TEMPORAL CHANGES IN BLACK-THROATED BLUE WARBLER (*Dendroica caerulescens*) DIET AND FORAGING DURING THE BREEDING SEASON

by
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(Under the direction of Robert J. Cooper)

ABSTRACT

For many migratory avian species there is a short period of time when conditions are suitable for reproduction. Many bird species face spatial and temporal changes in food availability during the breeding season. This study assessed temporal changes in foraging, food abundance, and nestling trophic position of a model migratory species, the Black-throated Blue Warbler (*Dendroica caerulescens*, Gmelin). Black-throated Blue Warbler diet is composed mostly of Lepidoptera larvae. Stable isotope analysis and a Bayesian isotope mixing model were used to estimate nestling diet composition and temporal dietary changes. Proportion of larvae in nestling diet decreased in nestlings hatched later in the breeding season. In addition to assessing nestling diet, adult male Black-throated Blue Warblers foraging choices were assessed. Male tree foraging tree species preferences did not change during the breeding season. This study provides evidence that temporal changes affect nestling diet composition, but do not affect male foraging tree species preference.

INDEX WORDS: Black-throated Blue Warbler, *Dendroica caerulescens*, nitrogen isotope, mixing model, temporal, trophic
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# TABLE OF CONTENTS

ACKNOWLEDGMENT ........................................................................................................ iv

LIST OF TABLES .............................................................................................................. vii

LIST OF FIGURES ......................................................................................................... viii

1 INTRODUCTION, LITERATURE REVIEW, AND STUDY OVERVIEW ................... 1

   INTRODUCTION ......................................................................................................... 1

   LITERATURE REVIEW ............................................................................................ 2

   STUDY OVERVIEW ................................................................................................. 5

   LITERATURE CITED ............................................................................................... 7

2 TEMPORAL TROPHIC EFFECTS ON DIET AND TROPHIC POSITION OF BLACK-
   THROATED BLUE WARBLERS (*Dendroica caerulescens*) .................................. 11

   ABSTRACT ............................................................................................................... 12

   INTRODUCTION ..................................................................................................... 13

   METHODS ................................................................................................................. 15

   RESULTS ................................................................................................................... 21

   DISCUSSION ............................................................................................................. 22

   LITERATURE CITED .............................................................................................. 26

3 TREE SPECIES PREFERENCE IN MALE BLACK-THROATED BLUE WARBLER
   (*Dendroica caerulescens*) FORAGING OVER A BREEDING SEASON ............... 36

TABLES ......................................................................................................................... 32

FIGURES ....................................................................................................................... 34
LIST OF TABLES

Table 2.1. Type 3 generalized linear model repeated measure results on the effect date, plot and tree species have on larval abundance.................................................................32

Table 2.2. Proportion of prey items contributing to carbon and nitrogen stable isotopes values in feathers of Black-throated Blue Warbler nestlings from two temporal periods......................33

Table 3.1. Names and abbreviations used for tree species in Chapter 3 graphs......................55

Table 3.2. Type 3 generalized linear model repeated measure results on the effect date, plot and tree species have on larval abundance.................................................................56
LIST OF FIGURES

Figure 2.1 Data including nestlings (before/after mean hatch date), spiders, Tipulidae, larvae and leaf species. Lines represent 95% confidence intervals…………………………………………34

Figure 2.2 Parameter estimates from repeated measures analysis of the influence date and tree species have on larval abundance (per 50 leaves) during the 2009 breeding season, with 95% confidence intervals………………………………………………………………………...…35

Figure 3.1. Foliar nitrogen concentrations in three tree species (red maple, striped maple and red oak) over the 2009 BTBW breeding season (± SE)………………..57

Figure 3.2. Parameter estimates from repeated measures analysis of the influence date and tree species have on larval abundance (per 50 leaves) during the 2009 breeding season, with 95% confidence intervals …………………………………………………………………...………...58

Figure 3.3 –Percent relative basal area from study sites of top nine tree and sapling species used for male foraging, and all other species combined………………………………………59

Figure 3.4. Tree sapling species preference and avoidance for foraging before nestling hatch. Positive values indicate greater usage than expected and negative values indicate less usage than expected, with 95% confidence intervals……………………………………………………60

Figure 3.5. Tree sapling species preference and avoidance for foraging after nestling hatch. Positive values indicate greater usage than expected and negative values indicate less usage than expected, with 95% confidence intervals…………………………………………………….61
CHAPTER 1
INTRODUCTION, LITERATURE REVIEW, AND STUDY OVERVIEW

INTRODUCTION

Conditions most favorable for reproduction occur over a relatively short time period for most avian species in temperate zones (Visser et al. 2004). Insectivorous migratory songbirds return to breeding grounds based on phenological cues and initiate breeding to coincide with peak insect emergence. For insectivorous species, the abundance of arthropods at the time of maximum food requirement of their young is a crucial determinant of reproductive success (Lack 1968, Visser 1998). Therefore, birds attempt to time their breeding to take advantage of this brief period of abundant food (Martin 1987). Seasonal decline in avian reproductive success has been well documented (Perrins 1970, Verhlust et al. 1995). However, birds sometimes are not successful which can lead to consequences such as higher energy expenditure by parents for foraging (Thomas et al. 2001), reduced nestling weight (Naef-Daenzer and Keller 1999), and reduced survival of fledglings due to lower weight prior to fledging (Naef-Daenzer et al. 2001).

For Black-throated Blue Warblers (Dendroica caerulescens), a migratory songbird, the timing of reproduction is very important because caterpillars, their main food source, are abundant for a short time during the breeding season. The Black-throated Blue Warbler is a model study species as it is similar to other insectivorous avian species in the region in which this study was conducted because it relies on Lepidoptera larvae to provision young (Goodbred and Holmes 1996). The system is a model study system because changing foliar quality and Lepidoptera abundance during the season have the ability to impact nestling diet and male
foraging. The overall goal of this study was to assess how temporal changes affect specific breeding aspects of Black-throated Blue Warblers. In this study, I use stable isotopes and a Bayesian isotope mixing model to assess Black-throated Blue Warbler diet and how diet changes over a breeding season. In addition to a diet analysis, I measured available food abundance and foliar nitrogen quality, and assessed foraging behavior in adult male Black-throated Blue Warblers throughout the breeding season.

LITERATURE REVIEW

Study species

The Black-throated Blue Warbler is a small insectivorous Neartic-Neotropical migrant that winters in the Greater Antilles and breeds from the southern Appalachian Mountains of Georgia northward into southern Canada (Holmes et al. 2005). This species is territorial, sexually-dichromatic, and exhibits strong site fidelity in both its breeding and wintering grounds (Holmes et al. 2005). Reproductive output in the Black-throated Blue Warbler is determined by food abundance (Holmes et al. 1992, Rodenhouse and Holmes 1992, Nagy et al. 2007). Black-throated Blue Warblers forage mainly in the understory, and Lepidoptera larvae (caterpillars) make up the majority of the prey items taken (>80%); (Robinson and Holmes 1982) and the majority of prey biomass (60-87%) brought to nestlings (Goodbred and Holmes 1996, Stodola unpublished data). Males and females both provision nestlings for 8–10 days, and provide virtually all food for fledglings during the first week after fledging (Holmes et al. 2005). In addition, nestling provisioning by male Black-throated Blue Warblers appears to be highly influential in determining offspring size (Stodola et al. 2009).
Study System

The research was conducted in the Nantahala National Forest in western North Carolina (latitude 35°03’N, longitude 83°25’W). This study is part of an eight-year long study monitoring a population of Black-throated Blue Warblers at the Coweeta Long-Term Ecological Research station. The forests in this area are dominated by communities of northern hardwood tree species and cove hardwoods, which include yellow birch (Betula alleghaneinsis, Britton), northern red oak (Quercus rubra L.), yellow poplar (Liriodendron tulipifera L.), black cherry (Prunus serotina Ehrh.), red maple (Acer rubrum L.), eastern hemlock (Tsuga Canadensis Carriere) and hickory (Carya) (Swank and Crossley 1998), and the understory is made up predominantly of rhododendron (Rhododendron maximum L.), mountain laurel (Kalmia latifolia L.) and striped maple (Acer pensylvanicum L.) (Swank and Crossley 1988).

