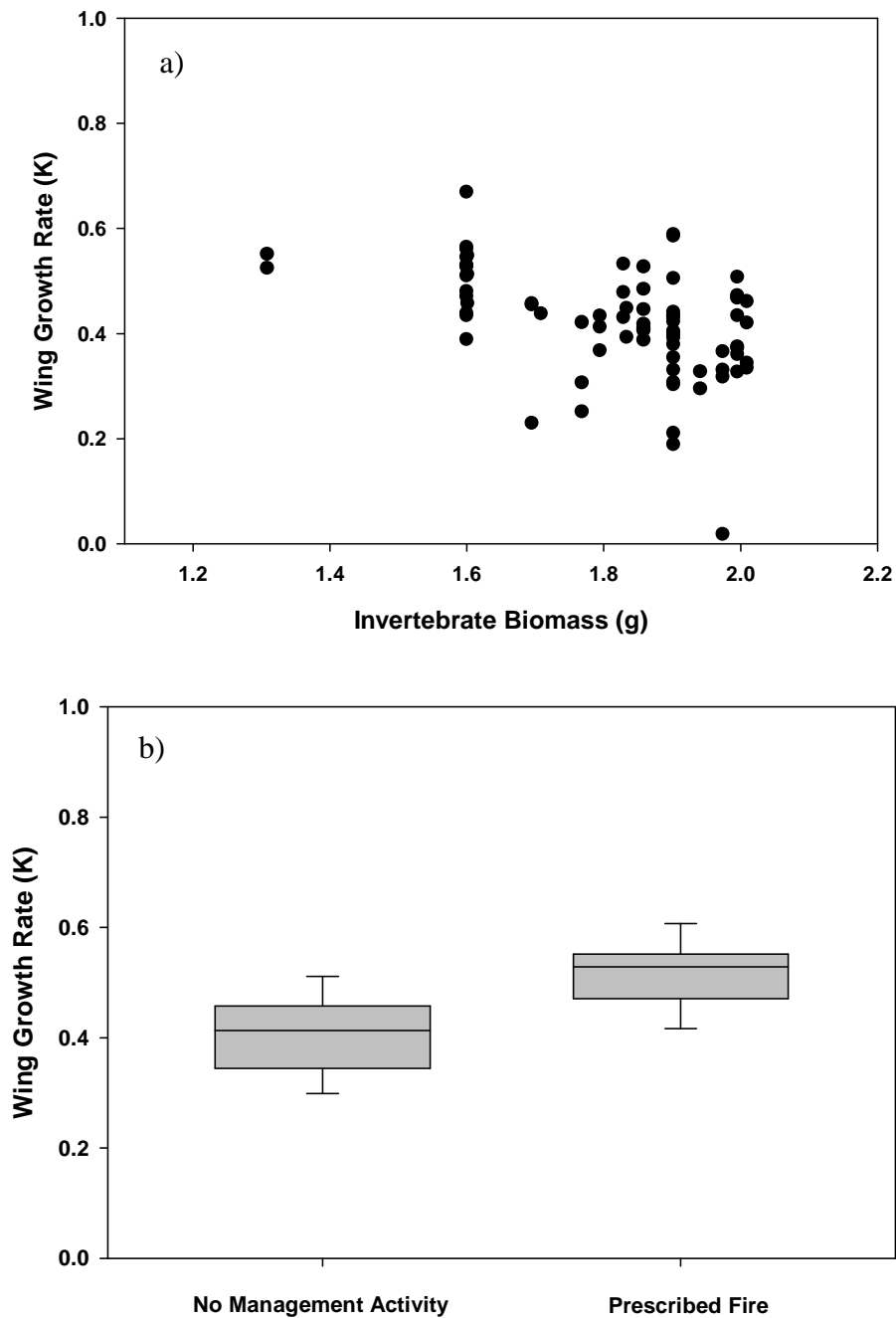


Figure 6. Relationship between red-winged blackbird nestling wing growth rate versus a) food resource availability (invertebrate biomass) and b) management activity measured in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

Table 1. Mean and standard deviation of vegetation, food resources, weather, and nest covariates included in models of nestling growth rates and measurements (mass, wing, tarsus) for red-winged blackbird nestlings in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. The covariates included were vegetation structure (visual obstruction in dm), Food Resources (total invertebrate biomass in grams), brood size (the number of nestlings in a nest), brood parasitism (presence of a Brown-headed Cowbird egg or nestling in a nest), temperature (average temperature during the 10 day nestling period), precipitation (total precipitation during the 10 day nestling period), management activity (0=no management, 1=managed by prescribed fire), and planting age (in years). Data for fields were only included if they contained at least one nest that survived to fledging.

<b>Covariates</b>	<b>Mean</b>	<b>Std. Dev.</b>	<b>Min.</b>	<b>Max.</b>
Vegetation Structure (dm)	3.87	1.14	0.80	6.98
Food Resources (g)	1.83	0.16	1.31	2.01
Brood Size	2.83	0.78	1.00	4.00
Brood Parasitism	7%	-	-	-
Temperature (degrees F)	67.88	4.04	57.45	74.09
Precipitation (cm)	0.39	0.34	0.00	1.02
Management Activity	0.16	0.37	0.00	1.00
Planting Age (years)	6.90	3.69	1.00	10.00

Table 2. Mean and standard deviation of habitat covariates included in models of invertebrate diversity and biomass in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. The covariates included were vegetation structure (visual obstruction in dm), vegetation diversity (Shannon Diversity Index of vegetation cover classes), native vegetation (percent cover of native grasses and native forbs), grass cover (percent cover of all grasses both native and exotic), planting age (in years; plantings  $\geq 10$  years were entered as 10 years), and management activity (0=none, 1=prescribed fire).

<b>Covariates</b>	<b>Mean</b>	<b>Std. Dev.</b>	<b>Min.</b>	<b>Max.</b>
Vegetation Structure	4.03	1.51	0.80	7.81
Vegetation Diversity	1.42	0.34	0.89	1.90
Native Vegetation				
% Cover Native Grasses	17.25	16.23	0.00	54.10
% Cover Native Forbs	9.95	11.45	0.00	35.00
Total Grass Cover	46.88	13.50	19.07	66.83
Planting Age	7.61	3.48	1.00	10.00
Management Activity	0.14	0.35	0.00	1.00



Table 3. Red-winged blackbird nestling growth rates and fledgling mass, wing, and tarsus in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. P-values are given for tests of differences between sexes.

	<b>Males</b>		<b>Females</b>		<i>P</i> -value
	Mean	S.E.	Mean	S.E.	
<b>Growth Rate (K) Mass</b>	0.549	0.032	0.546	0.032	0.877
<b>Fledge Mass (g)</b>	34.477	0.707	25.077	0.784	<b>&lt;0.001</b>
<b>Growth Rate (K) Wing</b>	0.427	0.020	0.442	0.020	0.268
<b>Fledge Wing (mm)</b>	54.904	1.046	50.752	1.157	<b>0.001</b>
<b>Growth Rate (K) Tarsus</b>	0.377	0.031	0.419	0.030	0.107
<b>Fledge Tarsus (mm)</b>	28.345	0.349	25.188	0.391	<b>&lt;0.001</b>

Table 4. Red-winged blackbird nestling growth rates and measurements (mass, wing, tarsus) measured in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. P-values are given for ANOVA tests for differences among planting types. Different lettered subscripts following means indicate significant differences among treatment types for pairwise comparisons.

	<b>Cool Season</b>		<b>Warm Season</b>		<b>High Diversity</b>		<i>P</i> -value
	Mean	S.E.	Mean	S.E.	Mean	S.E.	
<b>Growth Rate (K) Mass</b>	0.669	0.117	0.724	0.087	0.581	0.071	0.399
<b>Fledge Mass (g)</b>	31.501 <sup>a</sup>	1.483	26.773 <sup>b</sup>	1.277	31.056 <sup>a</sup>	0.912	<b>0.042</b>
<b>Growth Rate (K) Wing</b>	0.466	0.043	0.372	0.030	0.466	0.024	0.090
<b>Fledge Wing (mm)</b>	57.683 <sup>a</sup>	2.205	48.304 <sup>b</sup>	1.895	52.498 <sup>ab</sup>	1.358	<b>0.021</b>
<b>Growth Rate (K) Tarsus</b>	0.417	0.060	0.373	0.052	0.402	0.033	0.858
<b>Fledge Tarsus (mm)</b>	27.693	0.724	25.381	0.627	27.225	0.442	0.066

Table 5. Models of Red-winged blackbird nestling growth rates and measurements (mass, wing, tarsus) taken in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. Models were evaluated using Akaike's Information Criterion for small sample sizes ( $AIC_c$ ). The number of parameters in the model (including the intercept) is indicated in column K,  $\Delta AIC_c = AIC_c - \text{minimum}AIC_c$ , and  $w_i$  (model weight) =  $\exp[-\{\Delta AIC_c/2\}]/\sum \exp[-\{\Delta AIC_c/2\}]$ .

	<b>Best Supported Model(s)</b>	<b>K</b>	<b><math>\Delta AIC_c</math></b>	<b><math>w_i</math></b>
<b><u>Growth Rate (K) Mass</u></b>	Planting Age	2	0.00	0.19
	Brood Parasitism	2	0.20	0.17
	Vegetation Structure	2	0.80	0.13
	Temperature	2	1.00	0.11
	Brood Size	2	1.10	0.11
	Management Activity	2	1.20	0.10
	Precipitation	2	1.20	0.10
	Food Resources	2	1.40	0.09
<b><u>Fledge Mass (g)</u></b>	Vegetation Structure	2	0.00	0.48
<b><u>Growth Rate (K) Wing</u></b>	Food Resources	2	0.00	0.60
	Management Activity	2	1.10	0.35
<b><u>Fledge Wing (mm)</u></b>	Brood Parasitism	2	0.00	0.55
<b><u>Growth Rate (K) Tarsus</u></b>	Vegetation Structure	2	0.00	0.42
<b><u>Fledge Tarsus (mm)</u></b>	Planting Age	2	0.00	0.20
	Precipitation	2	0.80	0.13
	Vegetation Structure	2	0.90	0.13
	Brood Size	2	0.90	0.13
	Food Resources	2	1.00	0.12
	Brood Parasitism	2	1.20	0.11
	Management Activity	2	1.50	0.09
	Temperature	2	1.50	0.09

Table 6. Invertebrate biomass (in grams) and diversity (Shannon Diversity Index) sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. P-values are given for ANOVA tests for differences among planting types. Different lettered subscripts following means indicate significant differences among treatment types for pairwise comparisons.

