DFO Research, Education and Conservation Grant

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Title: Exploring the Effects of Non-native Tree Species on Chickadee Nestling Diet

Objective: This project seeks to explore the effects of urbanization on black-capped and mountain chickadee diet in Colorado's Front Range along a rural-urban gradient to determine how non-native tree populations affect the diet of native songbirds.

Significance: Increasing urbanization can shift provisioning behaviors of nature fauna via distribution changes of non-native trees in urban areas. Variation in tree composition, and therefore available arthropod populations, in chickadee breeding territories compared to the prey populations utilized by chickadees to feed their nestlings will provide insight into how small songbirds are navigating increasingly urban ecosystems in Colorado. Ultimately, this project will inform future urban land usage by evaluating the potential ecological costs and benefits of native and non-native trees on the reproductive success of native fauna in Colorado's Front Range.

Introduction: As urbanization has increased, humans have changed the composition of trees within urban areas to create a mosaic of both native and non-native tree species. Unsurprisingly, this rapid shift in community composition has altered the trophic cascades of organisms dependent on trees for survival (e.g., arthropods and the organisms that consume arthropods)(Oxbrough et al. 2016). Urban non-native trees are often planted by humans for their aesthetics or the fruit they bear, but these species can disrupt the population dynamics of native fauna reliant on trees or the arthropods that inhabit them, such as insectivorous birds (Hajdasz et al. 2019). Indeed, when native trees are replaced with ornamental non-native trees in urban settings, arthropod abundance drops (Burghardt and Tallamy 2013). However, the consequences of these shifts in arthropod abundances are poorly understood for bird communities which rely on them to successfully fledge young. I seek to clarify the relationship between non-native trees, nestling diet, and overall breeding success in resident backyard songbirds which rely on arthropods to provision their young. **Specifically**, **I will explore how urbanization impacts avian communities by documenting changes in avian diet driven by shifts in tree species composition and local arthropod communities, and the resulting impacts on reproductive success.** I hypothesize that the mosaic of native and non-native tree species that now exists in urban areas will alter trophic cascades of the birds dependent on specific tree species, and the arthropods they host, for survival. Specifically, birds breeding in urban territories (with more non-native trees) will have lower reproductive success than birds breeding in rural areas due to reduced arthropod numbers on non-native trees.

Study System: Black-capped (*Poecile atricapillus;* BC*)* and mountain (*P. gambeli;* MC) chickadees are small, insectivorous songbirds that readily breed in both urban and rural settings. Chickadees have small territories (~ 25 m) and rely heavily on surrounding foliage to glean arthropods for provisioning their nestlings (Narango et al. 2018). Because they glean insects to provision their nestlings, chickadees are middle-trophic species (consumers that occupy the middle positions in often complex food webs) and are

therefore reliable indicators for the effects of non-native trees in ecosystems (Schulze et al. 2004). The composition of tree species in this study system play a unique role due to the specialized adaptations that arthropod prey have evolved for feeding on specific (often native) plants (Burghardt and Tallamy 2013). Given that native trees increase arthropod abundance, which directly impacts chickadee breeding success, characterizing chickadee nestling diet will shed insight into how chickadee populations are responding to changing arthropod diversity and availability in a rapidly urbanizing world. **The main aim of this proposal is to characterize the diet of chickadee nestlings in urban and rural environments by sequencing fecal samples collected from wild chickadee nestlings to identify food items utilized compared to prey items in breeding territories.**