Stable Isotopes

Differences in isotopic signature are the result of different rates of reaction at an enzymatic level, which can result in slight variations in isotopic composition in nature, and these natural signatures can be used in ecological studies to trace food-web structure, migration patterns, and feeding preferences (Hobson & Clark 1992, Wassenaar & Hobson 1998, Fantle et al. 1999). Stable isotopes are heavier or lighter variations of an element because of a difference in the number of neutrons. Nitrogen has the heavy isotope of $^{15}$N. The ratio of $^{14}$N to its stable isotope $^{15}$N is used to measure the amount of assimilation of dietary proteins; the lighter $^{14}$N is lost to nitrogenous waste products (Michener & Kaufman 2007), and consumer tissues are enriched in $^{15}$N with respect to the diets from which they were synthesized. These trophic enrichment factors are typically between 2 and 4‰ at each trophic level (Post 2002). Hence, a
bird feeding its young a diet of caterpillars would have a different N\textsuperscript{15} signature than a bird feeding its young a diet of spiders or other predatory arthropods, because a predatory animal has a higher trophic position, thus it has accumulated a higher N\textsuperscript{15} signature.

Stable isotope composition of consumer tissues can often be related to the composition of an animals’ diet (Peterson and Fry 1987), and can be used to determine the relative contribution of isotopically distinct food sources (Hobson 1987, Ben David et al. 1997, Whitledge and Rabeni 1997, Romanek et al. 2000). Metabolically inert tissues, such as feathers, reflect diet only during growth and retain this information in a chronological manner (Darimont and Reichman 2002), and information about what the organism has been eating for weeks or months (Bearhop et. al. 2003, Pearson et. al. 2003).

**Bayesian Mixing Model**

In this study I employed a Bayesian stable isotope mixing model to infer prey composition of consumer diets (Black-throated Blue Warbler nestlings) while estimating variability in diet composition across a temporal scale in the consumer population structure (Semmens et al. 2009). The proportional contribution of each prey item to the nestling diet was evaluated using mixing models run in the programs R and JAGS using code from http://www.ecologybox.org (Semmens et al. 2009). These computational programs can provide estimates of the relative contributions of diet sources to an organism (Moore and Semmens 2008). This modeling approach reveals how individual and group diets shift during a breeding season, and the proportional contribution of prey items in nestling diet.
Avian Foraging

Avian foraging behavior has been studied for a variety of reasons, but often as an indication of habitat quality (Morrison et al. 1990). Foraging activity and success can serve as an index of prey availability (Lovette and Holmes 1995). Insectivorous birds foraging within a diverse temperate forest provide an opportunity to study the relationship between tree species availability and foraging behavior (Partridge 1974, 1976, Holmes and Robinson 1981, Robinson and Holmes 1982, 1984, Holmes and Schultz 1988, Parrish 1995). This study system provides an opportunity to assess the effect temporal changes in foliar nitrogen and arthropod abundance have on tree species choices of foraging male Black-throated Blue Warblers.

STUDY OVERVIEW

This study examines the effects temporal changes have on Black-throated Blue Warblers during the breeding season. Specifically, I assessed how Black-throated Blue Warbler nestling diet was affected by temporal changes during the 2009 breeding season using a Bayesian isotope mixing model. I also assessed the foraging tree species preference in adult male Black-throated Blue Warblers, and whether foraging choice was influenced by foliar quality or Lepidoptera abundance. Understanding the effect time has on nestling diet and male foraging allows a better understanding of how Black-throated Blue Warblers respond to environmental changes, such as climate change.

This thesis is divided into four chapters, with each chapter able to stand alone. The first chapter is an introduction into the background and reasoning behind my research. The second chapter examines whether temporal changes during a breeding season affect nestling diet. The third chapter examines foraging site choice by male Black-throated Blue Warblers over a
breeding season. Chapter four provides a summary and synthesis of my findings and gives recommendations for future research.
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CHAPTER 2
TEMPORAL EFFECTS ON DIET AND TROPHIC POSITION OF
BLACK-THROATED BLUE WARBLERS

1 B. Maley, K. Stodola, and R. J. Cooper. To be submitted.
ABSTRACT

Many bird species face spatial and temporal changes in food availability during the breeding season. In this study I examine temporal trophic changes in diet in Black-throated Blue Warbler (*Dendroica caerulescens*) nestlings to better understand their feeding ecology. Black-throated Blue Warbler nestling diet consists mainly of Lepidoptera larvae, however larval abundance declines during the breeding season in this ecosystem. Stable isotope analysis was used to estimate nestling trophic position and to more precisely describe a tritrophic system consisting of deciduous trees, Lepidoptera larvae, and insectivorous birds. $\delta^{15}N$ is enriched in trophic levels in a predictable manner, indicating the composition of nestling diet. Nestling diet composition and temporal dietary changes were estimated using a novel Bayesian isotope mixing model. Nestlings were divided into two groups, those sampled prior to mean hatch date (mean hatch date-June 17) and those sampled after mean hatch date. As predicted, the proportion of larvae in nestling diet decreased in nestlings hatched later in the breeding season. The results of this study provide evidence of a temporal change in diet of Black-throated Blue Warbler nestlings that coincides with a decline in their main food source, Lepidoptera larvae. Phenomena, such as nest predation or warmer springs, which cause birds to raise young later in the season relative to the early peak in food resources, have the potential to negatively affect insectivorous birds in deciduous forest ecosystems.
INTRODUCTION

Understanding seasonal variation in food sources and diet can lead to a better understanding of population dynamics (Dalerum and Angeborjn 2005). Conditions most favorable for reproduction occur over a relatively short time period for most avian species in temperate zones (Visser et al. 2004). Food abundance in these regions varies both spatially and temporally throughout the breeding season (Tremblay et al. 2005), affecting both the quantity and nutritional quality of food (Arnold et al. 2010). For insectivorous species, the abundance of arthropods at the time of maximum food requirement of their young is a crucial determinant of reproductive success (Lack 1968, Visser 1998). Therefore, birds attempt to time their breeding to take advantage of this brief period of abundant food (Martin 1987). However, they are not always successful in doing so, which can lead to fitness consequences such as higher energy expenditure by parents for foraging (Thomas et al. 2001), reduced nestling weight (Naef-Daenzer and Keller 1999), and reduced survival of fledglings due to lower weight prior to fledging (Naef-Daenzer et al. 2001).