	<b>Cool Season</b>		<b>Warm Season</b>		<b>High Diversity</b>		<i>P</i> -value
	Mean	S.E.	Mean	S.E.	Mean	S.E.	
<b>Invertebrate Diversity</b>	1.762	0.074	1.828	0.074	1.768	0.074	0.791
<b>Total Invertebrate Biomass</b>	0.358	0.052	0.259	0.052	0.219	0.052	0.197
<b>Acari Biomass</b>	<0.001	0.000	<0.001	0.000	<0.001	0.000	0.410
<b>Araneae Biomass</b>	0.022 <sup>a</sup>	0.002	0.014 <sup>b</sup>	0.002	0.011 <sup>b</sup>	0.002	<b>0.011</b>
<b>Coleoptera Biomass</b>	0.072	0.026	0.050	0.026	0.040	0.026	0.679
<b>Diptera Biomass</b>	0.041	0.010	0.048	0.010	0.062	0.010	0.321
<b>Ephemeroptera Biomass</b>	0.001	0.001	0.002	0.001	0.001	0.001	0.619
<b>Hemiptera Biomass</b>	0.089	0.016	0.062	0.016	0.040	0.016	0.148
<b>Hymenoptera Biomass</b>	0.015	0.004	0.027	0.004	0.017	0.004	0.170
<b>Lepidoptera Biomass</b>	0.072	0.029	0.028	0.029	0.014	0.029	0.363
<b>Neuroptera Biomass</b>	0.001	0.000	<0.001	0.000	<0.001	0.000	0.886
<b>Odonata Biomass</b>	0.010	0.004	0.013	0.004	0.009	0.004	0.723
<b>Orthoptera Biomass</b>	0.036	0.007	0.013	0.007	0.021	0.007	0.095

Table 7. Models of invertebrate diversity (Shannon Diversity Index) and biomass (in grams) sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. Models were evaluated using Akaike's Information Criterion for small sample sizes ( $AIC_c$ ). The number of parameters in the model (including the intercept) is indicated in column K,  $\Delta AIC_c = AIC_c - \text{minimum}AIC_c$ , and  $w_i$  (model weight) =  $\exp[-\{\Delta AIC_c/2\}]/\sum \exp[-\{\Delta AIC_c/2\}]$ .

	<b>Best Supported Model(s)</b>	<b>K</b>	<b><math>\Delta AIC_c</math></b>	<b><math>w_i</math></b>
<b>Invertebrate Diversity</b>	Management Activity	2	0.00	0.43
	Vegetation Structure	2	1.00	0.26
<b>Total Invertebrate Biomass</b>	Native Vegetation	3	0.00	0.51
<b>Araneae Biomass</b>	Native Vegetation	3	0.00	0.78
<b>Coleoptera Biomass</b>	Management Activity	2	0.00	0.28
	Vegetation Structure	2	0.80	0.19
	Grass Cover	2	1.20	0.15
	Planting Age	2	1.30	0.15
	Vegetation Diversity	2	1.40	0.14
<b>Diptera Biomass</b>	Planting Age	2	0.00	0.44
	Vegetation Diversity	2	1.40	0.22
	Grass Cover	2	1.90	0.17
<b>Lepidoptera Biomass</b>	Grass Cover	2	0.00	0.36
	Vegetation Diversity	2	0.50	0.28
<b>Odonata Biomass</b>	Vegetation Structure	2	0.00	0.59
<b>Orthoptera Biomass</b>	Vegetation Diversity	2	0.00	0.38
	Grass Cover	2	1.20	0.21

**CHAPTER FIVE: NESTLING CORTICOSTERONE AND BLOOD GLUCOSE  
LEVELS AS A MEASURE OF HABITAT QUALITY IN RESTORED GRASSLANDS**

A paper to be submitted to the *Journal of Wildlife Management*

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**ABSTRACT** Baseline levels of corticosterone in developing birds are good indicators of physiological condition, are associated with food resource availability, and have been used as indicators of habitat quality in birds. The objective of our study was to examine how restored grassland habitat type affects the physiological condition of nestling red-winged blackbirds (*Agelaius phoeniceus*) in grasslands. We collected blood samples from nestling red-winged blackbirds just prior to fledging and we measured whole blood glucose and assayed concentrations of plasma corticosterone. Baseline corticosterone levels were lower in the warm-season planting type than in either cool-season or high diversity plantings. We found no differences in baseline corticosterone levels or blood glucose levels between males and females. We found no relationship between baseline corticosterone levels and body mass or between baseline corticosterone levels and blood glucose for male or female nestlings. Blood glucose levels in male nestlings had a significant positive relationship with nestling mass, but not in female nestlings. Management activity and brood size had positive

relationships with baseline corticosterone, suggesting that more intensive management activity and larger brood sizes were related to increased stress levels. Nestling age and temperature during the nestling period were positively related to blood glucose levels.

**KEY WORDS** baseline corticosterone, grassland birds, food resource availability, red-winged blackbirds

## **INTRODUCTION**

Corticosterone is a steroid hormone that is released by the adrenal complex in response to stress in vertebrate animals (Siegel 1980). Elevated levels of corticosterone in the blood plasma of adult birds produce a variety of responses, both behavioral and physical, which allow individuals to survive the short term conditions that initiated the stress response (Siegel 1980; Sapolsky et al. 2000). However, long-term elevated corticosterone levels in adult individuals may have negative consequences, including reduced physical condition (Harvey et al. 1984) or reproductive success through reduced adult territory defense and food provisioning to young (Silverin 1986; Wingfield and Silverin 1986). In developing birds, the effects of elevated corticosterone levels may also have serious negative consequences. For example, zebra finches (*Taenopigia guttata*) that were exposed to elevated corticosterone levels during their nestling period had reduced growth and poor competitive abilities (Spencer and Verhulst 2007) and poor song quality (Spencer et al. 2003) as compared to control birds.

Baseline corticosterone levels in blood plasma are associated with food resource availability in birds (Kitaysky et al. 1999; Saino et al. 2003; Schoech et al. 2004; Pravosudov

and Kittaysky 2006; Jenni-Eiermann et al. 2008). Increased baseline corticosterone levels in adult and developing birds have been associated with poor feeding conditions (Saino et al. 2003; Pravosudov and Kitaysky 2006; Kempster et al. 2007; Jenni-Eiermann et al. 2008). In addition, plentiful food resource availability has been linked to lower baseline corticosterone levels. For example, experimental food supplementation of adult Florida scrub-jays (*Aphelocoma coerulescens*) resulted in decreased baseline corticosterone levels (Schoech et al. 2004). Further evidence for the relationship between baseline corticosterone levels and feeding conditions comes from a study of western scrub-jays (*Aphelocoma californica*), where experimentally induced food restrictions increased baseline corticosterone levels in nestlings (Pravosudov and Kitaysky 2006).

Baseline levels of corticosterone in developing birds are good indicators of their physiological condition (Pravosudov and Kitaysky 2006) and adult body condition. Muller et al. (2007) found that decreased body condition of adult blue tits (*Parus caeruleus*), was associated with elevated baseline corticosterone levels. The same negative relationship between body mass and baseline corticosterone level has been reported for black-legged kittiwakes (*Rissa tridactyla*) in Alaska (Kitaysky et al. 1999).

Baseline corticosterone levels have also been used as indicators of habitat quality in birds. In a study of American redstarts (*Setophaga ruticilla*), individuals in lower quality habitat had higher baseline corticosterone levels than those in higher quality habitat (Marra and Holberton 1998). Baseline corticosterone levels in black-legged kittiwakes were elevated in sub-optimal habitats when compared to those in higher quality habitats (Kitaysky et al. 1999).



In addition to baseline corticosterone levels in the blood plasma, blood glucose levels may provide an additional indicator of an individual's physiological condition. Blood glucose levels in birds are higher and more variable than in other vertebrates (Braun and Sweazea 2008; Lobban et al. 2010). It is unknown how birds can tolerate these higher and more variable blood glucose levels without experiencing the negative effects, such as tissue damage and death that can occur in other animals (Beuchat and Chong 1998). Blood glucose levels are a reflection of the diet and the recent level of food ingestion by birds (Davey et al. 2002) and blood glucose levels in birds have been shown to be affected by experimental food restrictions (Altan et al. 2005; Kempster 2007).

Habitat loss is one of the primary factors affecting population declines of grassland birds over the last several decades (Herkert 1995; Fletcher and Koford 2003; Herkert et al. 2003). Recovery efforts for grassland bird populations have focused on increasing the amount of grassland habitat in the landscape. However, it is unknown to what extent the ecological functions of these grassland plantings have been restored. Grassland bird food resources in the form of arthropods vary with plant diversity (Jamison et al. 2002; Benson 2003; Leathers 2003; Harveson et al. 2004; Sutter and Ritchison 2005). As a result, the choice of planting mix for a grassland restoration may affect food resource availability for grassland birds. In grassland restoration projects, plantings with higher plant species diversity are generally considered to be higher quality habitat than plantings of lower plant species diversity (McCoy et al. 2001).

Red-winged blackbirds (*Agelaius phoeniceus*) are one of the most common bird species in North America (Beletsky 1996). They nest in a variety of habitat types including marshes and uplands (Beletsky 1996; Swain et al. 2003; Sparling et al. 2007). Red-winged

blackbirds regularly forage for invertebrate food in upland habitats during the breeding season (Orians 1980). Because of their adaptability to different habitats, they provided an excellent opportunity to evaluate differences in baseline corticosterone and blood glucose levels among different habitat types. The objective of our study was to compare the physiological condition of nestling red-winged blackbirds among restored grassland habitat types. We also wanted to examine if habitat characteristics such as vegetation structure and food resource availability were related to nestling baseline corticosterone and blood glucose levels in restored grasslands.

## **METHODS**

### **Study Area**

The Spring Run Wetland Complex is a mix of more than 1600 ha of wetlands and reconstructed grasslands located in Dickinson County in northwest Iowa, USA. This area is managed by the Iowa Department of Natural Resources and is one of the largest prairie pothole remnants in the state.

We selected three planting types for our study to encompass the range of planting mixtures typically available to land managers. The habitat types we selected are (1) cool-season - plantings of non-native, cool-season grasses (e.g. smooth brome (*Bromus inermis*), timothy (*Phleum pratense*), reed canary grass (*Phalaris arundinacea*), and Kentucky bluegrass (*Poa pratensis*)) planted >10 years ago, (2) warm-season - a five species mix of native warm-season grasses (e.g. switch grass (*Panicum virgatum*), Indian grass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and side-oats grama (*Bouteloua curtipendula*)) planted >10 years ago and (3)

high diversity - a mixture of over 40 species of native grasses and forbs planted between 2005 and 2007. Within the study area, reconstructed fields were selected and/or planted in a block design, with each of the field types occurring in each block (Fig. 1). We will survey a total of 6 complete blocks.

### **Nest Monitoring and Blood Collection**

We located red-winged blackbird nests in upland vegetation from May through August in 2008-2009 using systematic searches and behavioral observations. Systematic nest searches consisted of observers walking systematically through the fields watching for birds to flush from nests. Once a bird flushed, we carefully searched through the vegetation near the flush location to find the nest. To aid in the relocation of nests, we recorded the Universal Transverse Mercator coordinates of nests using a Global Positioning System handheld unit. In addition, we tied flagging tape to a piece of vegetation 5m directly north and south of the nest location. At the time of nest location, we recorded information about the status of the nest including nest contents (number of eggs and/or nestlings, evidence of brood parasitism), nest condition, and ultimately the nest fate. We monitored nests approximately every 3 days.

We collected all blood samples in accordance with the Ornithological Council's Guidelines to the Use of Wild Birds in Research (Gaunt et al. 1999) and Iowa State University's Institutional Animal Care and Use Committee (permit # 8-06-6203-A). Starting on the first day after hatching; we individually marked nestlings by dyeing the down on the top of their heads with a non-toxic felt tipped pen. Once the nestlings were large enough, we banded them with a unique combination of color and numbered aluminum bands. We collected blood from nestlings just prior to fledging (8-10 days) using a 27 gauge needle. We

collected all blood samples just after sunrise within 3 minutes of approaching the nest. We collected blood into heparinized capillary tubes, with a maximum of 2 capillary tubes collected for each bird. Immediately after collection, we took blood glucose readings in the field using a portable blood glucose meter. After collection, we transferred the samples from the capillary tubes into labeled micro-centrifuge tubes and placed them into a cooler. Within 2 hours after collection, we centrifuged the samples and collected the plasma using a Hamilton syringe.