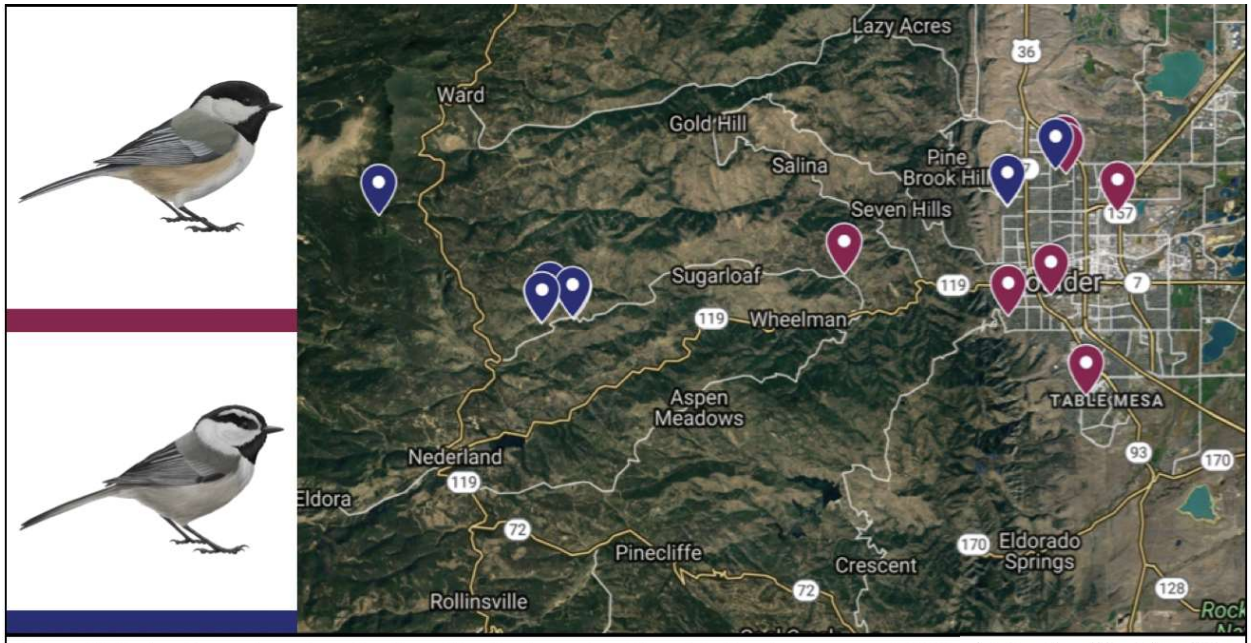

Figure 1. The location and species of each nestling fecal sample collected during the summer of 2019, with the city of Boulder, CO (right) and the CU Boulder Mountain Research Station located between Ward, CO and Nederland, CO (left). Multiple samples were opportunistically collected from the same nests during handling on Day 12. Plum represents black-capped chickadee (*Poecile atricapillus)* samples and navy represents mountain chickadee (*P. gambeli)* samples.

Methods: I monitored chickadee breeding along a 2000-meter elevational gradient from the urban terminus of Boulder, CO to its rural counterpart, CU Boulder's Mountain Research Station in Nederland, CO for one breeding season (April-July 2019). During the breeding season, I monitored chickadee nest boxes to collect breeding phenology and reproductive success data for adult chickadees (n=68). Nest boxes were checked weekly until incubation began. After incubation, nests were checked daily to confirm hatching (Day 0). The following methods were conducted during the previous breeding season (April-July 2019).

Completed Work:

a) *Fecal Sample Collection:* At Day 12, nestlings were removed from the nest, weighed, banded with a federal metal band, and had small blood samples (< 10 ul) collected for genetic samples. Fecal samples (BC: n=16; MC: n=7) were collected opportunistically from nestlings during handling. Once chicks had defecated, fecal sacks were placed in 1.5mL tubes using sterilized q-tips and frozen to preserve genetic material.

b) *Provisioning Surveys:* To measure feeding rate and visually confirm the prey items brought to the nest by parents, we conducted 1 hr long provisioning surveys at each nest (n=23) on Day 13 (1 day after banding). After a 15 min acclimatization period, observers began a 1 hr long observation period where they noted the visit time, the individual (male or female) visiting the nest based on color bands, and when possible, the prey item carried.

c) *Arthropod Surveys:* Following Narango et al. 2018, I conducted arthropod surveys to quantify the availability of prey items in chickadee breeding territories. I vigorously shook the branches of three trees in each cardinal direction (n=12 trees per survey; n=23 surveys) from a nesting box and collected dislodged arthropods in a catch net. Collected arthropods were stored in 70% ethanol for identification to Order in the lab and each tree species was recorded to determine native origin.