Variability in seasonal food abundance and parental energetic demands can influence the type of prey items fed to nestlings. Blue Tits (Parus caeruleans) diversified prey items when their main food supply (caterpillars) declined during the breeding season, and increased the proportion of spiders brought to the nest (Banbura et al. 1994, Naef-Daenzer et al. 2000). European Starlings (Sturnus vulgaris) adjusted their feeding strategy when faced with larger broods by feeding nestlings lower quality food items (Wright et al. 1998). Male Black-throated Blue Warblers take on a greater role in provisioning young when brood sizes increase (Stodola et al. 2009), and also increase provisioning in second broods to offset a decrease in female provisioning that occurs late in the breeding season (Stodola et al. 2010).
While studies have shown that insectivorous birds change prey items brought to nestlings and parental effort responds to changes during the breeding season, the dietary shifts that occur during a breeding season are notoriously difficult to quantify (Rosenberg and Cooper 1990). Traditional ways of analyzing diets such as identification of content in stomachs or feces, or direct observations, represent only a snapshot of an animal’s diet and do not assess diet as a whole over longer periods of time (Dalerum and Angeborjn 2005). An alternative method of assessing diet is to compare ratios of carbon and nitrogen stable isotopes in animal tissue (Darimont and Reichman 2002). The stable nitrogen isotope $\delta^{15}\text{N}$ is enriched relative to $\delta^{14}\text{N}$ in a predictable manner with each successive trophic level (Post 2002). Consequently, stable isotope composition of consumer tissues can often be related to the composition of an animals’ diet (Peterson and Fry 1987), and can determine the relative contribution of isotopically distinct food sources (Hobson 1987, Ben David et al. 1997, Romanek et al. 2000). Thus, a shift in $\delta^{15}\text{N}$ between a consumer and its food provides an approximate indication of trophic level (Post 2002).

I used the Black-throated Blue Warbler (*Dendroica caerulescens*), a migratory insectivorous songbird, to assess temporal changes in diet. Black-throated Blue Warblers are a good model species, because they are similar to most insectivorous songbirds in this region, relying largely on Lepidoptera larvae to provision young (Goodbred and Holmes 1996). However, they can also utilize numerous other types of insects during the breeding season to provision young (Holmes et al. 2005), potentially due to differences in availability. They breed in deciduous forests, where leaf quality of tree species present varies temporally throughout the year, generally declining in quality as the season progresses (Feeny 1970, Zehnder et al. 2009). Tree leaf quality influences the availability and abundance of Lepidoptera larvae, and
Lepidoptera abundance impacts nestling weight and survival (Naef Daenzer and Keller 1999), thus making this an excellent model system to study the effect temporal changes have on nestling diet.

The main objective of this study was to assess the dynamics of Black-throated Blue Warbler nestling diet throughout a breeding season. Previous studies in this system provide evidence that nestling weight decreases as the breeding season progresses (Stodola unpublished), and this weight loss may be due to declining Lepidoptera abundance. I analyzed δ15N and δ13C using a Bayesian stable isotope mixing model that incorporates individual nestling diet in the model (Semmens et al. 2009). I assessed primary producers, prey, and nestling feather δ15N and δ13C isotopes. I also tested if δ15N, or diet, changed throughout the breeding season using a Bayesian isotope mixing model. I predicted that temporal changes in the diet of nestling Black-throated Blue Warblers would reflect changes in food availability (i.e., fewer Lepidoptera larvae), and these dietary changes would be evident through stable isotope analysis.

METHODS

Study site

The research was conducted in 2009 in the Nantahala National Forest in western North Carolina (latitude 35°03’N, longitude 83°25’W). In this region we have established two study sites at approximate elevations of 1200 (low) and 1350 (high) m.a.s.l. The study plots are located within 6 km of one another. The 1200 m.a.s.l. site is approximately 40 hectares, and the 1350 m.a.s.l is approximately 20 hectares. The forests in this area are dominated by communities of northern hardwood tree species and cove hardwoods, which include yellow birch (Betula lutea), northern red oak (Quercus rubra), yellow poplar (Liriodendron tulipifera), black cherry (Prunus serotina), red maple (Acer rubrum), eastern hemlock (Tsuga canadensis) and
hickory (*Carya ssp.*) (Swank and Crossley 1998), and the understory is made up predominantly of saplings of the above species, plus striped maple (*Acer pensylvanicum*), rhododendron (*Rhododendron maximum*) and mountain laurel (*Kalmia latifolia*) (Swank and Crossley 1988).

**Study Species**

The Black-throated Blue Warbler is a small insectivorous Neartic-Neotropical migrant that winters in the Greater Antilles and breeds from the southern Appalachian Mountains of Georgia northward into southern Canada (Holmes et al. 2005). This species is territorial, sexually-dichromatic, and exhibits strong site fidelity in both its breeding and wintering grounds (Holmes et al. 2005). Reproductive output in the Black-throated Blue Warbler is largely determined by food abundance (Holmes et al. 1992, Rodenhouse and Holmes 1992, Nagy et al. 2007). Black-throated Blue Warblers forage mainly in the understory, and the majority of the prey items taken are Lepidoptera larvae (Robinson and Holmes 1982). Also, the majority of prey biomass brought to nestlings are caterpillars (Goodbred and Holmes 1996, Stodola unpublished data). Males and females both provision nestlings for 8–10 days, and provide virtually all food for fledglings during the first week after fledging (Holmes et al. 2005). In addition, nestling provisioning by male Black-throated Blue Warblers appears to be highly influential in determining offspring size (Stodola et al. 2009).

**Field Methods**

Fifteen Black-throated Blue Warbler pairs were monitored on one plot (low elevation), and fourteen pairs were monitored on the other plot (high elevation) throughout the breeding season. Pairs were banded with a unique set of color bands (small plastic bands that are attached to the legs of each adult bird), and a unique aluminum identification band also was attached to the leg of the bird. Territory maps were created through spot mapping and following pair
movement. I searched for nests for each pair and monitored them until the eggs hatched (or the nest failed), and the nestlings fledged. I removed nestlings from the nest six days after hatch, banded and weighed them, and removed several feathers for stable isotope analysis. Feathers were placed in plastic bags, to avoid contamination before analyses. Metabolically inert tissues, such as feathers, reflect diet only during their growth (Darimont and Reichman 2002). Nestlings can be removed on day six because they still can be safely returned to the nest without fear of causing premature fledging (Holmes et al. 1992).

**Food abundance surveys**

Food availability estimates were based on knowledge of the preferred food and foraging strategy of Black-throated Blue Warblers. This species feeds primarily on arthropods, especially caterpillars, on understory and sub-canopy foliage (Robinson and Holmes 1982, Holmes 1986, Rodenhouse and Holmes 1992, Goodbred and Holmes 1996, Nagy 2007). Black-throated Blue Warblers forage mostly in the lower strata of deciduous forests, but will also forage higher in trees (Holmes 1986, personal observation). Lepidoptera larvae do not appear to be strongly stratified in eastern deciduous forests (Cooper unpublished data, Holmes and Schultz 1988), so estimates of caterpillar abundance made from samples taken in the shrub layer were assumed to be representative of caterpillars available to foraging Black-throated Blue Warblers, an assumption previously made by Nagy et al. (2007).

Larval abundance surveys were performed on each plot to estimate changes in abundance over the breeding season. These surveys were performed bi-weekly starting 15 May and continuing until 15 July 2009 for a total of five surveys. Larvae were counted on sapling red maple, striped maple, and red oak, which are dominant understory deciduous trees in the study area. Four 500 meter transects were used in the lower elevation plot and five 500 meter transects
were used in the higher elevation plot (because of a disparity in available deciduous understory trees). Every 50 m, two food abundance counts were performed for each tree species with 50 leaves inspected for each count. All larvae were counted and their length measured in millimeters.

**Food/Leaf Quality**

I collected arthropod and leaf specimens from the 29 focal territories. These collections were performed on the same dates as the Lepidoptera surveys. I collected leaf specimens from three dominant deciduous understory trees: red maple, striped maple, and red oak. In each territory I haphazardly chose two trees of each species from 0.5-2 meters in height, and collected two leaves from each tree. The leaves were placed in plastic bags and frozen. Leaf samples were used to determine leaf quality (nitrogen content). I used one sample from a randomly selected territory for stable isotope analysis. Samples were dried at 64°C in a drying oven and ground using a ball mill grinder. Insect collecting was performed haphazardly in each territory for five minutes. All insects from 0.5-2 meters in height were collected and placed in plastic bags and frozen. Insects were kept in plastic bags for a few hours prior to freezing, which allowed them to clear their guts before isotope analysis. The insect samples were identified to order, weighed (dry weight), and used for isotope analysis. Samples for isotope analysis were chosen randomly from the larger insect samples.