### **Baseline Corticosterone Assay**

We assayed concentrations of plasma corticosterone with a double antibody I<sup>125</sup> radioimmunoassay kit (MP Biomedical, Orangeburg, NY, Catalog #07-120103). Although the kit was developed for measuring corticosterone levels in rats and mice, Washburn et al. (2002) validated this kit for use in measuring the plasma concentrations of corticosterone in birds. Following the protocols of Parsons (2009), plasma samples were diluted to 1:40 using the steroid diluent provided in the kit and all samples were analyzed in triplicate. In addition to the standards provided in the kit (25 ng/ml, 50 ng/ml, 100 ng/ml, 250 ng/ml, 500 ng/ml, and 1000 ng/ml), we diluted the 25 ng/ml standard with the steroid diluent to produce a 6 ng/ml standard and 12.5 ng/ml standard. We included control samples (provided with the kit) in every set of samples processed in the assay.

### **DNA Sexing**

We determined the sex of each nestling that reached fledgling age with DNA sexing. We sent blood samples on Pemacode sample cards to Animal Genetics, Inc., Tallahassee, FL for processing. Samples were assayed using Polymerase chain reaction (PCR) assays to amplify the DNA in each sample which contains the different sized W and Z bird sex

chromosomes. This PCR-based method has been used to sex many different species of birds in many different life stages (Santamaria 2010).

### **Habitat Characteristics – Vegetation Surveys**

We surveyed upland vegetation at 25m intervals along randomly located transects in each field twice each year in 2008 and 2009. The two vegetation surveys coincided with peak height of cool-season and warm-season grasses. The shape and size of the fields determined the number of transects and therefore the number of vegetation survey locations ranged from 24-30 survey locations. At each survey point along the vegetation transect, we estimated the percent cover of warm-season native grasses, cool-season native grasses, warm-season exotic grasses, cool-season exotic grasses, native forbs, exotic forbs, standing dead vegetation, woody vegetation, bare ground, and litter in 0.5m x 0.5m Daubenmire frames (Daubenmire 1959). We measured visual obstruction using a Robel pole in each cardinal direction at each survey location (Robel et al. 1970).

We recorded the management activity that occurred in each field during each nesting season. Fields in our study either had no management (0) or they were managed with mowing (2) or prescribed fire (3). We also recorded the planting age of each field at the start of each nesting season. Fields that were planted more than 10 years ago were listed as 10 years.

### **Weather Data**

We obtained daily weather data from the nearest National Oceanic and Atmospheric Administration's National Climatic Data Center weather station (number 14972). For each nestling in our dataset, we compiled the daily precipitation and temperature data for the 10

days corresponding to the days in the nest. We used the average daily temperature and total precipitation for each 10 day period.

### **Food Resource Availability - Invertebrate Biomass**

We sampled invertebrates using twelve inch diameter sweep nets in each field on 6 randomly selected 25m long sections of the vegetation transects described above. We conducted three rounds of invertebrate surveys, one in mid May, one in mid June and one in mid July in 2008 and 2009. These sampling periods coincided with important times for food resource availability for grassland birds. We conducted sweep net samples only on warm, sunny days between 1000 and 1800 hours. In order to avoid trampling the vegetation on the transects, sweep net sampling was off-set 5m to the left or right of the transect. During sweep net sampling, one observer walked at a pace of one sweep per meter, sweeping the vegetation within one meter of the ground. We placed invertebrate samples in 3.8 L sized zip-top bags at the completion of each survey. Immediately following sampling, invertebrate samples were sorted from vegetation debris using insect self-sorting tubes. Invertebrate samples remained in the tubes for 24 hours. During the 24 hour sorting time, invertebrates were drawn to the ends of the tubes and carried down a funnel into labeled whirl-pak sample bags filled with 70% ethyl alcohol for preservation. Invertebrate samples were identified to Order, counted, dried, and weighed to obtain estimates of biomass.

### **Data Analysis**

We tested for differences in baseline corticosterone and blood glucose levels among the different planting types using Analysis of Variance (ANOVA) with PROC MIXED in SAS (SAS Institute, Cary, NC, USA). We included Nest in a RANDOM statement to account for the non-independence of nestlings within a nest. We included Block, Year, Sex,

and Planting Type in the model statement. We conducted post-hoc pairwise comparisons among planting types using Tukey-Kramer adjustments for multiple testing. In addition, we wanted to examine the relationships between body mass, baseline corticosterone, and blood glucose levels. Because red-wing blackbird nestling mass at fledging is sexually dimorphic, with males having greater mass than females (Vogel 2011) we evaluated these relationships separately for males and females using PROC MIXED in SAS. Again, we included Nest in a RANDOM statement to account for the non-independence of nestlings within a nest.

We examined the effects of habitat, weather, and nest characteristics on baseline corticosterone blood glucose levels using a model selection procedure. We developed a set of covariates to compare models of factors affecting growth rates in nestling Red-winged Blackbirds (Table 1). We used Akaike's Information Criterion (AIC) to evaluate the support for each model and determine which models were best supported ( $\Delta AIC < 2$ ) by the data (Burnham and Anderson 2002). We report beta values where the 95% confidence interval does not include zero for covariates in the best supported models ( $\Delta AIC < 2$ ).

## RESULTS

We collected blood samples from 86 Red-winged Blackbird nestlings from 35 nests. There were 38 females and 48 males. Brood size ranged from 1 to 4, with an average brood size of 2.8 nestlings. Only 3 out of the 35 nests (9%) were parasitized by brown-headed cowbirds (*Molothrus ater*).

Baseline corticosterone levels were lower in the warm-season planting type than in either cool-season or high diversity plantings (Table 2). We found no evidence of differences in baseline corticosterone levels between males and females ( $F_{1,41}=0.88$ ,

$P=0.354$ ). In addition, baseline corticosterone levels did not differ between years ( $F_{1,41}=0.06$ ,  $P=0.807$ ). We found no relationship between baseline corticosterone levels and body mass or between baseline corticosterone levels and blood glucose for male or female nestling red-winged blackbirds (Figs. 2 and 4).

We found no evidence of differences in nestling blood glucose levels among the three planting types (Table 2). Additionally, we found no evidence of differences in blood glucose levels between males and females ( $F_{1,46}=1.49$ ,  $P=0.229$ ). However, we did find differences in blood glucose levels between years, with 2008 having higher levels than 2009 ( $F_{1,46}=7.14$ ,  $P=0.010$ ). Blood glucose levels in male nestlings had a significant positive relationship with nestling mass, but not in female nestlings (Figure 3).

Red-winged blackbird nestling baseline corticosterone levels were most associated with different management activity in our models (Table 3). We found strong evidence that management activity had a positive relationship with baseline corticosterone ( $\beta_{\text{management}}=1.208$ , 95% CI 0.220, 2.196), suggesting that management activity was related to increased stress levels (Fig. 5). We also found that brood size had positive relationship with nestling baseline corticosterone levels ( $\beta_{\text{brood size}}=1.695$ , 95% CI 0.181, 3.208), suggesting that larger brood sizes were related to increased stress levels (Fig. 6). Finally, we found that planting age was negatively related to baseline corticosterone levels ( $\beta_{\text{planting age}}=-0.338$ , 95% CI -0.662, -0.014), suggesting that more recently planted fields were associated with higher stress levels (Fig. 7).

Red-winged blackbird nestling blood glucose levels were most affected by nestling age at the time of blood collection in our models (Table 4). In fact, we found strong evidence that nestling age was positively related to blood glucose levels ( $\beta_{\text{nestling age}}=14.542$ , 95% CI



5.845, 23.238), suggesting that older nestlings had higher blood glucose readings (Fig. 8).

We also found evidence that temperature during the nestling period was positively related to blood glucose levels ( $\beta_{\text{temperature}} = 2.469$ , 95% CI 0.554, 4.384; Fig. 9).

## DISCUSSION

Nestling baseline corticosterone was lower in warm-season plantings than in either of the other two planting types. Both planting age and management activity of the high diversity fields (recently planted, managed) versus the warm-season fields (planted >10 years ago, no management) may have been factors in the differences we observed (Fig. 10).

Within the high diversity fields, only fields that were at least 4 years old were managed with prescribed fire. Because newly planted fields require time to accumulate litter, fields were not burned until they were at least 4 years old. In addition, newly planted high diversity fields were mowed to control weeds during year one. Because of increased management activity in the early years of restoration plantings, the effects of planting age and management activity on nestling baseline corticosterone levels may be linked. As a result, the differences we observed in nestling baseline corticosterone levels between high diversity and warm-season fields may be reduced or even reversed as the high diversity fields mature.

We found that larger brood sizes were related to increased stress levels in nestling Red-winged blackbirds. A similar relationship between brood size and nestling stress levels was reported in barn swallows (*Hirundo rustica*) in northern Italy (Saino et al. 2003). Larger brood sizes may induce stress in nestlings by reducing the amount of food available per nestling (Saino et al. 1997). Large brood sizes may also be associated with reduced nestling size (Parsons 2009) and increased ectoparasite loads (Saino et al. 2002). In contrast, no

relationship between brood size and baseline corticosterone was found in nestling tree swallows (*Tachycineta bicolor*) in central Iowa (Parsons 2009). We did not find a relationship between brood parasitism and either baseline corticosterone or blood glucose levels, however, only 7% of nests in our study were parasitized and this may not have been enough to allow us to detect an effect of brood parasitism.

Surprisingly, we found no apparent effect of food resource availability on red-winged blackbird nestling baseline corticosterone levels; this is different from what others have reported. In adult barn swallows, corticosterone levels increased with reduced aerial insect food availability (Jenni-Eirmann 2008). The same effects of food resource availability on corticosterone levels appear to occur with food supplementation. For example, Florida scrub-jays provided with supplemental food had lower baseline corticosterone levels when compared with birds that did not receive food supplementation (Schoech et al. 2004). Our findings were likely due to two factors 1) there were no differences in overall food resource availability (invertebrate biomass) among the planting types we studied (Vogel 2011) and 2) because our study fields are relatively close together geographically, adults may have the opportunity to forage in other fields beyond where their nests are located. In fact, previous work has suggested deficits in food resource availability may be compensated for by increased foraging time and/or foraging distance in adults (Adams et al. 1994; Tremblay et al. 2005; Zalik and Strong 2008).

While others have reported a negative relationship between body mass and baseline corticosterone for birds (Marra and Holberton 1998; Kitaysky et al. 1999; Pravosudov and Kitaysky 2006; Muller et al. 2007), we did not find this relationship in our study. Similarly, while a relationship between corticosterone and blood glucose levels has been reported by

others (Remage-Healy and Romero 2000, 2002), we found no relationship between baseline corticosterone and blood glucose for either male or female red-winged blackbird nestlings. The differences between our results and those reported by others could be because we sampled nestlings rather than sampling adult birds.

We found no differences in blood glucose levels between male and female nestling red-winged blackbirds. Similarly, others have found that blood glucose levels do not differ between the sexes. For example, no differences in blood glucose were found between males and females in captive adult European starlings (*Sturnus vulgaris*) and in adult short-tailed shearwaters (*Puffinus tenuirostris*) (Remage-Healy and Romero 2000; Davey et al. 2002).

There is evidence that the relationship between blood glucose levels and body mass among bird species is negative, meaning that bird species with larger body masses tend to have lower blood glucose levels (Braun and Sweasea 2008). However, within a species, the relationship between blood glucose levels and body mass is less clear. In our study, we found a relationship between blood glucose levels and body mass, but only for male nestlings. Others have found inconsistencies in the body mass/blood glucose relationship as well. For example, Davey et al. (2002) found that for adults, blood glucose levels were not related to body mass for Short-tailed Shearwaters. However, when they examined both adults and immature birds together, they found that blood glucose did vary as a function of body mass. Additionally, in a study of four bird species in southeast Australia, in only two of the four species was there an association between blood glucose levels and adult body mass (Lill 2011).

