Urbanization affects environmental conditions for developing songbirds

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ABSTRACT

Urbanization introduces a set of novel stressors for animals in these modified habitats. Developing young, in particular, can be at risk due to increased predation, exposure, and high food intake requirements. Song sparrows (*Melospiza melodia*) are a model wild songbird system that persist in both urban and rural habitats, and have young that develop very quickly. I investigated how urbanization changes the arthropod community, how that in turn affects nestling diet, and ultimately if there were any differences in nestling amino acid profiles or body condition between habitat types. Prior work found that urban habitats also have far higher rates of brood parasitism, which can additionally restrict food intake for urban nestlings. However, this prior work has also found that urban song sparrow adults avoid consequences for living in urban habitats, so I wanted to see if nestlings were also able to avoid the consequences of urban living. In chapter one, I found our urban arthropod communities had lower average arthropod abundance, biomass, and diversity when compared to rural arthropod communities. Song sparrow nestling diets differed somewhat in composition of arthropod prey items by habitat, but urban and rural nestlings had the same average biomass of stomach contents. In chapter two, I investigated whether different habitat types or brood parasitism altered body size and amino acid concentration of song sparrow nestlings. Only gamma-aminobutyric acid differed, being significantly lower in urban, parasitized nestlings. No groups differed in metrics of body condition. In chapter three, I performed a meta-analysis to investigate how brood-parasitic brown-headed cowbirds (*Molothrus ater*) affect host clutch size and nest success. Host clutch sizes were lower when parasitized, but surprisingly non-parasitized nests were more likely to fail. Despite lower arthropod food availability and high levels of brood parasitism, urban song sparrow nestlings are not compromised, supporting the conclusion that urbanization does not necessarily have negative consequences for individuals of this species.

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GENERAL AUDIENCE ABSTRACT

Urbanization introduces a set of new challenges for animals. Young animals, in particular, can be at risk due to increased predation, exposure, and lack of food. Song sparrows (*Melospiza melodia*) are a model wild songbird system that persist in both urban and rural habitats, and have young that develop very quickly and rely on parents providing a diet of arthropods, including spiders and insects. I investigated how urbanization changes the arthropod community, how that in turn affects nestling diet, and ultimately if there were any differences in nutrition or body condition. Urban habitats also have higher rates of cowbirds laying their eggs in the nests of other species, which can make it even harder for urban song sparrow nestlings to get enough food. However, this prior work has also found that urban song sparrow adults avoid consequences for living in urban habitats, so I wanted to see if nestlings were also able to avoid the consequences of urban living. In chapter one I found our urban arthropod communities had lower average number and mass of arthropods, when compared to rural arthropod communities. Song sparrow nestling diets differed somewhat in composition of arthropod prey items by habitat, but urban and rural nestlings had the same average biomass of stomach contents. In chapter two, I investigated whether different habitat types or having cowbird 'siblings' resulted in different nutrition and body size of song sparrow nestlings. Out of nearly 30 measures of nutrition only one differed, being significantly lower in urban nestlings with siblings. No groups differed in body size. For chapter three I analyzed the literature to investigate how brown-headed cowbirds (*Molothrus ater*) affect the number of eggs host birds lay and host nest success. Host clutch sizes were lower when cowbirds were present, but surprisingly, it was nests without cowbirds that were more likely to fail. Despite lower arthropod food availability and high levels of cowbird presence, urban song sparrow nestlings avoided negative consequences, supporting the conclusion that urbanization does not necessarily harm individuals of this species.

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Attribution

Chapter 1 was Co-authored by Korin R. Jones, Taylor E. Fossett, Samuel J. Lane, and Kendra B. Sewall. **Korin R. Jones:** Formal analysis, Writing - review and editing. **Taylor E. Fossett:** Investigation, Writing - review and editing. **Samuel J. Lane:** Investigation, Writing - review and editing. **Kendra B. Sewall:** Conceptualization, Methodology, Investigation, Writing - review & editing, supervision, funding acquisition. All co-authors provided comments and approved the manuscript.

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Dissertation Introduction

We are currently living through the sixth mass extinction, brought about by extensive habitat destruction, climate change, and exploitation of natural resources (Cowie et al., 2022). While the loss of species has driven massive amounts of research in the last few decades, there has also been a concerted effort to understand how such drastic changes to the landscape are affecting the plants and animals that are able to persist under such conditions. One particularly drastic way native habitat is modified is through urbanization. As we increasingly modify habitats to meet human needs, it continues to be vital to understand the effects of urbanization on various organisms- from community shifts down to an individual's physiology.