**Stable Isotope Analysis**

Leaf, insect, and nestling feather samples were used for stable isotope analysis. The samples were frozen and then oven dried at 64°C for 48 hours. Leaf samples were ground in a mill and I placed 2.0-3.0 milligrams into tin capsules for analysis. Insect samples were placed whole in tin capsules for analysis. Nestling feathers were weighed separately and placed whole
in tin capsules for analysis. All samples were analyzed at the University of Georgia Analytical Chemistry Lab. Stable isotope ratios are expressed as the deviation from standards in parts per thousand (‰) according to the following equation: \( \delta^{13}C, \delta^{15}N = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \times 10^3 \), where \( R_{13C}/12C \) or \( 15N/14N \). Stable isotope ratios of carbon \( (\delta^{13}C) \) and nitrogen \( (\delta^{15}N) \) were measured using a Delta Plus isotope-ratio mass spectrometer (ThermoFinnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA).

**Statistical Analysis**

*Larval abundance*

A repeated measures Poisson linear regression analysis was used to examine the effect that sample date, plot, and tree species had on larval abundance, and type 3 analysis results are presented. This analysis includes the maximum likelihood ratio and p-values based on chi-squared distributions (SAS Institution 2006). Model parameter estimates are presented with 95% confidence intervals.

*Bayesian Isotope Mixing Model*

I employed a Bayesian stable isotope mixing model to infer prey composition of consumer (Black-throated Blue Warbler nestlings) diets while estimating variability in diet composition across a temporal scale in the consumer population structure (Semmens et al. 2009). I analyzed \( \delta^{13}C \) and \( \delta^{15}N \) stable isotope data from Black-throated Blue Warbler nestlings, using two temporal periods during the breeding season with multiple individuals from each temporal period. The proportional contribution of each prey item to the nestling diet was evaluated using mixing models run in the programs R and JAGS using previously developed code (http://www.ecologybox.org; Semmens et al. 2009). These computational programs can provide estimates of the relative contributions of diet sources to an organism (Moore and Semmens...
This modeling approach reveals how individual and group diets shift during a breeding season, and the proportional contribution of prey items in nestling diet. To assess trophic relationships in the study system, I assessed probable prey (based on videotapes of prey delivery to nests; Stodola et al. 2009, 2010), which included Lepidoptera larvae, spiders, and crane flies (Diptera: Tipulidae). I combined spiders and Tipulidae in this analysis because there was no statistical difference in $\delta^{15}N$ between them ($F_{1,21}=0.003, p=0.96$). I assessed diet composition in all individuals, and divided these individuals into two groups. The first group consisted of all individuals hatched prior to the mean hatch date (17 June, Julian date= 168) of nestlings analyzed in this study. The second group consisted of all individuals hatched after the mean hatch date of nestlings analyzed in this study. Nestlings were separated into these groups because of the decline in larval abundance after this sampling date. For modeled contributions, I assumed the isotopic discriminations factor for $\delta^{13}C \pm 1.5$ to 2.2% (Pearson et al. 2003) and $\delta^{15}N$ 1 to 6% (DeNiro and Epstein 1978, Pearson et al. 2003). I used a Bayesian framework, designed to estimate the probability distributions of source contributions to a mixture, while explicitly accounting for uncertainty with multiple sources, fractionation, and isotope signatures (Rush et al. 2010). Using uninformative priors and estimates of uncertainty associated with mixing model inputs, the model ran for $10 \times 10^5$ iterations, resulting in convergence on the posterior source contributions of the different prey items of the diet of nestling Black-throated Blue Warblers (Rush et al. 2010).
RESULTS

Larval abundance

Larval abundance varied by date and tree species as indicated by the significant interaction (Table 2.1). Parameter estimates indicate that this interaction was due to the general decline in larval abundance on red oak and red maple throughout the season, while abundance on striped maple increased slightly (Figure 2.1). Specifically, larval abundance was greatest on red maple and most pronounced during the first three time periods in the breeding season (Figure 2.2). The largest decrease in larval abundance occurred after the June 15 sampling date (Figure 2.2). The model results show that there was no plot effect on larval abundance ($\chi^2=1.91$, df=1, p=0.168)(Table 2.1), but there were differences in larval abundance among tree species ($\chi^2=16.41$, df=2, p=0.0003)(Table 2.1). Date also had an effect on larval abundance ($\chi^2=16.76$, df=4, p=0.002)(Table 2.1), and there was an interaction effect of plot and date on larval abundance ($\chi^2=18.76$, df=8, p=0.016)(Table 2.1).

Stable Isotope Analysis – Study System

Leaves

All primary producers had the lowest $\delta^{15}$N. Red oak had the highest $\delta^{15}$N signature of the three tree species sampled and striped maple had the lowest $\delta^{15}$N value (Figure 2.1). All tree species had similar $\delta^{13}$C signatures, however red oak and red maple were more depleted in $\delta^{15}$N than striped maple (Figure 2.1).

Arthropods

Arthropods had higher $\delta^{15}$N than primary producers and lower $\delta^{15}$N than nestlings. Lepidoptera larvae were more depleted in $\delta^{15}$N than spiders and Tipulidae (Figure 2.2). Larvae were also more depleted in $\delta^{13}$C than spiders and Tipulidae (Figure 2.2).
**Nestlings**

Nestlings hatched prior to the mean hatch date were more depleted in $\delta^{15}N$, indicating a diet of lower trophic level arthropods (Figure 2.1). Nestlings hatched after the mean hatch date had higher $\delta^{15}N$, indicating a diet of higher trophic level arthropods (Figure 2.2).

**Bayesian Isotope Mixing Model**

Model estimates of diet composition of nestling feathers from nestlings hatched prior to mean hatch date identified the mean diet composition to be 99.35% larvae (Table 2.2) and the mean composition of spiders and Tipulidae to be only 0.64% (Table 2.2). Model estimates of diet composition of nestling feathers from nestlings hatched after mean hatch date identified a mean portion of diet composed of spiders and Tipulidae to be 8.82% (Table 2.2), with lower mean proportion of larvae than the nestlings hatched prior to mean hatch date at 91.24% (Table 2.2).

**DISCUSSION**

The Bayesian mixing model analysis showed clear evidence of a dietary shift in Black-throated Blue Warbler nestlings during the breeding season. The main food delivered to Black-throated Blue Warbler nestlings was Lepidoptera larvae, a finding consistent with earlier studies by Goodbred and Holmes (1996), and Stodola (unpublished). The remainder of their diet consisted of other prey, especially spiders and Tipulidae (Stodola unpublished data). The proportion of spiders and Tipulidae in nestling diet increased in nestlings hatched later in breeding season while larval abundance declined. Nestling diet appears to be responding to temporal declines in Lepidoptera abundances that occur during the breeding season. However, it is unclear whether temporally caused changes in diet adversely affect nestling survival.
This study is part of a long-term study of the breeding ecology of Black-throated Blue Warblers in the southern Appalachians. Nest initiation, larval abundance, and nestling weight have been measured in this area since 2003, and nestling weight declines throughout the breeding season (Stodola unpublished). However, in the 2009 breeding season nestling weight was not affected by date ($r^2 = 0.001$, $p=0.47$). Year to year changes occur in breeding systems, however the long term data combined with this analysis provide evidence that nestling diet lower in Lepidoptera larvae may result in lower nestling weight.