Red-winged blackbird nestling blood glucose levels appeared to increase with nestling age in our study. In fact, two other Australian bird species, welcome swallows

(*Hirundo neoxena*) and spotted doves (*Streptopelia chinensis*), had nestling blood glucose levels increased with nestling age (Lill 2011). A possible explanation offered for this trend was increased metabolic rates as nestlings grew larger (gained mass) and were more active in the nest (Lill 2011).

Higher temperatures during the nestling period were associated with higher blood glucose levels in our study. Reductions in ambient temperature have been shown to reduce the availability of insect food resources for birds (Jenni-Eiermann et al. 2008). Warmer temperatures may increase invertebrate activity and therefore lead to increased food availability. However, we did not find an effect of food resource availability on blood glucose levels in our study. In spite of this, invertebrates may be more active during warmer temperatures making them more available to foraging birds.

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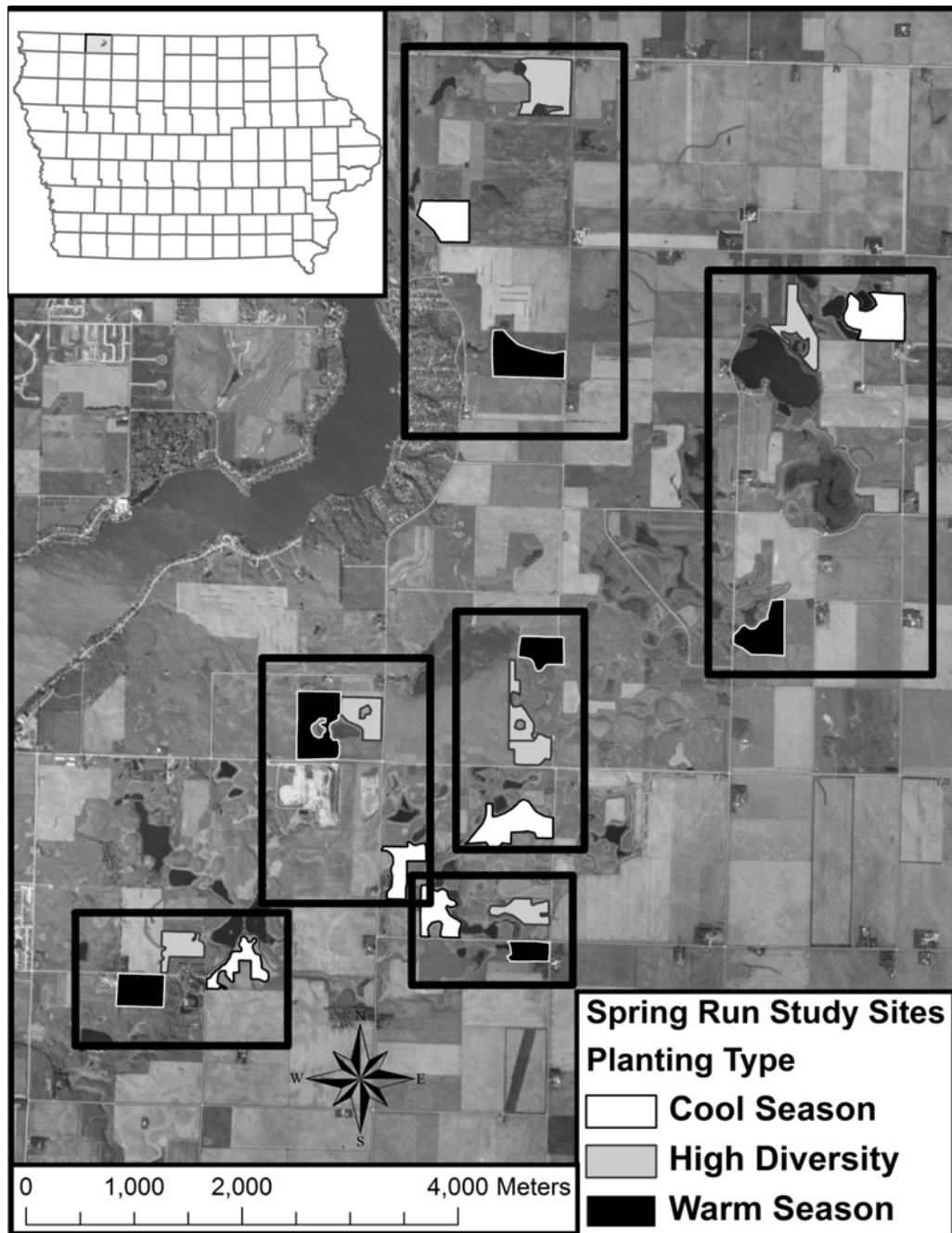


Figure 1. Study sites were located in the Spring Run Complex in Dickinson County, Iowa, USA in 2008, and 2009. Solid white polygons indicate sites that were planted with cool-season grasses  $\geq 10$  years ago, solid black polygons indicate sites that were planted to warm-season grasses  $\geq 10$  years ago, and solid light grey polygons indicate sites that were planted with a high diversity seed mix between 2005 and 2007. Black rectangles indicate study site blocking.

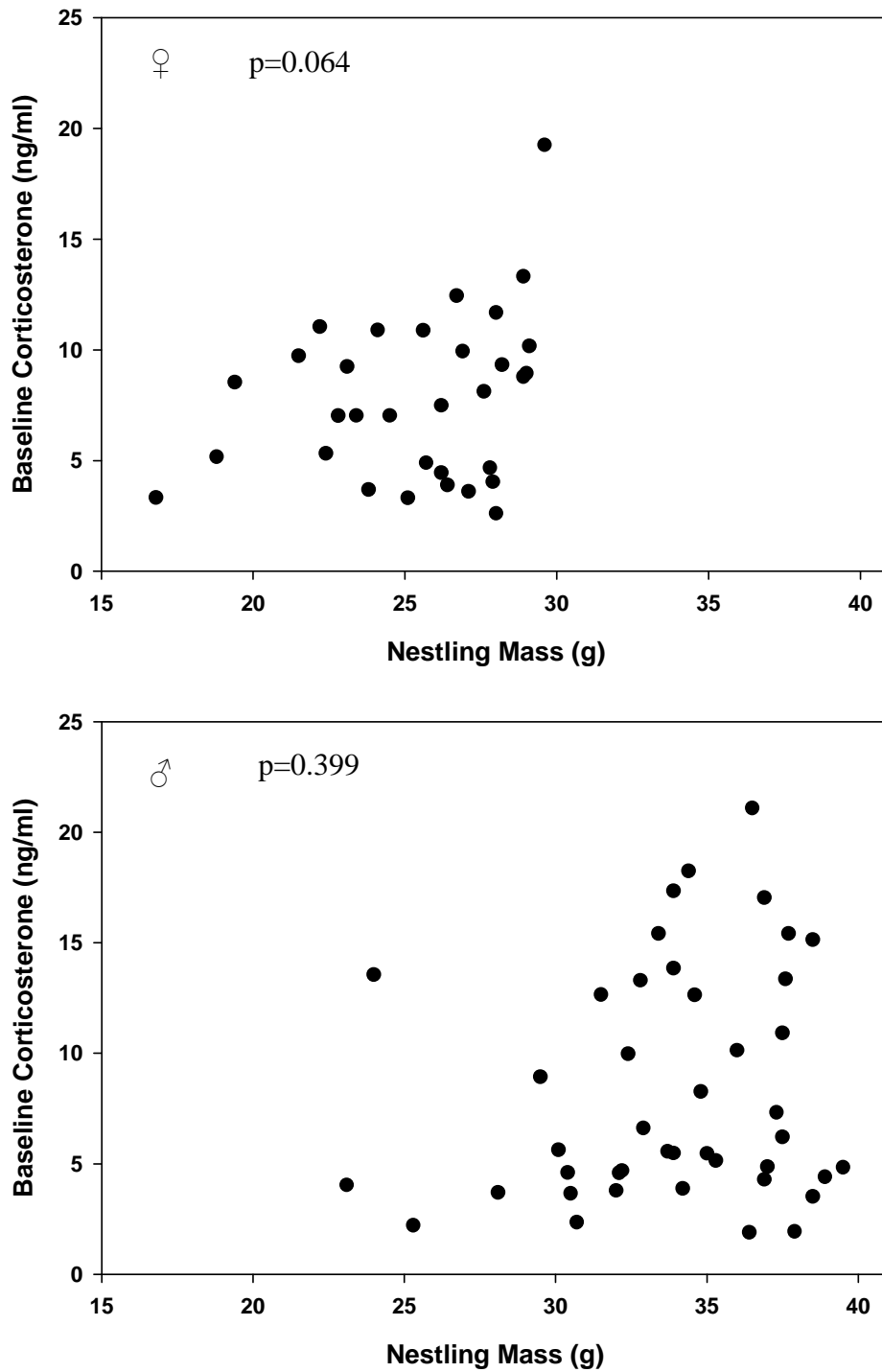


Figure 2. Relationship between baseline corticosterone and nestling mass for female (top) and for male (bottom) red-winged blackbird nestlings sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

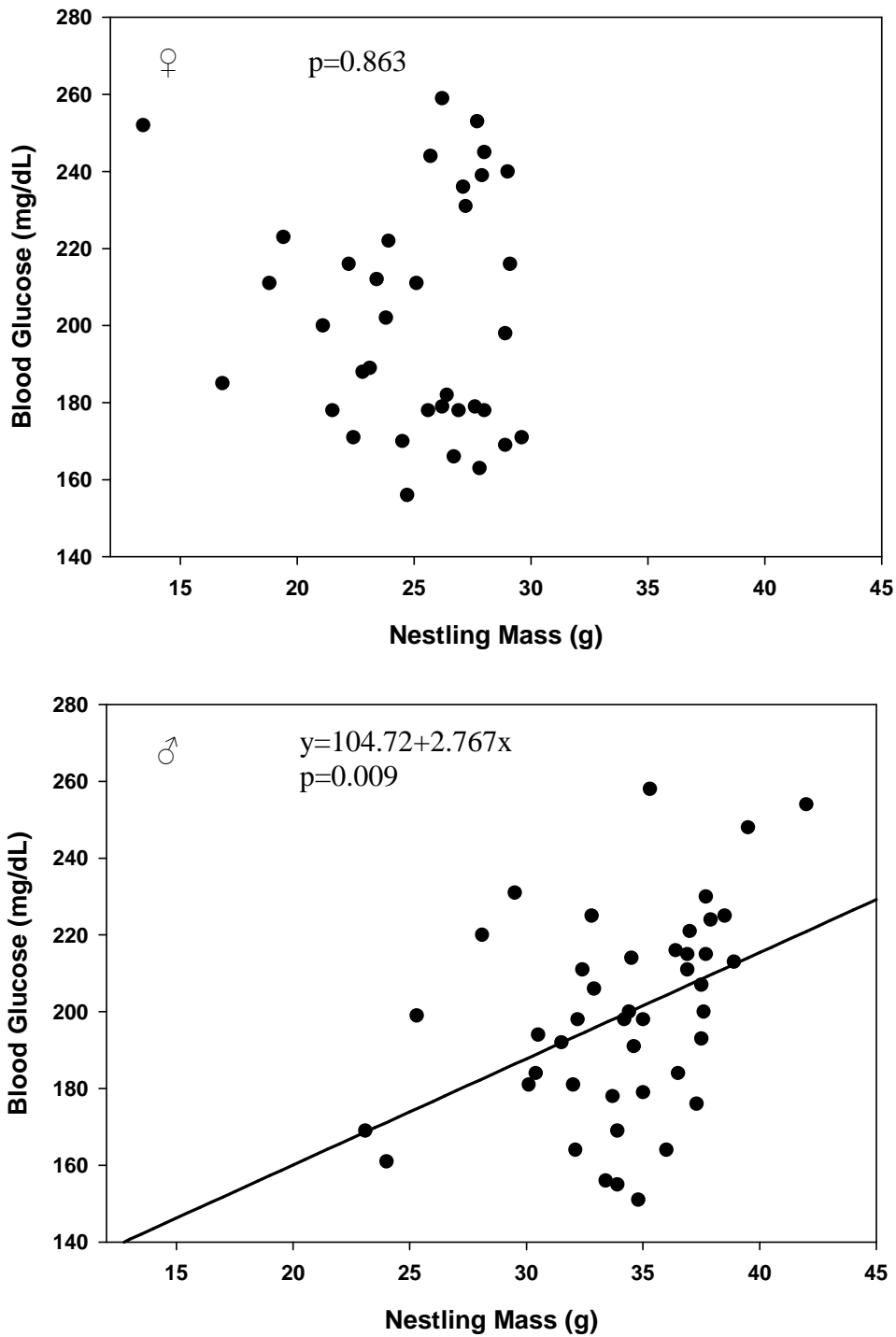


Figure 3. Relationship between blood glucose and nestling mass for female (top) and for male (bottom) red-winged blackbird nestlings sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

