Title: Assessing Environmental Heterogeneity Among Barn Swallow Nesting Sites

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Introduction. The world is currently undergoing the largest wave of urban growth in history with over 56% of the world's populations living in urban areas. Urbanization is inherently a mechanism of landscape structure and compositional change. These manufactured landscapes continue to change as cities develop and in turn result in large amounts of environmental heterogeneity in factors ranging from air pollution to resource abundance 1–3. Identifying and being able to study variation of abiotic and biotic elements in these spaces is increasingly important. Urbanized habitats can produce urban heat island effects with striking increases in temperature, light, noise and air pollution as well as increased human activity, all of which have been shown to have direct impacts on wildlife4–7. All of these factors have been found to have impacts across various avian species ranging from body condition and size, to gut microbiota, and even migration behavior and patterns8–12 Understanding how environmental abiotic conditions vary across environments is especially important, not just in the context of environmental heterogeneity, but in the context of various scales—site-level to regional. More work is needed to assess the connection between urbanization and the ability for wildlife to adapt to rapidly changing environmental elements. Barn swallows (Hirundo rustica) are an excellent study system for exploring environmental variation and physiological responses to urbanization. Barn swallows are one of the most widespread of all songbirds and can be found in Colorado during their breeding season across both rural and highly urbanized areas. Barn swallows nest exclusively in mudcup nests that they construct on human-made structures, including barns, bridges, culverts, and the eaves of buildings. As human commensals and insectivores, they are well adapted to human disturbance and unlike many other urban-dwelling avian species, they do not rely on human food13– 15. A previous study on barn swallows in China found that urbanization can lead to body size decrease in barn swallows 13. Yet, much remains unknown about the degree of variation of abiotic factors and the effects of urban-associated environmental factors on barn swallows.

Objective: To investigate barn swallow populations along an urban-rural gradient in Boulder County, I will analyze whether and how site-level and nest-level environments and associated nestling development, vary across an urban-rural gradient in Boulder County (Table 1.). Preliminary data collected during May – September 2022 already reveals variation across nesting sites related to fluctuations in nest-level temperature, however collecting more nest- and sitelevel data for various abiotic factors is necessary to understand the degree of variation across these microenvironments. 1. What specific environmental factors vary between rural and urban environments? H1a. Environmental features differ at barn swallow nest locations across the urban-rural gradient differ. H1b. Environmental features do not differ at barn swallow nest locations across the urban-rural gradient.

Methods. March-October 2023. March- April. Scoring urban-rural gradient. As barn swallows return to Boulder County for the breeding season, we will choose sites based on active nests in Boulder, Co (downtown) across Boulder County to Longmont, CO. After these sites have been selected, I will score an urbanization index using Image Processing Laboratory's Urbanization Index which combines vegetation, building, and paved landscape-cover variable scores into a PCA and generates an urbanization score for each site based on geographical coordinates. MayOctober. Characterizing environmental differences across an urban-rural gradient. I propose to measure environmental variation across urban and rural areas in Boulder County at active barn swallow nesting sites. For site-level

comparisons of environmental features I will measure temperature (C), light intensity (lux), and air quality/pollution (No2, NoX, NO) for ten urban and ten rural sites and at an estimated 30 nests. For nest-level measures, I will measure fine scale temperature (C) within the nest. I will record nest and site-level temperatures (nest-level ibuttons; site-level Hobo-loggers), light intensity hourly (site-level Hobo-loggers), and air quality (passive diffusion sampling tubes, two-week period after hatching) at standardized times to derive systematic and comparable measures for each breeding location. iButtons will be sewn into the bottom of nests and will record nest-temperature in 10 minute intervals (following the protocol I developed during summer 2022), Hobo-loggers will be placed alongside nests to measure ambient temperature and light levels to assess site-level variance and diffusion tubes will be installed within 15 feet of nests to measure ambient nitrogen oxide readings at the site level. May-October 2023. Characterizing reproductive outcome differences. All nests at each breeding site will be monitored for activity. Clutch initiation dates will be recorded, and hatching activity will be monitored closely. Just after hatching, nestlings will be individually marked using sharpies and non-toxic nail polish (Sally Hansen) to track individual growth rates by taking measures of mass and wing length every other day during the 12-day nestling period. Significance for Colorado birds. Colorado has experienced massive urban expansions over the last 60 years and has more recently fallen into comparison with cities like Los Angeles, Phoenix, and Dallas, some of the biggest cities in the US6,19. Denver, by itself, has had over a 2% increase in population in less than half a decade. As cities and their surroundings become increasingly more urban, it is critical to understand how species that reproduce and live in these environments are being impacted. Barn swallows are especially relevant for studies related to urbanization as they are highly mobile and prolific within the human built environment. As aerial insectivores, they are highly sensitive to climate change as their environments change and have been found to be decreasing in numbers. 20,21 Boulder County has had an almost 600% increase in population over the last 70 years, with a projected city growth increase of 1% per year over the next 50 years.22,23 These are drastic indicators of increasing urbanization, showing how dire it is to understand whether and how wildlife in Boulder County will respond to increasingly changing environmental conditions. Especially relevant, assessing these responses and the degree of urbanization in cities like Boulder can lead to more intentional conservation and infrastructure policy surrounding avian species that breed, migrate, or winter in urban environments.