The results of this study are similar to those found in Blue Tits, where adults altered prey brought to nestlings when caterpillar abundance declined (Banbura et al. 1994, Naef-Daenzer et al. 2000) and Blue Tit nestlings were fed caterpillars that reflected approximate caterpillar abundance, with fewer caterpillars and more spiders entering the diets of late broods compared to earlier broods (Arnold et al. 2010). Caterpillars are the single most important prey item for most forest-dwelling, insectivorous breeding birds (Greenberg 1995). They require shorter handling times, are easier to ingest than spiders, contain less chitin, and contain more water - 85% for caterpillars, and 73% for spiders (Edney 1977), and so allow for maximum chick growth (Banbura et al. 1999, Tremblay et al. 2005). Spiders also contain no alpha tocopherol as well as significantly lower levels of carotenoids than caterpillars (Arnold et el. 2010), making them lower quality food items. Blue Tit nestlings hatched later in the breeding season are predicted to have poorer antioxidant defenses and higher levels of oxidative damage than early hatchlings, because they received a diet higher in spiders than caterpillars (Arnold et al 2010). A diet higher in larvae may be more beneficial and may lead to higher survival rates for nestlings, and later hatch dates may have a negative impact on nestling survival due to temporal changes in food availability.
Although analyses of stable isotopes can reveal temporal shifts in diets, there are limitations to the methods (Dalerum and Angebjorn 2005). In this study I was only able to assess a relatively small sample of larvae (n=31) for stable isotope analysis. Larvae size could impact the $\delta^{15}$N signature as well as the type of primary producer the prey is feeding on (i.e., tree species may affect larval $\delta^{15}$N signature). However, I found no relationship between larvae size and $\delta^{15}$N ($r^2=0.11, p=0.08$). More isotope analysis of larvae, and their host tree species on which they are found, should be performed to examine whether declining $\delta^{15}$N in leaves affects Lepidoptera $\delta^{15}$N, and how tree species affects $\delta^{15}$N in larvae. Gannes et al. (1997) recognized three processes that complicate dietary reconstruction from stable isotopes: animals assimilate with different efficiencies, animal tissue fractionates differently, and animals allocate nutrients to different tissues. In this study I only analyzed nestling feathers, because removing feathers is the least invasive tissue sampling technique. Although there are unknowns with stable isotope analysis, the results still provide insight into Black-throated Blue Warbler nestling diet, and more information than traditional methods alone.

To my knowledge, no other studies have used stable isotope analysis to assess temporal changes in the diet of a temperate forest dwelling insectivorous migratory nestling. However, nestling $\delta^{15}$N signature has been used to examine avian trophic relationships in coastal ecosystems (Knoff et al. 2001, Knoff et al. 2002, Rush et al. 2010). Isotope analysis is a widely accepted method used to discern diet differences between terrestrial and marine biomes (Inger and Bearhop 2008), but I believe they can be useful in assessing any study system. This study provides evidence of how isotope analysis and a Bayesian mixing model would be useful in studying forest passerine food webs, and a way to estimate temporal changes in diet.
There are several important implications of this study. Reproductive success in avian species depends on timing of reproduction with respect to food abundance (Lack 1968, Visser et al. 1998). Black-throated Blue Warblers must synchronize initiation of breeding with food abundance to successfully breed, and factors that lead to a mismatch could cause decreased fecundity. For example, failure of initial nest attempts because of nest predation or other factors is usually followed by a second nest attempt, which will not be timed to coincide with peak caterpillar abundance. Perturbations that increase nest predation, such as habitat fragmentation (Robinson et al. 1995), may therefore have synergistic effects with food availability to further decrease fecundity. A shifting climatic regime also has the potential to disrupt the synchrony between different trophic levels (Visser and Both 2005), and cause the mismatch of phenological processes. A combination of traditional diet assessment and isotope analysis provides a powerful tool in studying effects of these perturbations involving seasonal shifts in animal’s diets.
LITERATURE CITED


Knoff, A.J., S. A. Macko and R. M. Erwin. 2001. Diets of nesting Laughing Gulls (Larus atricilla) at the Virginia Coast Reserve: observations from stable


TABLES

Table 2.1- Type 3 generalized linear model repeated measure results on the effects of date, plot, and tree species on larval abundance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
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</tr>
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<td>Plot</td>
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Table 2.2- Proportion of prey items contributing to carbon and nitrogen stable isotope values in feathers of Black-throated Blue Warbler nestlings from two temporal periods.

<table>
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<th>Temporal period</th>
<th>Larvae (mean)</th>
<th>±SD</th>
<th>Spiders and Tipulidae (mean)</th>
<th>±SD</th>
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</thead>
<tbody>
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<td>Nestling diet before mean hatch date</td>
<td>99.35</td>
<td>0.08</td>
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<td>Nestling diet after mean hatch date</td>
<td>91.24</td>
<td>0.87</td>
<td>8.82</td>
<td>0.90</td>
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</table>
FIGURES

Figure 2.1- Data including nestlings (before/after mean hatch date), spiders, Tipulidae, larvae and leaf species. Lines represent 95% confidence intervals.
Figure 2.2- Parameter estimates from repeated measures analysis of the influence date and tree species have on larval abundance (per 50 leaves) during the 2009 breeding season, with 95% confidence intervals.
CHAPTER 3

TREE SPECIES PREFERENCE IN MALE BLACK-THROATED BLUE WARBLER FORAGING OVER A BREEDING SEASON¹

¹ B. Maley, K. Stodola, and R. J. Cooper. To be submitted.
ABSTRACT

Foraging behavior by insectivorous birds can be a predictor of habitat quality and prey availability. In this study I observed foraging behavior in male Black-throated Blue Warblers (*Dendroica caerulescens*) to assess if nestling stage, Lepidoptera abundance, and foliar nitrogen content affects male Black-throated Blue Warbler foraging site (tree species) over a breeding season. I quantified foraging behavior by observing twenty-nine individuals repeatedly over the breeding season. I measured leaf quality and Lepidoptera abundance in common understory tree species, to assess if these variables influenced foraging. Of the tree species examined for foliar quality, red oak (*Quercus rubra* L.) had higher foliar nitrogen concentration than red maple (*Acer rubrum* L.) and striped maple (*Acer pensylvanicum* L.). Larval abundance was highest in red maple in May and June and in striped maple in July. There were some subtle changes in tree species preferences from before to after hatching, but males mostly preferred the same species regardless of breeding status and changing caterpillar abundance over time. Red maple, red oak, and hemlock (*Tsuga Canadensis* Carriere) were tree species preferred by males for foraging. Species consistently avoided by males were striped maple, witch hazel (*Hamamelis virginiana*), and rhododendron (*Rhododendron maximum* L.). Overall, although foliar quality and food abundance seemed to influence foraging choices, males still tended to prefer or avoid specific tree species regardless of breeding status and other time-dependent variables.
INTRODUCTION

Habitat quality can be defined as the ability of the environment to provide conditions appropriate for individual and population persistence (Hall et al. 1997). A direct measure of habitat quality is reproductive output, which is related to fitness (Lambrechts et al. 2004, Holmes et al. 1996). A number of other factors, such as vegetative and other structural habitat characteristics (Morrison et al. 2006), presence or absence of predators (Rodenhouse et al. 2003), and food abundance (Barnes et al. 1995), also presumably influence reproductive output and therefore habitat quality. Food supply is often considered the resource that limits bird populations and determines productivity (Lack 1966); however it can be difficult measuring food supply for birds (Cooper and Whitmore 1990).