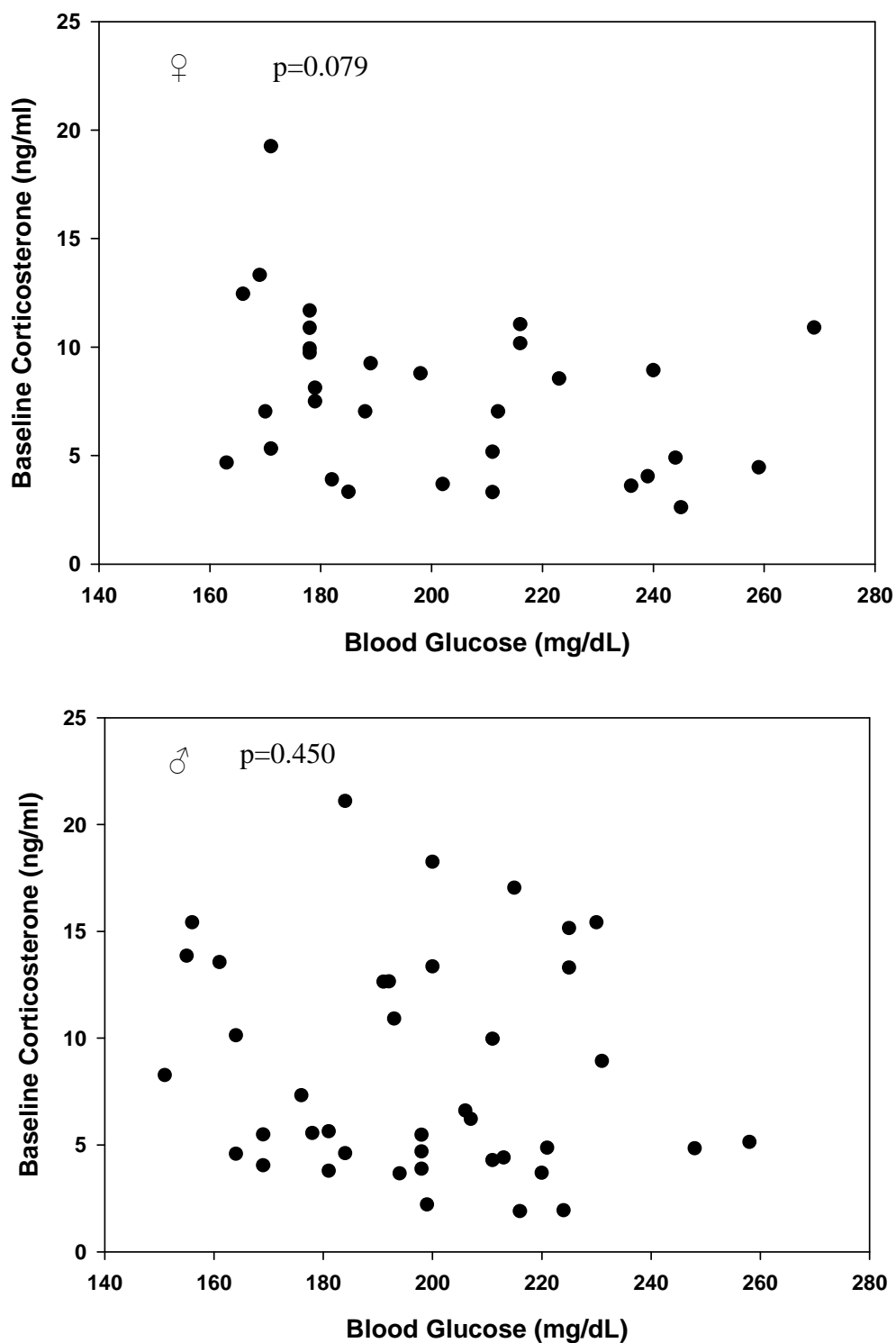


Figure 4. Relationship between baseline corticosterone and blood glucose for females(top) and for male (bottom) red-winged blackbird nestlings sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

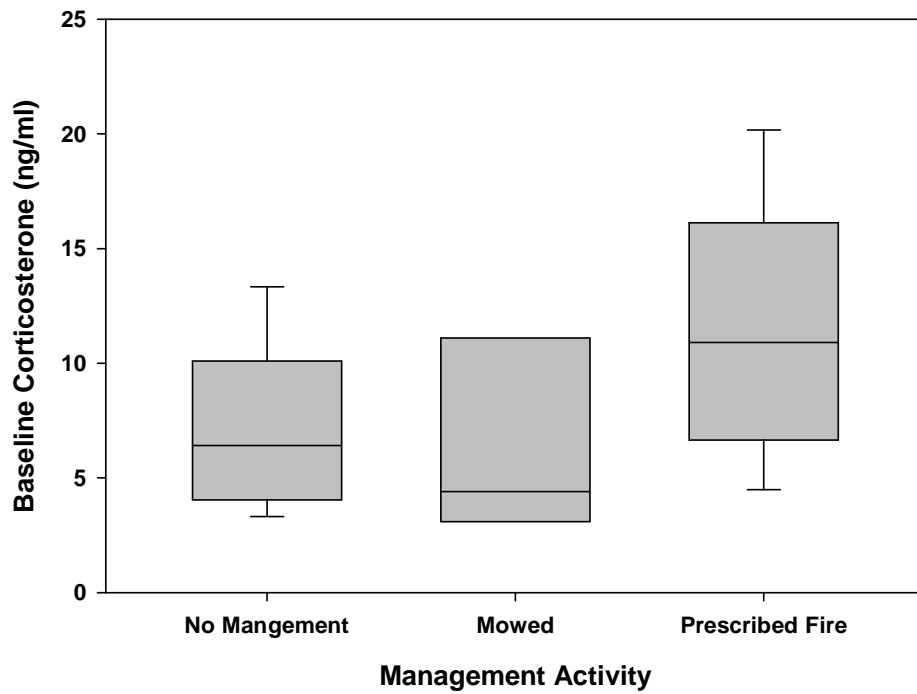


Figure 5. Relationship between baseline corticosterone and management activity for red-winged blackbird nestlings sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

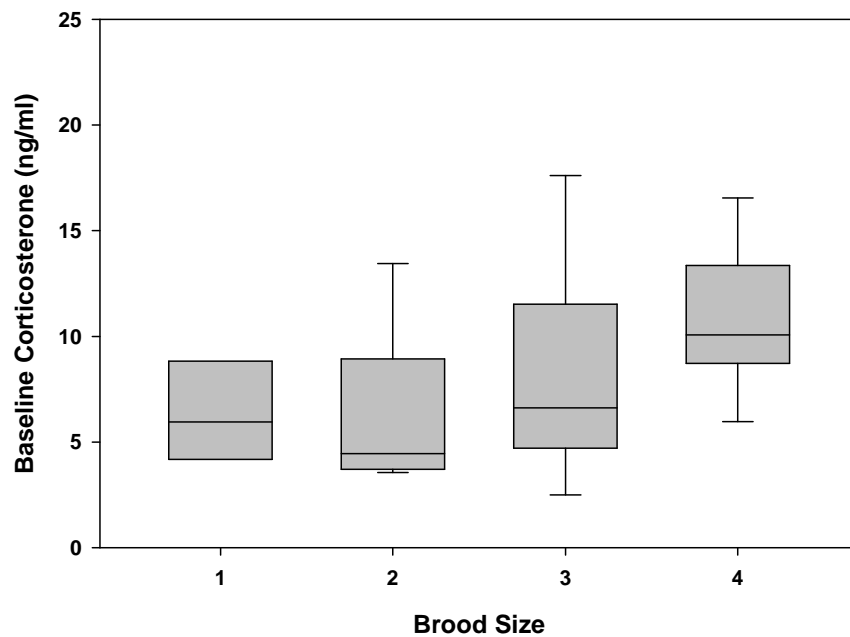


Figure 6. Relationship between baseline corticosterone and brood size for red-winged blackbird nestlings sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.



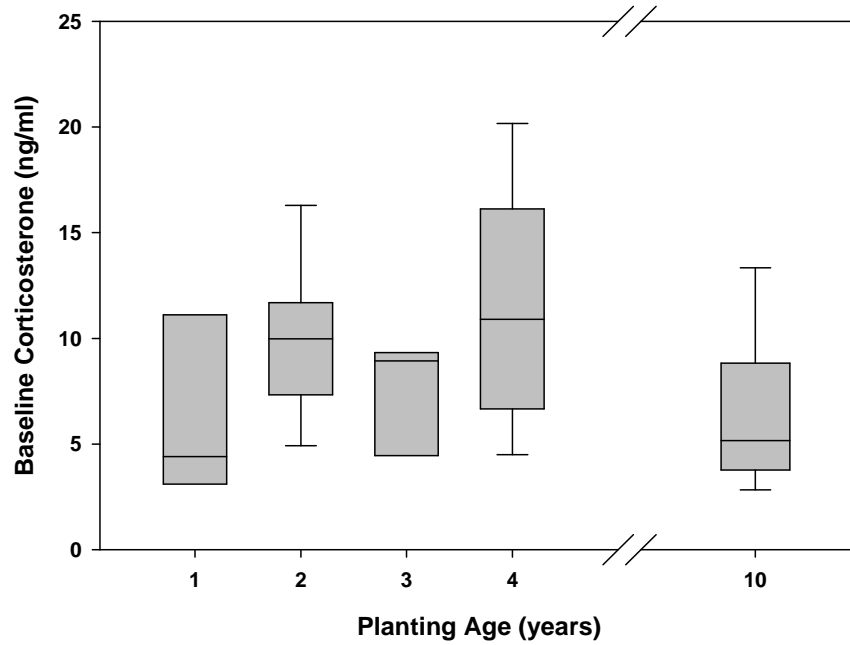


Figure 7. Relationship between baseline corticosterone and planting age for red-winged blackbird nestlings sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