Work to be completed:

d) *Genetic Analyses:* I will sequence the fecal samples collected during the 2019 field season for non-avian DNA to 1) describe the diet of chickadee nestlings in urban versus rural environments and 2) examine the variation between BC and MC diet. Fecal samples will be sequenced using metabarcoding to assess prey items consumed by chickadee nestlings. This DNA sequence data in conjunction with the provisioning surveys and arthropod surveys conducted, will reveal the impacts of non-native tree populations on chickadee provisioning behavior. Diet composition information (i.e., non-avian DNA sequenced in fecal samples) will be compared to metrics of reproductive success, such as clutch size and fledgling success rates, to assess the correlation between nestling diet and chickadee reproductive success.

Project Timeline:

Season	Objective
Spring 2020	-Sequence Summer 2019 Samples for Non-Avian DNA -Analyze fecal samples, provisioning surveys, and arthropod data
Summer 2020	-Collect Summer 2020 Diet Composition Data
Fall 2020	-Sequence Summer 2020 Samples for Non-Avian DNA -Analyze sequence data, arthropod data, and provisioning data
Spring 2021	-Analyze project data set and finalize statistical analyses
Fall 2021- Spring 2022	-Write and defend Honors Thesis, prepare Honors Thesis for publication and graduate

Project Completion: The majority of this project has been completed under my own direction. This past summer (April-July 2019), I opportunistically collected fecal samples from chickadee nestlings, and conducted arthropod and provisioning surveys. This semester, I will analyze the provisioning survey data and sort collected arthropods to Order. The final step of this project is sequencing the fecal samples and preparing the results for publication. This research will be done under the guidance of University of Colorado, Boulder graduate student Kathryn Grabenstein, my direct mentor, and faculty advisor Dr. Scott Taylor. The field component of this project, which has already been conducted, occurred at field sites in Boulder County, CO (n=54). The second field season, planned for Summer 2020, will be conducted at the same field sites. The remaining lab work will be done in the Taylor Lab at the University of Colorado Boulder. My direct mentor, Kathryn Grabenstein, in addition to myself, will ensure the completion of this project. The Taylor Lab at CU Boulder possesses all necessary permits required for capturing, banding, and collecting fecal samples from chickadees (Federal Bird Banding and Marking Permit 24169; Scientific Collection License-Colorado Parks and Wildlife- 19BD2307). Our experimental methods have been approved by CU, Boulder (IACUC Protocol #2520).

Budget: Funds Requested from Denver Field Ornithologists Research, Education and Conservation Grant

	Budget Item	Amount	Subtotal
a	**Sequence Summer 2020 Samples (n=10) for non-avian DNA**		
	Sequence 10 samples @ $135.00/sample	10	$1,350.00
	TOTAL REQUEST FROM DFO RESEARCH GRANT:		**$1,350.00**

Total Projected Cost of Research Project

	Budget Item	Subtotal	Funding Source
a	**Sequence Summer 2019 Samples**		
	Sequence 23 samples @ $135.00/sample	$3,105.00	AOS and CFO Research Award
b	**Sequence Remaining Summer 2020 Samples**		
	Sequence 10 samples @ $135.00/sample	$1,350.00	BCNA Research Grant
	TOTAL PROJECT COST	$5,805.00	

Budget Justification: Funding from the Denver Field Ornithologists will help pay for the final component of this project: sequencing half of the fecal samples to be collected from BC and MC nestlings in 2020 for non-avian DNA. I will use the funds awarded from the DFO to determine variation in chickadee diet. These data will provide the missing link between tree composition, available arthropods, and breeding success by shedding insight into what prey items adult chickadees are provisioning to their young, and the impacts diet has on reproductive success. To finance the remainder of this project, I have applied for research grants from the Colorado Field Ornithologists and American Ornithological Society, and I will apply for a research grant from the Boulder County Nature Association to fund sequencing the remaining fecal samples from 2019 and the fecal samples to be collected in the summer of 2020.

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