Avian foraging behavior has been studied for a variety of reasons, but often as an indication of habitat quality (Morrison et al. 1990). Tremblay et al. (2005) showed a clear habitat-related difference in foraging distance and provisioning rates of breeding Blue Tits (Parus caeruleus), indicating that the differences were related to food abundance. Birds returned to foraging sites that provided large prey size and high density of prey (Naef-Daenzer and Keller 1999, Naef-Danezer 2000). Blue Tits also diversified prey items brought to the nest when abundance of high-quality prey (caterpillars) was low, increasing the proportion of spiders by 50% and decreasing the proportion of caterpillars (Banbura et al. 1994). Birds may adjust their foraging behavior in response to changing resource conditions as well as changing resource demands during the breeding season. Foraging activity and success can serve as an index of prey availability (Lovette and Holmes 1995), and sites with increased foraging activity generally indicate higher habitat quality. Bird species may use certain tree species preferentially, possibly due to differences in arthropod abundance, but perhaps also due to other factors such as foliage
structure (Holmes and Robinson 1981, Gabbe et al. 2002). Forests with a high tree species and structural diversity present birds with diverse macro-and microenvironments for foraging (MacArthur and MacArthur 1961, Whelan 2001). For example, insectivorous birds inhabiting a northern hardwood forest will preferentially utilize the foliage of different species of trees depending on arthropod abundance and foliage structure (Holmes and Robinson 1981, Robinson and Holmes 1982), but the profitability of different tree species for foraging may also fluctuate over time (Whelan 1989, Parrish 1995). In a diverse southern bottomland hardwood forest, McCasland (1997) found no consistency in tree species preferences by three species of insectivorous songbirds over a breeding season. Thus, a forest with high tree species diversity may, at any given time during the breeding season, provide birds with diverse combinations of foliage structure and prey availability (Steele 1993). Insectivorous birds foraging within a diverse temperate forest provide an opportunity to study the relationship between tree species availability and foraging behavior (Partridge 1974, 1976, Holmes and Robinson 1981, Robinson and Holmes 1982, 1984, Holmes and Schultz 1988, Parrish 1995).

An aspect of the foraging behavior of insectivorous birds that has been seldom studied is the influence of foliar leaf quality on foraging. That is, a diverse forest may provide insectivorous birds with diverse arthropod assemblages through the effects of differing foliar quality on tree species throughout a breeding season. Nitrogen is the limiting factor in many species of plants and arthropods, and increased insect density has been linked to higher foliar nitrogen concentrations (Mattson 1980). Foliar nitrogen concentrations generally decline in tree species during a season, and leaf chewers were more abundant on trees with high foliar nitrogen concentration (Feeny 1970, Zehnder et al. 2009). Increased bird foraging on some herbivorous arthropod guilds has also been observed in response to experimentally increased foliar nitrogen
levels (Forkner and Hunter 2000). If tree species have different temporal patterns of quality and profitability, then birds may utilize tree species differently during the breeding season because of changes in foliar quality, food abundance, and types of prey during a breeding season.

The overall goal of this study was to assess tree species preference through the foraging of male Black-throated Blue Warblers during a breeding season. Specifically, I wanted to assess whether males utilized particular tree species for foraging more than others. In addition, I wanted to assess whether this preference for particular foraging sites (tree species) changed over the course of the breeding season relative to nesting stage (pre and post hatch, a time dependent variable). I also measured larval abundance and foliar nitrogen concentration in three dominant understory tree species. I predicted that male Black-throated Blue Warblers would utilize a variety of tree species for foraging, and that preferences would change over time, because tree species may provide differing foraging opportunities for them during a breeding season due to temporal changes in leaf quality and insect abundance. I also predicted nesting stage would affect foraging sites because nestlings require increased foraging activity, and parents may utilize specific tree species because of insect abundance.

**METHODS**

**Study site**

The research was conducted in 2009 in the Nantahala National Forest in western North Carolina (latitude 35°03’N, longitude 83°25’W). In this region we have established two study sites at approximate elevations of 1200 (low) and 1350 (high) m.a.s.l. The study plots are located within 6 km of one another. The 1200 m.a.s.l. site is approximately 40 hectares, and the 1350 m.a.s.l is approximately 20 hectares. The forests in this area are dominated by communities of northern hardwood tree species and cove hardwoods, which include yellow birch
(Betula lutea), northern red oak (Quercus rubra), yellow poplar (Liriodendron tulipifera), black cherry (Prunus serotina), red maple (Acer rubrum), eastern hemlock (Tsuga canadensis) and hickory (Carya ssp.) (Swank and Crossley 1998), and the understory is made up predominantly of rhododendron (Rhododendron maximum), mountain laurel (Kalmia latifolia) and striped maple (Acer pensylvanicum) (Swank and Crossley 1988).

Study Species

The Black-throated Blue Warbler is a small insectivorous Neartic-Neotropical migrant that winters in the Greater Antilles and breeds from the southern Appalachian Mountains of Georgia northward into southern Canada (Holmes et al. 2005). This species is territorial, sexually-dichromatic, and exhibits strong site fidelity in both its breeding and wintering grounds (Holmes et al. 2005). Reproductive output in the Black-throated Blue Warbler is determined by food abundance (Holmes et al. 1992, Rodenhouse and Holmes 1992, Nagy et al. 2007). Black-throated Blue Warblers forage mainly in the understory, and Lepidoptera larvae make up the majority of the prey items taken (Robinson and Holmes 1982) and the majority of prey biomass brought to nestlings (Goodbred and Holmes 1996, Stodola unpublished data). Males and females both provision nestlings for 8–10 days, and provide virtually all food for fledglings during the first week after fledging (Holmes et al. 2005). In addition, nestling provisioning by male Black-throated Blue Warblers appears to be highly influential in determining offspring size (Stodola et al. 2010).

Foraging Observations

Fourteen male Black-throated Blue Warblers were monitored on the high elevation plot and fifteen male Black-throated Blue Warblers were monitored on the low elevation plot throughout the breeding season. Males were used because they are less elusive than females. In
New Hampshire, foraging behaviors of male and female Black-throated Blue Warblers were very similar (Holmes 1986); nevertheless, I caution the reader that intersexual differences in foraging behavior can exist, so results observed here for males may not be readily extrapolated to females. Plots were gridded using plastic flagging on a series of transect lines that ran 90 degrees to each other throughout entire plot. Flagging was placed on trees every 25 meters and marked by letter and number, so that territory maps could be created for pairs of birds. Each male was color banded with a unique combination of plastic color bands, permitting repeated observations on individual birds, and territory maps were created for each focal male from these observations. Foraging data were gathered on each focal male for up to ten minutes twice per week from May-July 2009. Males were found by systematically searching the territory. I then followed the male through its territory for as long as possible (up to 10 minutes) while it was actively foraging to obtain foraging observations. Tape recorders were used to record all foraging observations, which were later transcribed to a computer database. Observations began when the observer correctly identified the color bands on the bird, and observations were stopped if a male was lost in the canopy and restarted once they were re-sighted. Foraging observations were performed between 06:00-12:00. Foraging tree, time spent (sec) in each tree, and all foraging maneuvers (i.e., prey attacks) performed in each tree species were recorded.

**Food abundance surveys**

Larval abundance surveys were performed on each plot to estimate changes in abundance over the breeding season. I was interested in estimating Lepidoptera larvae abundance because they make up the majority of prey items brought to nestlings (Goodbred and Holmes 1996, Stodola unpublished data). These surveys were performed bi-weekly starting 15 May and continuing until 15 July 2009 for a total of five surveys. Larvae were counted on red maple,
striped maple, and red oak, which are dominant understory deciduous trees in the study area. Four 500 meter transects were used in the lower elevation plot and five 500 meter transects were used in the higher elevation plot (because of a disparity in available deciduous understory trees). Every 50 m, two food abundance counts were performed for each tree species with 50 leaves inspected for each count. All larvae were counted and their length measured in millimeters.