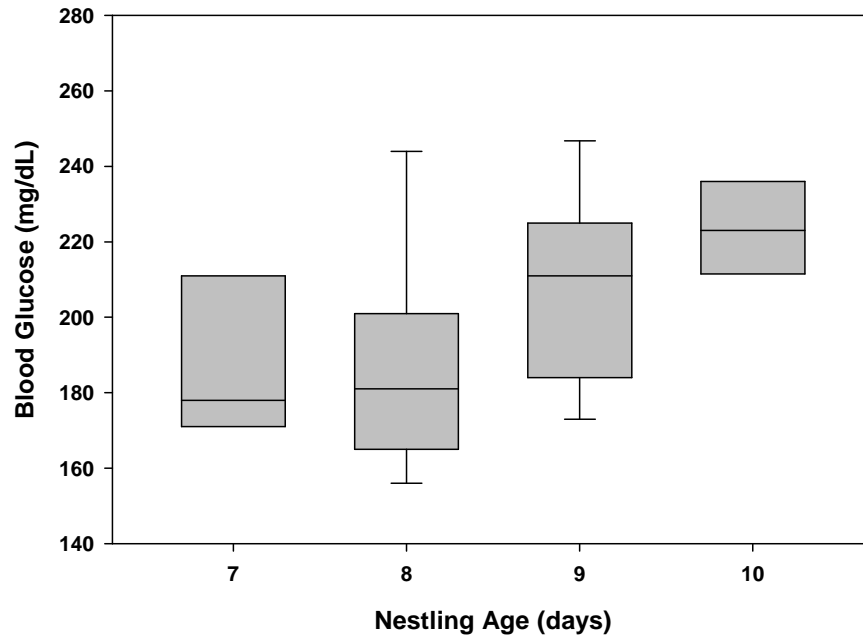


Figure 8. Relationship between blood glucose and nestling age for red-winged blackbird nestlings sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

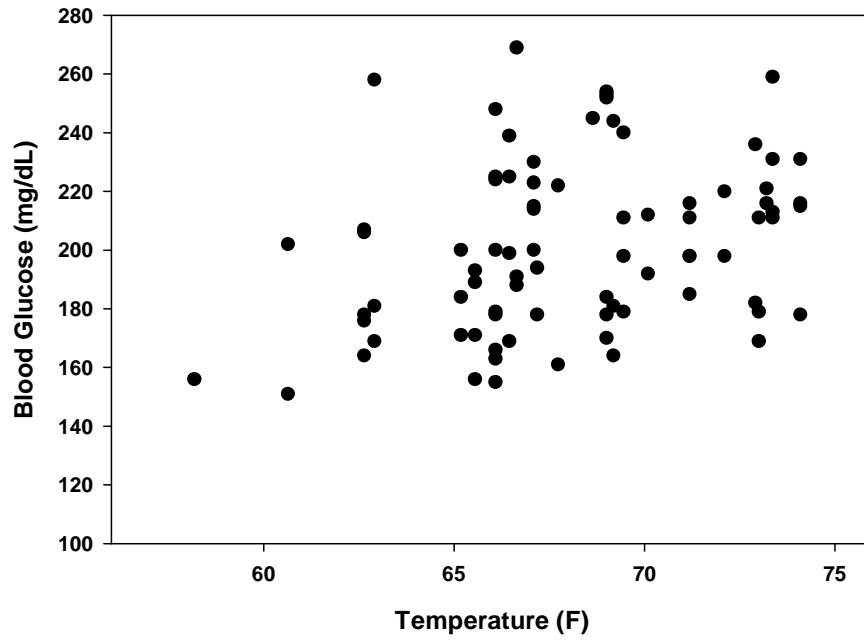


Figure 9. Relationship between blood glucose and temperature for red-winged blackbird nestlings sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA.

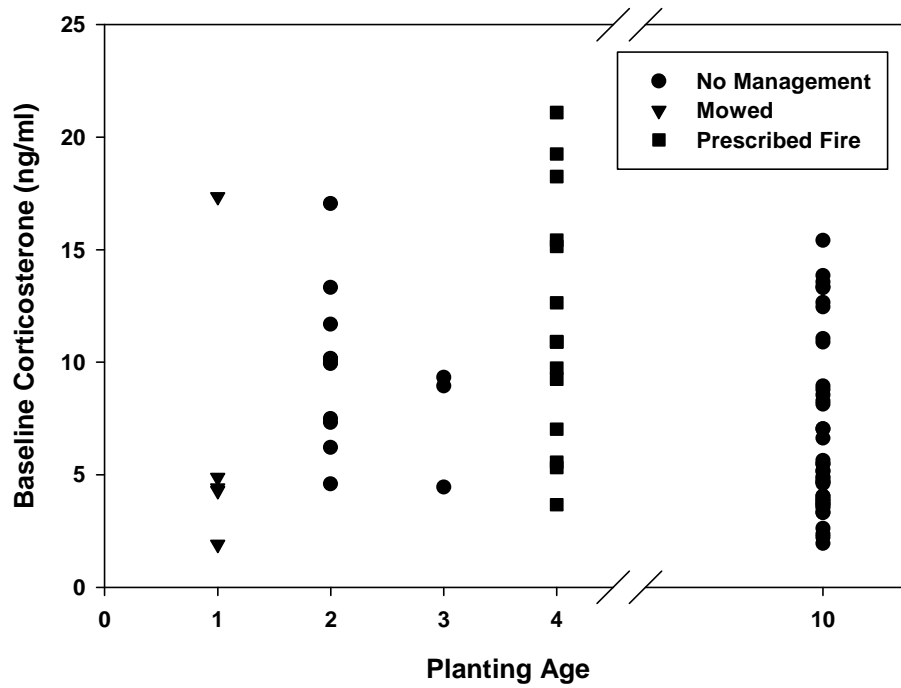


Figure 10. Relationship between baseline corticosterone and planting age (with management activity) for red-winged blackbird nestlings sampled in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. Management activity is indicated by the shape of the symbol, circles indicate no management, triangles indicate mowing, and squares indicate prescribed fire.

Table 1. Mean and standard deviation of habitat, weather, and nest covariates included in models of baseline corticosterone and blood glucose levels for red-winged blackbird nestlings in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. The covariates included were vegetation structure (visual obstruction in dm), Food Resources (invertebrate biomass in grams), Management Activity (0=none, 2=complete mow, 3=prescribed fire), planting age (in years – plantings  $\geq 10$  years were entered as 10 years), brood size (the number of nestlings in a nest), temperature (average temperature during the 10 day nestling period), precipitation (total precipitation during the 10 day nestling period), brood parasitism (presence of a brown-headed cowbird egg or nestling in a nest), and nestling age (age of nestling at the time of blood collection).

<b>Covariates</b>	<b>Mean</b>	<b>Std. Dev.</b>	<b>Min.</b>	<b>Max.</b>
Vegetation Structure (dm)	3.81	1.14	0.80	6.98
Food Resources (g)	1.82	0.16	1.31	2.00
Management Activity	0.71	1.20	0.00	3.00
Planting Age (years)	6.70	3.72	1.00	10.00
Brood Size	2.80	0.79	1.00	4.00
Temperature (degrees F)	68.18	3.74	58.18	74.09
Precipitation (cm)	0.15	0.14	0.00	0.40
Brood Parasitism	9%	-	-	-
Nestling Age (days)	8.62	0.70	7.00	10.00

Table 2. Red-winged blackbird nestling baseline corticosterone (ng/ml) and blood glucose levels (mg/dL) measured in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. P-values are given for ANOVA tests for differences among planting types. Different lettered subscripts following means indicate significant differences among treatment types for pairwise comparisons.

	<b>Cool Season</b>		<b>Warm Season</b>		<b>High Diversity</b>		<i>P</i> -value
	Mean	S.E.	Mean	S.E.	Mean	S.E.	
<b>Baseline Corticosterone</b>	10.14 <sup>a</sup>	1.73	3.37 <sup>b</sup>	1.50	10.47 <sup>a</sup>	1.24	<b>0.004</b>
<b>Blood Glucose</b>	207.73	11.30	214.75	9.53	211.39	7.03	0.915

Table 3. Models of red-winged blackbird baseline corticosterone levels taken in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. Models were evaluated using Akaike's Information Criterion for small sample sizes ( $AIC_c$ ). The number of parameters in the model (including the intercept) is indicated in column **K**,  $\Delta AIC_c = AIC_c - \text{minimum}AIC_c$ , and  $w_i$  (model weight) =  $\exp[-\{\Delta AIC_c/2\}]/\sum \exp[-\{\Delta AIC_c/2\}]$ .

<b>Model</b>	<b>K</b>	<b><math>\Delta AIC_c</math></b>	<b><math>w_i</math></b>
Management Activity	2	0.00	0.37
Brood Size	2	0.80	0.25
Planting Age	2	1.50	0.18
Precipitation	2	4.00	0.05
Brood Parasitism	2	4.60	0.04
Nestling Age	2	4.70	0.04
Temperature	2	5.20	0.03
Vegetation Structure	2	5.40	0.03
Food Resources	2	5.40	0.03

Table 4. Models of red-winged blackbird blood glucose levels taken in 2008 and 2009 on the Spring Run Complex in Dickinson County, Iowa, USA. Models were evaluated using Akaike's Information Criterion for small sample sizes ( $AIC_c$ ). The number of parameters in the model (including the intercept) is indicated in column K,  $\Delta AIC_c = AIC_c - \text{minimum}AIC_c$ , and  $w_i$  (model weight) =  $\exp[-\{\Delta AIC_c/2\}]/\sum \exp[-\{\Delta AIC_c/2\}]$ .

<b>Model</b>	<b>K</b>	<b><math>\Delta AIC_c</math></b>	<b><math>w_i</math></b>
Nestling Age	2	0.00	0.83
Temperature	2	3.90	0.12
Precipitation	2	8.30	0.01
Vegetation Structure	2	9.70	0.01
Food Resources	2	9.90	0.01
Brood Size	2	9.90	0.01
Brood Parasitism	2	10.00	0.01
Management Activity	2	10.20	0.01
Planting Age	2	10.20	0.01



**CHAPTER SIX: ASSESSING THE ROLE OF CONSPECIFIC ATTRACTION IN  
HABITAT RESTORATION FOR HENSLOW'S SPARROWS IN IOWA**

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**ABSTRACT** The presence of conspecific individuals may provide important cues about habitat quality for territorial songbirds. We tested the ability of a conspecific song playback system to attract Henslow's sparrows to previously unoccupied restored habitat. We successfully attracted Henslow's sparrows to three out of seven treatment plots using conspecific song playbacks and we found no Henslow's sparrows in control plots. The addition of social cues using playback systems in restored grassland habitats may aid conservation efforts of Henslow's sparrows to available habitat.

**KEYWORDS** *Ammodramus henslowii*, conspecific attraction, Henslow's sparrow, Iowa.

## INTRODUCTION

Many grassland-bird populations have declined over the past several decades (Knopf 1994, Herkert 1995). The primary factor thought to be associated with declining grassland-bird populations is habitat fragmentation and destruction (Herkert 1995, Fletcher and Koford 2003, Herkert et al. 2003). The tallgrass-prairie region of North America is one of the most endangered ecosystems on Earth (Smith 1981; Noss et al. 1995) and in Iowa, less than 0.01% of the original 12 million hectares of prairie remains (Sampson and Knopf 1994). Loss of habitat over the past century restricted grassland-dependent species to small isolated remnants.

Recent habitat restoration efforts focused on mitigating external environmental threats alone, such as habitat destruction, may not be enough to conserve imperiled songbird species (Ward and Schlossberg 2004, Ahlering and Faaborg 2006). Animal behavior has recently been recognized as playing an important role in species conservation (Ward and Schlossberg 2004, Ahlering and Faaborg 2006). Social information and conspecific attraction may be important for many species. In fact, a recent review found that in 20 out of 24 studies examining conspecific attraction in songbirds, birds were successfully attracted using social cue manipulation (Ahlering et al. 2010).

