The "urban-*adapted*" bird: Does dietary plasticity allow some species of birds to thrive in urban habitats?

1. BACKGROUND: Urbanization has altered habitats, restructured avian communities, and influenced the range sizes and population dynamics of bird species [1-3]. While urban environments comprise just 5.6 percent of the total land cover of the United States[4], the shift from rural to urban land use is considered to be the second leading cause of species endangerment and extinction during the twentieth century[5]. As the proportion of developed land is projected to increase by as much as 63 percent in the first half of this century[6], urbanization is expected to become the primary driver of species extinction and thus understanding how organisms respond to urban habitats is of paramount importance[2]. Despite the urgency to minimize the impacts of urbanization, our ability to apply effective conservation strategies is hindered by a limited understanding of the mechanisms by which urban habitats influences bird populations [7].

The interaction between human land use and the life history traits of species determines whether a given species will be a "winner" (urban-adapted species) or "loser" in human-dominated landscapes (urban-avoiding species)[1,8]. It is hypothesized that bird populations and communities in anthropogenic environments are largely structured by the distribution of resources accessible to a given dietary guild[9,10]. Species with specialist dietary requirements, especially obligate insectivores[11], are expected to be sensitive to human-induced habitat modification and may therefore experience high rates of local extinction across the urban habitat matrix[12]. Conversely, species with generalist dietary habitats and those that have the ability to utilize anthropogenic resources (e.g., granivores utilizing bird feeders[13]) are expected to be positively associated with urban environments[1]. For example, urban landscapes dominated by ornamental and non-native species provide essential resources for frugivorous birds, especially during winter months[14], but are expected to negatively influence insectivores due to reduced abundances of insects during the growing season[15]. While studies of avian community composition[16] and survival[10] suggest the influence of resources on avian response, the direct connection between avian health and the utilization of resources across the rural-to-urban gradient has not yet been empirically tested.

2. RESEARCH OBJECTIVE: To assess the influence of dietary resources on bird health across the rural-to-urban gradient. Here, I seek to examine the influence of urban land cover on avian diet and the effects of dietary habits on the health of birds across the rural-to-urban gradient at local and regional scales in metropolitan Washington D.C. I expect that the composition and abundance of avian food resources vary temporally and spatially along this gradient with resource subsidization as the primary driver of higher food availability at intermediate degrees of urbanization[17,18]. As such, I expect that the maintainance of populations in urban environments is contingent on the ability to utilize these resources. Specifically, the proposed research will address: 1) Do birds vary their diet across the rural-to-urban gradient? 2) Do birds exhibit increased omnivory with increasing urban land cover? 3) What are the consequences of diet on bird health in urban environments? 4) Do local-scale habitat features moderate the influence of urbanization on avian diet and health?

To address these questions, I will sample feather and claw of birds across the rural-to-urban gradient and measure the stable isotope ratios of Carbon and Nitrogen to assess avian diet and concentrations of the stress hormone corticosterone (CORT) to approximate the condition of individuals. CORT has been found to be predictive of several biologically important indicators of bird health, with low CORT values associated with breeding success[19], body condition[20], and survival[21]. Analysis of stable isotope ratios of δ^{13}C and δ^{15}N has been applied to estimate the trophic position of prey items in feather[22,23] and claw [24] material. Tail feathers are molted immediately following the end of the breeding cycle and thus δ^{13}C and δ^{15}N ratios represent a snapshot of an individual's diet during the growth of the feather. While claw material is grown continuously, samples collected at the start of the breeding season are representative of bird diet prior to the breeding period. As feather and claw samples are representative of avian diet at different points in a bird's annual cycle, assessing isotopic ratios within both claw and feather samples provides a key temporal signature of avian diet [del Rio et al. 2009] and allows researchers to assess the width of a bird's dietary niche [24, 25]. Moreover, the combined analysis of δ^{13}C, δ^{15}N, and CORT in feather and claw samples allows us to evaluate the influence of urbanization on dietary niche position and width and the relationship between diet and avian health [26].

3. PROPOSED RESEARCH: I propose to assess variation in diet and condition across the rural-to-urban gradient in two species of birds: the Northern Cardinal (NOCA, *Cardinalis cardinalis*) and Carolina Chickadee (CACH, *Poecile carolinensis*). Differences and similarities in the life histories of these species, both of which are common to the rural and suburban habitats of metropolitan Washington, D.C., provide an ideal study system with which to assess the influence of urbanization on avian diet and health. Both species exhibit some degree of omnivory, are non-migratory, and utilize bird feeders in suburban and urban habitats. Key differences between the life history of the species include nesting habits (NOCA are cup nesting and CACH cavity nesting species) and response to urbanization (NOCA are found at more urban sites than CACH). Moreover, these species have been shown to exhibit a differential response to urban land cover in terms of survival (Evans et al. 2015) and abundance (Evans et al. 2015b), with NOCA experiencing greater abundance and higher survival in suburban and urban habitats than CACH – it is expected that these differences are driven by the ability to utilize resources in the human-built environment.