**Leaf Quality**

In each territory I haphazardly chose two trees of each species from 0.5-2 meters in height, and collected two leaves from each tree. The leaves were placed in plastic bags and frozen. I used one sample from a randomly selected territory for stable isotope analysis. Samples were dried at 64°C in a drying oven and ground using a ball mill grinder. They were weighed into tin capsules with a with a Mettler UMT2 microbalance (Mettler Toledo, Greifensee, Switzerland) and analyzed with a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan, Italy) for nitrogen concentration.

**Tree availability**

On each of the two gridded plots, flagged points were chosen for measurement of vegetation characteristics. Thirty-five points were selected on the high elevation plot, and 68 points on the low elevation plot. Each point was separated by approximately 70 m and arranged in a checkerboard pattern that encompassed the area where Black-throated Blue Warblers nested. Basal area for all tree species less than 3-10 cm in diameter at breast height (dbh) within an 11.3-m-radius were measured and defined as saplings. Between 3-5 points were used to measure tree availability (percent basal area) in each male territory. I used saplings to measure tree availability because Black-throated Blue Warbler’s forage mainly in the understory (Holmes and Robinson 1981, Holmes et al. 2005).
Statistical Analysis

To assess differences in foliar nitrogen concentrations between tree species I used analysis of variance in the R package (R Development Core Team 2008). A repeated measures Poisson linear regression analysis was used to examine the relationship sample date, plot, and tree species had on larval abundance, and type 3 analysis results are presented (SAS Institution 2006). Model parameter estimates are presented with 95% confidence intervals. Available tree species were determined for each male territory by averaging 3-5 points found in or around the territory. For all foraging analyses I took the top nine tree species utilized in foraging, and created another category representing the remaining tree species. I created a variable before and after hatch to determine how nestling phase affects parental foraging, serving as a time-dependent variable. Unlike previous studies of foraging behavior that used nest stage in models (Sakai and Noon 1990), I was able to pinpoint the exact time when hatching occurred for each nest rather than using a mean hatch date for all nests. Values were generated by calculating the difference between percent foraging events (number of attacks, gleans, etc.) for one sample period and percent basal area of the male’s territory. A preference index (PI) was calculated by taking the average of the avoidance and preference values of each male sampled before hatch and after hatch and presented with 95% confidence intervals (Holmes and Robinson 1981, Gabbe et al. 2002, Strode et al. 2009).

RESULTS

Leaf Quality

Foliar nitrogen concentrations were highest for all tree species at the earliest sampling date and decreased over time (Figure 3.1). Red oak had the highest foliar nitrogen content for all sampling dates (May: $F_{2,45}=5.32$, $p=0.008$, June: $F_{2,43}=3.22$, $p=0.05$, July: $F_{2,51}=18.72$, $p<0.001$)
There was a plot effect in the May and July sampling dates. In the May sampling date the high elevation plot showed a higher foliar nitrogen concentration in red maple than the low elevation plot (high elevation $\bar{x} = 2.61$, low elevation $\bar{x} = 2.03$, $F_{1,16} = 8.34$, $p = 0.01$). In the July sampling date the higher elevation showed a higher foliar nitrogen concentrations in red oak and striped maple (red oak: high elevation $\bar{x} = 2.36$, low elevation $\bar{x} = 1.97$, $F_{1,15} = 12.58$, $p = 0.003$), striped maple: high elevation $\bar{x} = 1.95$, low elevation $\bar{x} = 1.62$, $F_{1,18} = 7.37$, $p = 0.0142$).

**Larval abundance**

Larval abundance varied by date and tree species as indicated by the significant interaction (Table 3.2). Parameter estimates indicate that this interaction was due to the general decline in larval abundance on red oak and red maple throughout the season, while abundance on striped maple increased slightly (Figure 3.2). Specifically, larval abundance was greatest on red maple and most pronounced during the first three time periods in the breeding season (Figure 3.2). There was no plot effect on larval abundance ($\chi^2 = 1.91$, df=1, $p = 0.168$)(Table 3.2), but there were differences in larval abundance among tree species ($\chi^2 = 16.41$, df=2, $p = 0.0003$)(Table 3.2). Date also had an effect on larval abundance ($\chi^2 = 16.76$, df=4, $p = 0.002$)(Table 3.2), and there was an interaction effect of plot and date on larval abundance ($\chi^2 = 16.76$, df=8, $p = 0.016$)(Table 3.2).

**Foraging**

Red oak, red maple, hemlock, black birch, and “other” species had the highest basal area of trees larger than ten cm diameter in this study system (Figure 3.3). Rhododendron, red oak, witch hazel, striped maple and yellow birch had the highest basal area of trees less than ten cm and greater than 3 cm (saplings) in this study system (Figure 3.3). Regardless of time of the breeding season, males generally showed a preference for hemlock, red maple, black birch,
yellow birch, red oak and beech (Figures 3.4-3.5). Males avoided rhododendron, witch hazel, and striped maple throughout the breeding season (Figures 3.4-3.5). Although slight shifts were observed over the breeding season, males generally did not change preference or avoidance for tree species due to nesting stage (Figures 3.4-3.5). All other species fell in between preference and avoidance as indicated by strong overlap of zero by 95% confidence intervals.

**DISCUSSION**

Choices made by foraging insectivorous birds reflect a combination of preferred foraging substrates and prey on the one hand, and opportunism on the other. Insectivorous songbirds have demonstrated an ability to switch from preferred prey (caterpillars) to other prey types when caterpillars declined in abundance (Cooper et al. 1990, Banbura et al. 1994). For this reason, plus the need to forage more actively (and perhaps opportunistically) when feeding young, I predicted that males would use different tree species for foraging throughout the breeding season because tree species profitability would shift during the breeding season. While male Black-throated Blue Warblers may take advantage of tree species that temporarily provide resources to themselves and young, they appear to mainly utilize specific tree species for foraging during the breeding season.

There was also some evidence that male Black-throated Blue Warbler preference for different tree species is influenced by larval abundance and foliar nitrogen concentration. Of the three tree species examined, red oak had the highest foliar nitrogen concentration and was a preferred foraging substrate. Red maple had the highest caterpillar abundance in May and June and also was a preferred substrate. Striped maple, however, had the highest caterpillar loads in July but was generally avoided by foraging males during this study. Striped maple supported a suite of generalist, leaf-chewing Lepidoptera larvae. Many of the Lepidoptera species that feed
on striped maple are highly polyphagous (Marquis and Passoa 1989) and may chose other tree species that have higher-quality leaves (Zehnder et al. 2005). Differences in larval abundance were often two-fold between the species with the highest caterpillar density and the next-highest species, but differences were estimated to be only 0.2 to 0.5 larvae per 50 leaves. It is difficult to assess if this constitutes a biologically significant difference.

Black-throated Blue Warblers are one of the most intensively studied insectivorous birds in North America. Foraging behavior has been described in breeding populations in New Hampshire; these studies showed that Black-throated Blue Warblers mostly use hovering in foraging, and they showed a preference/avoidance for specific leaf structures (Holmes et al. 1979, Holmes and Robinson 1981, Whelan 2001). To my knowledge no one has studied the effects of nesting stage on Black-throated Blue Warbler foraging tree choice, and there are no data on their foraging in this study system. While this study is limited because I focused on foraging observations on males and I do not have food abundances for all tree species used by males, I believe it does provide useful information on habitat use in this study system.