In territorial songbirds, the presence of conspecific individuals may provide important cues about habitat use. For some bird species, research has demonstrated that settlement decisions are likely influenced by the presence of conspecifics (e.g. Danchin et al. 1998, Ward and Schlossberg 2004, Fletcher 2007). Most of these studies have focused on forest species (e.g. Ward and Schlossberg 2004, Fletcher 2007) or colonial nesting species (e.g. Danchin et al. 1998). Past research on the effects of conspecific attraction in grassland

species has focused on the establishment of new lek sites for re-introduced or translocated gallinaceous birds (Rodgers 1992). More recently, however, the role of conspecific attraction in the settlement decisions of grassland songbird species has been explored (Ahlering et al. 2006, Nocera et al. 2006). For example, successful attraction of Baird's sparrows (*Ammodramus bairdii*) by use of song playbacks in previously unoccupied sites has been demonstrated (Ahlering et al. 2006).

The Henslow's sparrow (*Ammodramus henslowii*) has been recognized as a species of particular conservation concern by the U.S. Fish and Wildlife Service (U.S. Fish and Wildlife Service 2002) and is listed as threatened in the state of Iowa (Iowa Department of Natural Resources 2005). We were interested in evaluating the efficacy of using social cues to aid in the recovery of Henslow's sparrow populations. Specifically, our objective was to test the ability of a conspecific song playback system to attract Henslow's sparrows to previously unoccupied restored habitat.

## **STUDY AREA**

The Spring Run Wetland Complex is a mix of over 1600 hectares of wetlands and reconstructed grasslands located in Dickinson County in northwest Iowa, USA (Figure 1). The area is managed by the Iowa Department of Natural Resources and is one of the largest prairie pothole complexes in the state. Historically, the region was characterized by a mix of mesic to dry tallgrass prairies. The vegetation community of the area was dominated by several species of grasses such as big bluestem (*Andropogon grardii*), Indian grass (*Sorghastrum nutans*), little bluestem (*Schizachyrium scoparium*), and side-oats grama (*Bouteloua curtipendula*). Forbs included lead plant (*Amorpha canescens*), compass plant

(*Silphium laciniatum*), rattlesnake master (*Eryngium yuccifolium*), pale purple coneflower (*Echinacea pallida*), and gray-headed coneflower (*Ratibida pinnata*) (Thompson 1992, Ladd 1995, Christiansen and Muller 1999). Land use in Iowa is currently 94% agricultural, with corn (*Zea mays*) and soybeans (*Glycine max*) as the primary crop types (Jackson et al. 1996). Iowa's climate consists of warm, humid summers and cold winters. The average annual precipitation of Iowa is approximately 81cm and the average growing season length is 158 days (Iowa Department of Natural Resources 2005). The average annual temperature in Iowa is approximately 9.4° C (Thompson 1992) with an average summer temperature of approximately 22° C (Iowa Department of Natural Resources 2005).

## **METHODS**

Within the Spring Run Complex, we located seven fields with available habitat for Henslow's sparrows (Figure 1). All of the fields contained mature grassland vegetation (age of planting > 10 years). Four of the fields were planted to a cool-season grass mixture of smooth brome (*Bromus inermis*), timothy (*Phleum pratense*), reed canary grass (*Phalaris arundinacea*), and Kentucky bluegrass (*Poa pratensis*), with scattered forbs of Canada thistle (*Cirsium arvense*), common milkweed (*Asclepias syriaca*), and alfalfa (*Medicago sativa*). The other three fields were planted to a warm-season grass mixture of switch grass (*Panicum virgatum*), Indian grass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and side-oats grama (*Bouteloua curtipendula*), with several forb species of Canada thistle (*Cirsium arvense*), common milkweed (*Asclepias syriaca*), and goldenrod (*Solidago* sp.). Recent records of Henslow's sparrows in Iowa are rare, although it was once a common species in the state (Jackson et al. 1996, Melde and

Koford 1996). Habitat for Henslow's sparrows in Iowa consists of fields with moderate vegetation height (45cm-85cm), a small forb component (5% -20%), and dense litter comprised of previous years' growth (Melde and Koford 1996). All of the proposed fields met these criteria. Extensive line transect surveys of the proposed study sites conducted weekly from 4 June to 12 July 2007 revealed that Henslow's sparrows were not present (J.Vogel, unpublished data), however, a single male was heard singing within a few (0.75 to 6.2) kilometers before 2007 (R. Koford, unpublished data).

We divided each of the seven study fields into two plots (plots were equal in size to one-half of the overall size of the field or approximately four hectares). Henslow's sparrows tend to have relatively small territories of less than one hectare (Herkert 1998, O'Leary and Nyberg 2000, Monroe and Ritchison 2005). We randomly assigned one plot on each field to the treatment and the other as a control plot. On the treatment plots, we established a playback station using pre-recorded songs (Elliot et al. 1997) of Henslow's sparrows only. Observations of Henslow's sparrows have indicated that individuals are responsive to song playbacks (Zimmerman 1988, Melde and Koford 1996), making it a good candidate for this experiment.

We constructed playback stations after Ahlering et al. (2006). Each station consisted of a portable compact disc player connected to a programmable (Borg General Controls TA0005) timer (Figure 2). The timers were connected to rechargeable 12 volt batteries and solar panels. We mounted playback stations in aluminum boxes for protection from the elements. Large holes approximately the same size as the speaker diameter were drilled in front of the speakers to allow for sound transmission. The drilled holes were covered by a thin screen (to keep insects, etc. out of the boxes) and the speakers were placed right up

against the openings so that sound transmission was directly from the speakers through the openings. Boxes were mounted to posts at approximately one meter high, the typical perching/singing height for Henslow's sparrows in each field (Hanson 1994). We located each playback station (one playback station per plot) at the far edge of each plot (away from the control plot) and broadcast toward the interior of the experimental plot. Song playbacks could not be heard from the control plots.

In mid-May 2008, we constructed and erected playback stations on each of the seven sites to test their operation and reliability (Figure 1). Playback stations remained on the study sites during the equipment test period until the beginning of August 2008. We modified the design of the playback stations slightly for the 2009 field season to increase the song volume by using computer software to digitally amplify the songs. In addition, we enlarged the holes to allow for greater sound transmission. We placed playback stations in each of the treatment plots during the first week of April 2009 to coincide with the arrival time of the first Henslow's sparrow individuals (Herkert 1998). Playback stations were programmed to broadcast songs starting one hour before sunrise and ending at 0930 CST and again in the evening just before sunset. Broadcasts were played for one hour at a time, with 30 min intervals in between for a total of four hours in the morning and two hours in the evening. Playbacks were continued through the beginning of August 2009. We checked and maintained the playback stations weekly and parts were replaced as necessary for continuous operation throughout the study period.

We monitored study plots weekly by walking 100m long transects placed throughout each field to record observations of Henslow's sparrows on each plot from 2 June – 18 July 2008 (equipment test period) and from 1 June – 10 July 2009. We chose locations for bird

survey transects to maximize the number of transects in each field. We placed transects only in upland vegetation, and we did not locate transects near field edges or wetlands.

We conducted six rounds of bird surveys in 2008 and 2009. We repeated bird surveys once each week along the same transects within each field during each round of surveys. We conducted bird surveys between sunrise and 10:00am. We did not conduct bird surveys on days where weather conditions could have impeded visibility or audibility (rain, fog, or wind in excess of 30km/hr). Surveys consisted of one observer walking along the transect at a constant pace identifying birds by sight and sound within 35m on either side of the transect. The distance of the bird(s) from the observer and the compass bearing was recorded using laser rangefinder binoculars.

### **Data Analysis**

The recommended minimum sample size is 60-80 individuals for using line transect methods to adjust for imperfect detectability and estimate density (Buckland et al. 1993). Unfortunately, we recorded a total of only 10 Henslow's sparrows during our surveys, and therefore we chose a presence/absence response for our statistical analysis. Using McNemar's Test (McNemar 1947) for paired data, we tested the null hypothesis that the number of control/treatment pairs where birds were present in the treatment but absent in the control was equal to or less than the number of control/treatment pairs where birds were present in the control but absent in the treatment (SAS Version 8.2, SAS Institute, Cary, NC, USA). McNemar's Test is a non-parametric test based on a Chi-square distribution with one degree of freedom (Park 2002). McNemar's Test is used to test for marginal homogeneity in 2x2 contingency tables (McNemar 1947, Park 2002). We used the asymptotic test (Park 2002) because of our small sample size and considered the one-tailed p-value to evaluate the

significance of the test. A 2x2 contingency table containing zeros is problematic because calculations produce an undefined test statistic (Park 2002). To deal with zeros in our contingency table, we added a small constant (0.00001) to each cell containing a zero (Park 2002). Given our small sample size, the resulting low power of the test increases the chance of a Type II error; therefore we chose an alpha level of 0.1 instead of 0.05 to decrease the possibility of a Type II error.

## RESULTS

Henslow's sparrows were not detected in any surveys during the 2008 equipment test period. We successfully attracted Henslow's sparrows to some treatment plots in 2009 using conspecific song playbacks. Henslow's sparrows were more likely to be found in treatment plots than in control plots ( $\chi_1^2=3.0, P=0.08$ ). Specifically, we found Henslow's sparrows in three out of the seven treatment plots during our 2009 surveys and in none of the control plots in 2009. Two of the treatment plots where we found Henslow's sparrows were cool-season grass fields and one was a warm-season grass field (Figure 1). In two fields (one cool-season and one warm-season) we found only males in the treatment plots, but in one field (cool-season) we found both males and females. We did not observe Henslow's sparrows perching on the playback structures at any time during the study.

## DISCUSSION

Although our sample size was small, we successfully attracted Henslow's sparrows to previously unoccupied habitat using conspecific song playbacks. Our results are similar to those reported by Ahlering et al. (2006) for another grassland songbird, the Baird's sparrow,



and by Harrison et al. (2009) for a shrub-steppe obligate, the Brewer's sparrow (*Spizella breweri*). For Baird's sparrows, half of the experimental playback plots (three out of six) in their study were successful in attracting Baird's sparrows, whereas none of the control plots were (Ahlering et al 2006). Similarly, more Brewer's sparrows were attracted to the playbacks plots than the control plots (Harrison et al. 2009). In contrast, an examination of conspecific attraction in the Nelson's sharp-tailed sparrow (*Ammodramus nelsoni*) had opposite results and no evidence of a treatment response to song playbacks was reported (Nocera et al. 2006).

The influence of social cue manipulation may have unintended effects on both target and non-target species (Betts et al. 2008, Fletcher 2008, Betts et al. 2010). For target species, the addition of song playbacks may attract individuals to poor quality habitat (Betts et al. 2008, Fletcher 2008). In fact, it is possible to mislead individuals of some species into settling in poor quality habitat simply by broadcasting their songs in sink areas (Betts et al. 2008). In addition, manipulation of social cues can affect non-target species through both attraction and avoidance of heterospecifics (Fletcher 2008). Avoidance behavior in heterospecifics has been demonstrated as a response to social cue manipulation and in one case, resulted in a reduction of non-target species richness of 30% (Fletcher 2008).

Henslow's sparrows have very specific habitat and nesting requirements with regard to vegetation height, vegetation density, and litter depth (Zimmerman 1988, Herkert 1994, Melde and Koford 1996, Skipper 1998, Cully and Michaels 2000). As a result, Henslow's sparrows may have low site fidelity caused by changing grassland habitat conditions due to regular management activities, such as prescribed burning and mowing (Hands et al. 1989). For managers, this presents a difficult problem of maintaining Henslow's sparrow populations

under constantly changing grassland conditions (Mills et al. 2006). Future studies should include collection of vegetation conditions in association with social behavior.