GENERAL METHODS

Field sampling: This study will utilize data collected as a part of the Neighborhood Nestwatch Program (NN), a citizen science project run by the Smithsonian Migratory Bird Center. NN has established a network of over 200 sampling sites within metropolitan Washington D.C., with sampling predominantly located at the homes of project participants. Habitats represented by study sites range from rural open and forested areas, to suburban and urban environments. From the NN network, I will select 60-80 sites with recorded CACH and/or NOCA captures, using the proportion of impervious surface to maintain as broad a representation of the rural-to-urban gradient as possible (see local and regional habitat variables, below). To avoid potential pseudoreplication, no more than one individual of each species will be sampled per site and a minimum distance of 1 km will be maintained between sites. Technicians will visit participant properties at the start of avian breeding season (April and May) and set two to eight mist nets for a period of three to five hours of mark-recapture. Birds (n = 60 of each species) will be captured using target netting with playback of mobbing calls or conspecific song. We will measure body mass (to the nearest 0.01 g), unflattened wing chord (to the nearest 0.25 mm), and age individuals into juvenile and after hatch year (AHY) age classes using plumage, skull ossification, or molt criteria (methodologies vary by species, see Pyle 1997). To limit unwanted variation, only AHY male birds will be sampled. Prior to release, a tail feather and claw sample will be taken and individuals will be marked with a US Fish and Wildlife aluminum band and a unique combination of colored plastic bands. All potential dietary sources, including samples of native (e.g., American Pokeweed, *Phytolacca americana*) and non-native fruits (e.g., Amur Honeysuckle, *Lonicera maackii*), ground and foliar arthropods, and supplemental food sources, will be collected from sites and their surrounding areas ($\leq$ 100 m) at three periods over the course of a year − during the banding visit, at the end of the breeding season (15 July - 15 Aug), and during winter months (Dec - Feb).

Stable isotopes: Samples of potential dietary sources will be dried in ovens prior to analysis. All samples will be washed in a 2:1 chloroform:methanol solution to remove surface oils. For each tissue and prey item, a 0.3 - 0.4 mg sample will be combusted at 1350°C in an elemental analyzer and analyzed in a continuous-flow isotope ratio mass spectrometer following the methods of Rushing et al. (2014). Isotopic ratios ^{13}C and ^{15}C will be evaluated relative to the Pee Dee Belemnite and atmospheric nitrogen standards, respectively, and reported per mil using δ notation using the equation:

$$\delta X = \left[\frac{R_{sample}}{R_{standard}}\right] \times 1000$$

Accuracy and precision of δ^{13}C and δ^{15}N is expected to be within ±2.0‰ (Bearhop et al. 2002). We will use the Bayesian stable isotope mixing model of Hopkins and Ferguson (2012) to evaluate the proportional composition of dietary sources for each individual.

Corticosterone: To examine the adult condition of birds, we will measure CORT in feather and claw samples using a methanol based extraction, modified from Bortolotti et al. (2008), and a commercial ELISA kit (Corticosterone ELISA kit; Neogen Corporation, Ayr, UK) following Carbajal et al. (2014).

Local and regional habitat variables: I will assess the variation in δ^{13}C, δ^{15}N, and CORT as a function of local and regional scale habitat features. Regional habitat features include the proportion of impervious surface and canopy cover within 500 and 1000 m of a given site to approximate the position of the site along the rural-to-urban gradient. These proxy variable and scales of analysis have been shown to be predictive of a demographic response in birds to urban land cover (see Ryder et al. 2010, Evans et al. 2015). Local habitat features, sampled at NN sites between 2010 and 2015, include shrub cover, canopy height and proportional cover, and ground cover. Additional local-scale habitat features will be derived from participant surveys and include supplemental feeding habits (i.e., presence and maintenance of bird feeders) and the presence or absence of outdoor cats and dogs. It is expected that some local habitat features (e.g., shrub cover) buffer the response of birds to urbanization while others (e.g., free-roaming cats) negatively influence avian health in urban habitats [Belaire 2014])

Analysis: I will use Structural Equation Modeling (SEM; McCune and Grace 2002) to examine hypothesized relationships between habitat features, bird health, and dietary habits (below). SEM is a multivariate technique that allows us to simultaneously analyze the complex, and often correlated, inter-relationships among measured (e.g., CORT and land-cover) and latent variables (i.e., those estimated from measured values such as niche width). I will use a Bayesian framework to develop and test *a priori* SEM in JAGS, implemented in Program R (see Plummer et al. 2003). I will evaluate the influence of structural linkages between local and regional scale environmental attributes, CORT, and dietatry traits using Markov chain Monte Carlo sampling and use Bayesian model selection to compare *a priori* models (see Raferty 1993).