Black-throated Blue Warblers used a number of tree species while foraging that I did not sample for food abundance. Of these, males showed a strong avoidance for rhododendron, possibly due to the low abundance of arthropods, especially larvae. Rhododendron leaves have high lignin and low nitrogen concentrations compared to other tree species (Strickland et al. 2010), and these qualities may lead to leaves that are more resistant to damage and deter insect feeding (Zehnder et al. 2009). Male Black-throated Blue Warblers showed a foraging preference for hemlock, red maple, yellow and black birch, red oak and beech. Males showed a strong preference for hemlock throughout the breeding season. I did not collect larval abundance for hemlock, but the high preference value indicates that either (1) there is a food source they are
cuing in on, or (2) hemlock allows for higher detection level because of the foliage structure and/or many of the trees are losing foliage. Hemlock could be an important tree species for male foraging, but it is in decline in this study system.

An introduced insect, the hemlock woolly adelgid (*Adelges tsuagae* Carriere) has caused the decline in hemlock across the eastern United States. The woolly adelgid kills trees of all sizes and ages within 4-15 years of infestation (Orwig et al. 2002). Hemlock could functionally disappear from eastern forests in the next several decades (Ellison et al. 2005), and be replaced by hardwood species such as birch, oaks, and maples (Orwig et al. 2002). The inevitable loss of hemlock in the southern Appalachians may alter the foraging ecology of male Black-throated Blue Warblers. There is a high probability that hemlock will be replaced by rhododendron in riparian areas in the southern Appalachians (Roberts et al. 2009). If hemlock is replaced by rhododendron then Black-throated Blue Warblers may face serious foraging consequences.

Previous studies have demonstrated that choices of foraging substrates made by insectivorous warblers reflect a number of factors. Whelan (2001) showed that three species of northern hardwoods warblers preferred certain leaf structures over others (Whelan 2001). Different guilds of insectivorous birds in a northern hardwoods forest exhibited tree species preferences and aversions for foraging, based on leaf morphology and food availability (Holmes and Robinson 1981). In a southern bottomland forest ecosystem with high tree species diversity, McCasland (1997) found that arthropod loads for different tree species fluctuated greatly over space and time, and three species of insectivorous birds had no consistent preferences regarding tree species used for foraging. In this study, which was conducted in a deciduous forest with intermediate tree species diversity compared with the above two systems, I found that male Black-throated Blue Warbler choice of foraging substrates was specific, and they consistently
relied on particular tree species for foraging during the breeding season. Interestingly, the tree species chosen were different from those observed by Robinson and Holmes (1981), likely indicating site-specific substrate preferences. Rules regarding consistent tree species preferences by insectivorous birds across different sites remain difficult to demonstrate, likely because they may not exist.
LITERATURE CITED


SAS INSTITUTE. 2006. SAS 9.1.3 help and documentation. SAS Institute, Cary, NC.

Steele, B. B. 1993. Selection of foraging and nesting sites by Black-throated Blue


### TABLES

Table 3.1- Names and abbreviations used for tree species in Chapter 3 graphs.

<table>
<thead>
<tr>
<th>Tree species abbreviations</th>
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<tr>
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<tr>
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<td>black birch</td>
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Table 3.2- Type 3 generalized linear model repeated measure results on the effects of date, plot, and tree species on larval abundance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>4</td>
<td>16.76</td>
<td>0.002</td>
</tr>
<tr>
<td>Plot</td>
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<td>1.91</td>
<td>0.168</td>
</tr>
<tr>
<td>Tree</td>
<td>2</td>
<td>16.41</td>
<td>0.0003</td>
</tr>
<tr>
<td>Date*tree</td>
<td>8</td>
<td>18.76</td>
<td>0.016</td>
</tr>
</tbody>
</table>
FIGURES

Figure 3.1- Foliar nitrogen concentrations in three tree species (red maple, striped maple, and red oak) over the 2009 Black-throated Blue Warbler breed season (± SE).
Figure 3.2 – Parameter estimates from repeated measures analysis of the influence date and tree species have on larval abundance (per 50 leaves) during the 2009 breeding season, with 95% confidence intervals.
Figure 3.3 - Percent relative basal area from study sites of top nine tree and sapling species used for male foraging, and all other species combined.
Figure 3.4 – Tree sapling species preference and avoidance for foraging before nestling hatch. Positive values indicate greater usage than expected and negative values indicate less usage than expected, with 95% confidence intervals.
Figure 3.5 – Tree sapling species preference and avoidance for foraging after nestling hatch. Positive values indicate greater usage than expected and negative values indicate less usage than expected, with 95% confidence intervals.
CHAPTER 4

CONCLUSIONS

CONCLUSIONS

Conditions most favorable for reproduction occur over a relatively short time period for most avian species in temperate zones (Visser et al. 2004), including Black-throated Blue Warblers. This study examined the effects temporal changes had on specific breeding aspects of Black-throated Blue Warblers. Specifically, I assessed nestling diet composition changes during the breeding season, and how tree species foraging preferences in male Black-throated Blue Warblers changed during the breeding season. The seasonal decline in avian reproductive success has been well documented (Perrins 1970, Verhlust et al. 1995), however this study examined two breeding aspects that are not well documented in this study system.

I provide evidence that Black-throated Blue Warbler nestlings experience a temporal diet shift during the 2009 breeding season. Larval abundance declined during the breeding season, which appears to affect Black-throated Blue Warbler nestling diet composition. Nestling weight has declined in this breeding system during the breeding season (Stodola unpublished), however during the 2009 breeding season nestling weight was unaffected during the breeding season. Nestling weight may not have declined because the 2009 breeding season was an unusually good food year for Black-throated Blue Warblers. Spiders and Tipulidae are lower quality food items than Lepidoptera larvae, which may explain the decline in nestling weight that usually occurs during the breeding season. The results from this study and previous studies indicate that Black-throated Blue Warbler nestlings hatched later in the breeding season are more susceptible to a
change in diet because of the decline in larval abundance, and these nestlings may experience a lower chance of survival.

I found that male Black-throated Blue Warbler choice of foraging substrates was specific, and they consistently relied on particular tree species for foraging during the breeding season. Here I show how foraging observations in concert with food abundance surveys can estimate how profitable specific tree species can be for insectivorous birds. Foliar nitrogen content does not seem to be the main indicator of larval abundance in this study system, and did not impact male foraging choice. To accurately assess how foliar quality affects larval abundance, more analysis need to be performed to measure phenolics, lignin, and cellulose content in leaf species.

Stable isotope analysis was used to assess nestling trophic position, and a Bayesian isotope mixing model was used to assess how date of hatch affected diet composition. Other studies have used isotope analysis to assess trophic position, but to my knowledge this was the first study to use stable isotope analysis to assess a migratory insectivorous forest dwelling nestling diet. While isotope analysis is not a perfect measure and has many unknowns, it proved to be a useful tool in this study.

This study is part of a long term monitoring program of Black-throated Blue Warblers, and I wanted to build on the knowledge of Black-throated Blue Warbler nestling diet and male foraging. In this study I show that temporal changes can impact nestling diet, which in turn may affect nestling quality and survival. Climate change has the ability to exacerbate temporal changes, causing a mismatch in bud burst (van Asch et al. 2007), and insect emergence (Visser et al. 2004), and consequently Black-throated Blue Warbler reproduction. Studying trophic ecology will allow a better understanding of how future environmental changes, such as climate
change, will impact Black-throated Blue Warblers and other insectivorous migratory songbirds in the southern Appalachians.
LITERATURE CITED


