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. Results from this study will directly inform future urban land usage regarding non-native tree introduction and cultivation. 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 plants within urban areas to include both native and non-native 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). Urban non-native trees are often chosen 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 plants are replaced with ornamental non-native trees in urban settings, arthropod abundance drops (Burghardt and Tallamy 2013). However, while non- native trees likely alter complex urban food webs (Oxbrough et al. 2016), the direct connections between these trophic shifts and native bird species are poorly understood. I hypothesize that the mosaic of native and non-native tree species that now exists in urban areas will alter trophic cascades of the organisms dependent on specific tree species, and the arthropods they host, for survival. Many bird populations live in close contact with humans in urban areas and rely both directly and indirectly on trees for food. However, information on how non-native trees in urban areas have shifted the diets of urban bird populations is lacking. I seek to explore how urbanization changes avian diet through shifts in tree species composition and subsequent changes to local arthropod communities. Study System: Black-capped (Poecile atricapillus; BC) and mountain chickadees (P. gambeli; MC) are small, insectivorous songbirds that occur along elevational and urban-rural gradients. Chickadees have small territories (~ 25 m) and rely heavily on surrounding foliage to glean arthropods, which they provision to their nestlings (Narango et al. 2018). Because they glean insects to provision their nestlings, chickadees are middle-trophic species, and therefore reliable indicators for the effects of non-native trees DFO Grant Application Cori Carver on native ecosystems (Schulze et al. 2004). The arthropods that chickadees use to provision their nestlings have evolved specialized adaptations for feeding on specific (often native) plants. 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 available prey items. Methods: The methods used in 2020 represent a continuation of a two-year study that began in 2019 and was previously funded by the DFO. Nestling fecal samples sequenced in 2019 indicate diet variation between BC and MC nestlings, with more arthropod diversity in BC diet but more dependence on Dipterans (true flies) and Lepidopterans (butterflies and moths) in MC diet. The final data samples for this study were collected in 2020. We monitored chickadee breeding along a 2000-meter elevational gradient from the urban epicenter of Boulder, CO to its rural counterpart, CU Boulder's Mountain Research Station in Nederland, CO for one year (April-July). During the breeding season, I monitored chickadee nest boxes to collect breeding phenology and reproductive success data for adult chickadees. Nest boxes were checked weekly until incubation began. After incubation, nests were checked daily to confirm hatching (Day 0). o 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 (2019: n=23; 2020: n=46) were collected opportunistically during handling. Once chicks had defecated, fecal sacks were placed in 1.5mL tubes using sterilized q-tips and frozen to preserve genetic material. o Provisioning Surveys: To measure feeding rate and visually confirm the prey items brought to the nest by parents, we conducted provisioning surveys at each nest (2019: n=23, 2020: n=39) on Day 13 (1 day after banding). o Arthropod Surveys: Following Narango et al. 2018, we conducted arthropod surveys to quantify the availability of prey items in chickadee breeding territories. We vigorously shook the branches of three trees in each cardinal direction (n=12 trees per survey) from a nesting box and collected dislodged arthropods in a catch net. Collected arthropods were stored in 70% ethanol for later identification in the lab. o Genetic Analyses: I sequenced the fecal samples collected during 2019 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 were sequenced using metabarcoding to assess prey items consumed by chickadee nestlings. The fecal samples collected in 2020 will be sequenced using the same methods as the 2019 samples and 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.