HYPOTHESES AND PREDICTIONS

Hypothesis 1. The dietary habits of conspecific birds vary in composition and breadth along the rural-to-urban gradient. Across bioregions, urban environments have been shown to favor dietary generalists and granivores and exclude birds with insectivorous diets. Indeed, in an assessment of avian community composition across the rural-to-urban gradient in metropolitan Washington, D.C. we observed a sharp decline in the proportional abundance of obligate insectivores (Evans et al. 2015). (Note: Bearhop 2004 suggested that isotopes can be a proxy for niche width)

Prediction 1.1: *The ratios of $\delta^{13}C$ and $\delta^{15}N$ in feathers will reflect dietary shifts from insect to plant-based and anthropogenic food sources.* Analysis of d13C and d15N in feathers (and possible diet items including bird food?) will give us a sense of the the proportional composition of plant-based foods (Im hoping we can pick up a distinct signature for bird seed) and insects in the diet. We expect that with increasing urbanization diets will become more omnivorous and the ratio of insects to plant-based foods (including anthropogenic food sources) will decline. To test whether increased omnivory is advantageous to bird health, we use corticosterone as a proxy of avian condition. This last bit is the awkward one however, survival was higher for NOCA in urban habitats and we assumed that this was driven by resources. Using enhanced survival of NOCA as our guide, we might expect that a species exhibiting high plasticity (NOCA) would benefit from this dietary shift while those exhibiting low plasticity (e.g., CACH) would by negatively influenced by a shift from insectivory.

Prediction 1.2: *The dietary niche width among conspecifics, as measured as variance in $\delta^{13}C$ and $\delta^{15}N$ in feathers, will increase with increasing urban land cover.* Populations with higher degrees of dietary specialization are expected to exhibit low variance in δ-values (Bearhop et al. 2004). CChange this bit, see Newsome's paper – Shannon-Weiner metric ... Further, we predict that the dietary niche width among NOCA will be greater than that of CACH.

Prediction 1.3: *Avian condition, as measured by corticosterone concentrations in feathers, decreases with increasing omnivory for NOCA but not CACH.* We expect ... We will analyze CORT concentrations as a function of neighborhood and yard-scale habitat and management practices within a generalized linear model framework, using model selection to determine the variables most predictive of avian condition (see Burnham and Anderson 2002). Preliminary analysis of CORT in Carolina Chickadee (Poecile carolinensis) feathers at NN sites (n = 22) exhibit elevated CORT concentrations in sites dominated by non-native plants (β = -3.2 ± 1.4, p-value = 0.04) but no response to the degree of urbanization at a given site (proportion of impervious surface within 500 m, β = 0.2 ± 0.3, p-value = 0.47). These results suggest that CORT concentrations may be highly reflective of the biological response of birds to yard-scale habitat features.

Hypothesis 2. Urban habitats minimize starvation pressure by providing a more consistent temporal distribution of resources. This is a test of Shochats credit-debt hypothesis that we mentioned in the survivorship paper. Shochat suggested that the more natural environments have greater temporal variation which would increase starvation pressures. Under this scenario anthropogenic food sources and winter fruiting of non-native species would enhance survival. Hobson (1999) suggested that d15N enrichment is representative of nutritional stress. To test this, we would have to sample birds during the non breeding season (perhaps early spring?) and expect higher d15N ratios in rural habitats (relative to more urban ones). To test whether there is an influence of nutritional stress on bird health, we use corticosterone as a proxy of condition.

Prediction 2.1: *Birds will exhibit enhanced levels of $\delta^{15}N$, as measured in avian blood, along* *the rural-to-urban gradient.* text lorem ipsum yada yada.

Prediction 2.2: *Concentrations of corticosterone in avian blood will increase proportionally with $\delta^{15}N$.*

4. RELEVANCE OF THE PROPOSED RESEARCH: My previous research has addressed the influence of urbanization on avian dispersal (dissertation citation), community composition (dissertation citation), and survival (Ryder et al. 2010, Evans et al. 2015) in metropolitan Washington, D.C. Despite emergent patterns that have clearly demonstrated the influence of urban habitat on these biological processes, the mechanisms driving these patterns remain largelly unknown. By addressing the distribution and influence of resources in avian diet, the proposed research project builds on my previous work by addressing the mechanism by which urbanization is expected to have the greatest impact on bird populations.

5. ROLE OF THE SMITHSONIAN INSTITUTION: Research in urban environments is often limited by accessibility and adequate coverage of the urban gradient (Cooper et al. 2007). To alleviate this problem we will utilized sites (n = 242) that were part of Neighborhood Nestwatch (hereafter referred to as NN), which is an ongoing citizen science project run by the Smithsonian Migratory Bird Center. NN is the ideal sampling framework for studying the effects of urbanization on avian demography because it provides access to residential properties within core urban and suburban environments as well as forested and agricultural land cover types (Figure 3.1). By incorporating privately owned land within our study design, we are able to capture portions of the urban and suburban matrix not normally monitored in avian survivorship studies.

WORKS CITED