Social information has been included resource selection models for Bobolinks (*Dolichonyx oryzivorus*) and Savannah sparrows (*Passerculus sandwichensis*) (Nocera and Forbes 2010). For some species, social information can be more influential than habitat cues, such as vegetation structure, in settlement decisions (Betts et al. 2008). Traditional habitat models that do not consider social factors may not be adequate for informing conservation strategies for some species (Harrison et al. 2009), including Henslow's sparrows.

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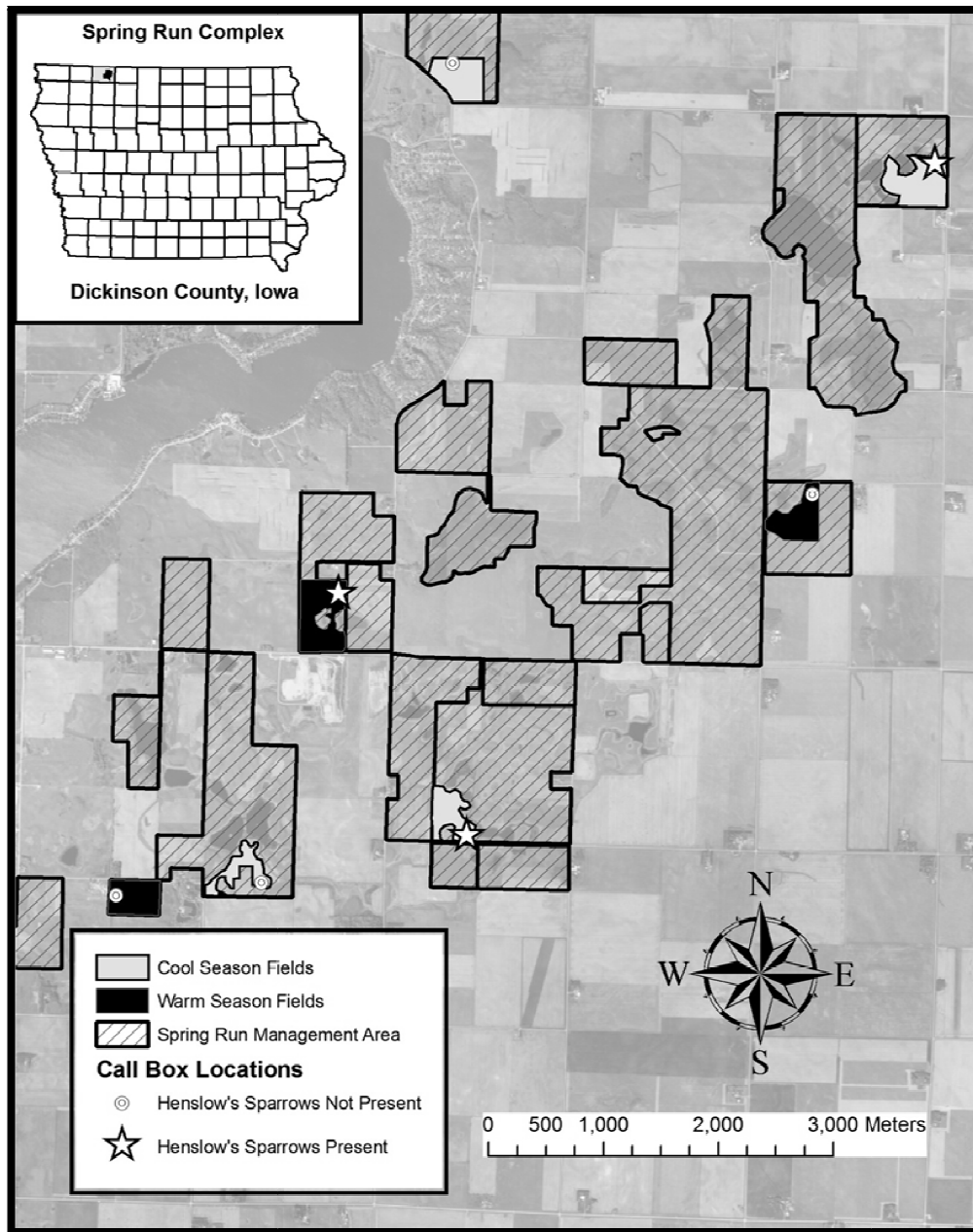


Figure 1. Field locations of Henslow's sparrow playback stations during the 2008 and 2009 seasons on the Spring Run Complex, Dickinson County, Iowa, USA. Black polygons indicate fields planted to warm-season grasses and light gray polygons indicate fields planted to cool-season grasses. Stars indicate the locations where Henslow's sparrows were observed during 2009 surveys.





Figure 2. Henslow's sparrow playback stations established on the Spring Run Complex in 2008 and 2009. Playback stations consisted of a portable compact disc player connected to a programmable timer. Timers were connected to rechargeable 12 volt batteries and solar panels. Playback stations were mounted in aluminum boxes for protection from the elements. The aluminum boxes were drilled out in front of the player speakers to allow for sound transmission. Boxes were mounted to 4 x 4 posts at the typical perching/singing height for Henslow's sparrows in each field.

## CHAPTER SEVEN: GENERAL CONCLUSIONS

The most common bird species we encountered on the Spring Run Complex were Bobolink (*Dolichonyx oryzivorus*), Common Yellowthroat (*Geothlypis trichas*), Red-winged Blackbird (*Agelaius phoeniceus*), and Sedge Wren (*Cistothorus platensis*). We saw an average of 32 bird species during our annual surveys (38 species in 2007, 28 species in 2008, and 31 species in 2009). We did not find bird densities were consistently higher or lower in any one of the 4 planting types. Both vegetation characteristics and food resources were important in explaining grassland bird densities and that different species, even those within a species category, were influenced by different habitat characteristics. Therefore, management of grassland habitat for grassland birds will not be successful under a “one size fits all” approach. Instead, we conclude a variety of planting types and management strategies may be necessary to conserve grassland birds. Negus et al. (2010) advocated for management practices that would enable the inclusion of a mosaic of vegetation characteristics to provide the variety of habitat types necessary for multiple grassland bird species. Additionally, Ribic et al. (2009) found that no single grassland habitat type would be adequate to conserve all grassland birds and that a variety of grassland habitat types would be required. We found that obligate species densities, and in particular, Bobolink densities were higher in cool-season fields than in any of the other field types. Recommendations about grassland habitat plantings have advocated the use of native grass species over non-native grasses (Allen 1993) and avoiding single-species plantings of native or non-native grasses (McCoy et al. 2001). However, given the importance of cool-season, non-native grass plantings for Bobolinks in our study, consideration must be given to the impact that elimination of these plantings may have on the future of Bobolink populations.

Measuring reproductive success is a key element in assessing the value of habitat restoration to grassland birds. We found overall nest survival for Red-winged Blackbirds in mature warm-season fields was more than twice as high than in cool-season fields. While overall nest survival in our high diversity planting type was not significantly different from the other two planting types, this may be in part because of the recent planting age of these fields ( $\leq 4$  years). Although our results suggested that daily nest survival differed among planting types, adult Red-winged Blackbird density was not different across the same four planting types.

Red-winged Blackbird clutch size ranged from 1 to 5 with an average clutch size of 3.4. Nests fledged an average of  $2.4 \pm 0.98$  young. We found evidence of nestling starvation in 48% of successful nests and in 36% of all nests containing nestlings. Brood parasitism by Brown-headed Cowbirds occurred in 11% of all nests and in 5% of successful nests. Thirty percent of all nests we monitored fledged young and predation was the most common cause of nest failure.

We found a relationship between Red-winged Blackbird nest survival and visual obstruction suggesting that nests had higher survival in fields with taller vegetation. We also found that Red-winged Blackbird nests located in fields with more variation in invertebrate biomass over the nesting season may have lower nest survival than those with less variation in invertebrate food availability over the nesting season. Our data suggest that terrestrial invertebrate food availability, and particularly consistency in invertebrate food availability over the nestling period, is potentially important in nest survival.

Male Red-winged Blackbird nestlings were larger than females at fledging with regard to mass, wing, and tarsus measurements. Although male Red-winged Blackbird nestlings are

larger than females, we found that growth rates did not differ between the sexes. Nestling growth rates did not differ among grassland restoration planting types. There were also no differences among years for growth rates or nestling measurements. The sex ratio of the nestlings was 36 males and 35 females.

Red-winged Blackbird nestlings were smaller in the warm-season planting type than in either high diversity or cool-season plantings. In addition, we found that vegetation structure (visual obstruction) was negatively associated with nestling mass at fledging, yet warm-season fields in the Spring Run Complex did not have higher visual obstruction readings than either cool-season or high diversity fields.

We found that brood parasitism by Brown-headed Cowbirds may have an effect on red-winged blackbird nestling primary length at fledging, however, only 7% of our nests were parasitized. Others have found that Red-winged Blackbird nestlings were of similar size (mass and tarsus) in nests parasitized by Brown-headed Cowbirds and in unparasitized nests (Clotfelter and Yasukawa 1999). Red-winged Blackbird nestlings are larger than Brown-headed Cowbird nestlings and therefore can potentially out-compete them for food (Beletsky 1996).

Food resource availability was negatively related to nestling wing growth rates, however, invertebrate diversity did not differ among the planting types. Only the Order Araneae (spiders) had differences in biomass among the planting types. Araneae biomass was highest in cool-season fields than in either warm-season fields or high diversity fields. We found that the amount of native vegetation in a grassland restoration was negatively related to Araneae biomass. It is unclear why spiders in the Order Araneae would prefer exotic cool-season fields to the other planting types. Cool-season fields in the Spring Run Complex have

significantly lower variation in visual obstruction readings than warm-season or high diversity fields, resulting in more homogenous cover. Perhaps the more homogenous nature of cool-season fields results in better web construction locations, less web obstruction due to vegetation variation, and therefore increased prey capture.

Red-winged Blackbird nestling baseline corticosterone levels were lower in the warm-season planting type than in either cool-season or high diversity plantings. Planting age of the high diversity fields (recently planted) versus the warm-season fields (planted >10 years ago) was likely a factor in the differences we observed. Management activity and brood size had positive relationships with baseline corticosterone, suggesting that more intensive management activity and larger brood sizes were related to increased stress levels. Partially related to their recent planting age, high diversity fields received more management than warm-season fields which likely contributed to the higher corticosterone levels in nestlings from high diversity fields.

Blood glucose levels in male nestlings had a significant positive relationship with nestling mass, but not in female nestlings. A possible explanation offered for this trend is increased metabolic rates as nestlings grow larger (gained mass) and are more active in the nest (Lill 2011). However, in our study, increasing nestling mass was only related to increased blood glucose levels in male nestlings. Nestling age and temperature during the nestling period were also positively related to blood glucose levels. Reductions in ambient temperature have been shown to reduce the availability of insect food resources for birds (Jenni-Eiermann et al. 2008). However, we did not find an effect of food resource availability on blood glucose levels in our study. In spite of this, invertebrates may be more active during warmer temperatures making them more available to foraging birds.

Although our sample size was small, we successfully attracted Henslow's Sparrows to previously unoccupied habitat using conspecific song playbacks. For some species, social information can be more influential than habitat cues, such as vegetation structure, in settlement decisions (Betts et al. 2008). Traditional habitat models that do not consider social factors may not be adequate for informing conservation strategies for some species (Harrison et al. 2009), including Henslow's Sparrows. The addition of social cues using playback systems in restored grassland habitats may aid conservation efforts of Henslow's Sparrows to available habitat.

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