As urbanization continues to intensify and expand with more of the human populations around the world moving to urban areas (Seto et al., 2012), further investigating how this land use change alters animal communities, populations, and individuals is crucial to future conservation and research efforts. For example, communities across diverse taxonomic and trophic groups differ between urban and native habitats, from arthropods to birds, mammals, reptiles, amphibians, and bacteria. In many cases, this difference is a loss of diversity and abundance for each of these taxonomic groups. This, in turn, has also shifted interspecific interactions, from predator-prey relationships to parasitism, to direct competition over resources. Understanding how these relationships have been altered is vitally important for conservation purposes, as well as for research on how habitat type differentially affects different organisms. For example, it was known that birds across North America were declining (Wilcove and Terborgh 1984), and also that arthropod populations across the world were suffering precipitous declines (Seibold et al., 2019; Lister and Garcia 2018; Møller 2019), but Tallamy and Shriver (2021) connected these concepts. They found birds relying on insects as an essential part of their

diets have seen an average decrease in species-wide populations by approximately 9 million, while bird species that do not rely on insects as an important food source have actually seen slight population increases, highlighting the importance of understanding specific drivers of decline. Songbirds have been a focal group for understanding land-use changes on populations and community interactions such as predator-prey interactions and parasitism (Planillo et al., 2020; Lister and Garcia 2018; Devictor et al., 2007; Rodewald 2009; Knutie 2024). However, there are many responses to urbanization that do not result in population crashes and extirpation, but rather induce sublethal effects for individuals, altering diverse aspects of their physiology.

Individuals are not isolated from their environment, and any questions being asked of their physiology must take their habitat into account. On this individual level, landscape changes can have a wide range of effects on everything from immune response to hormone changes, oxidative stress, neural measures, erythrocyte metrics, growth and development rates, and multiple facets of nutrition (Isaksson 2015; Isaksson 2020; Sewall and Davies 2017; Andersson et al, 2015). Urbanization in particular can negatively affect these metrics in songbirds (Seress et al., 2018; Toledo et al., 2016; Heiss et al., 2009; Corsini et al., 2020; 1 species in Salmón et al., 2018; Meillère et al., 2015). However, across such a broad range of species and locations, there have also been many studies that find no differences in these physiological metrics by habitat type (Iglesias-Carrasco et al., 2020; Goodchild et al., 2022; 3 species in Salmón et al., 2018). Further clarification of how urban living affects individuals, especially across different life history stages, is necessary.

Urbanization does not affect all life stages equally. Developing young in particular require high levels of crucial nutrients to meet the high energetic and nutritional demands that rapid development requires. A restriction on nutrients during this time can lead to death in severe

cases (Almquist 1952; Baker 2009; Alagawany et al., 2021; Murphy et al., 1993), but sub-lethal effects can result in compromised development that persists into adulthood (Boonekamp et al., 2014; Searcy et al., 2004). Altricial songbirds, whose nestlings are fed by their parents for a period of time before they are able to leave the nest under their own volition, are a group of animals particularly well suited to evaluate the effects of restricted food access on development due to their rapid pace of growth, doubling their body mass multiple times before leaving the nest in only weeks (Ricklefs et al., 1973; Ricklefs et al., 1979). Understanding how populations can persist in urban environments requires evaluating how the most at-risk life stages are affected.

We used an established population of wild song sparrows (*Melospiza melodia*), to investigate how urbanization affects prey communities these birds rely on, and ultimately how that may affect nestling nutrition and development. Song sparrow nestlings need to grow from ~2g at hatch to ~15g at fledge in 10 days, and have decreased body mass in adulthood when their diets are experimentally restricted as nestlings (Searcy 2004). A key component of song sparrow diets during the breeding season, particularly for their nestlings, is arthropods. Many songbird species time their breeding seasons with arthropod emergence (Dunn 2004) because arthropods are very protein rich compared to other options, such as grains or fruit (Payne et al., 2016). A substantial amount of work has been done to understand how urbanization affects arthropod communities, and while there were general trends captured: urban areas typically reduce the abundance and diversity of their arthropod communities, and specifically reduce the number of high trophic level arthropods (Fenoglio et al., 2020; McIntyre 2000; Faeth et al., 2005; Bennett and Lovell 2014; Chatelain et al., 2023), other studies have found either no effect or even cases

of increased abundance in urban habitats for certain arthropod taxa (Chatelain et al., 2023; Benedict et al., 2021).

Not only do urban song sparrows have to contend with potentially poorer arthropod prey availability, they also have to tolerate brood parasites restricting the amount of food their song sparrow nestlings receive. Brood parasites are birds that forgo raising their own young in favor of laying their eggs in host nests, thereby shifting all of their parental care costs onto the host (Hamilton and Orians 1965). This parasitism ultimately has direct consequences for the host young, who are less likely to survive to fledge, or may fledge at a lower body mass compared to individuals from nests that are not parasitized, due to the increased competition for resources (Lorenzana and Sealy, 1999; Payne 1977). Prior work in our system has shown that rates of brood parasitism by the native brown-headed cowbird (*Molothrus ater*) are far higher at our urban sites, potentially acting as an additional level of resource restriction on developing urban sparrow nestlings that rural nestlings avoid (Lane et al., 2023).

Potentially reduced prey availability, dietary differences, and increased brood parasitism rates could result in compromised nutrition and growth for developing song sparrows in urban habitats. Adequate protein levels are necessary for tissue creation and maintenance in addition to many other biological functions, and therefore it is crucial for developing young to receive substantial amounts of protein. Understanding how the levels of amino acids, the building blocks of protein, may differ by habitat type in nestlings provides insight into the potential costs of environmental food restriction on developing song sparrows. A lack of essential amino acids, in particular, has been tied to compromised development and even death in other bird species (Alagawany et al., 2020; Hendriks 2003). Additionally, many amino acids have different roles, so quantifying a panel of them gives better insight into what nestlings may be lacking, which is

more precise compared to evaluating differences in whole protein. The majority of amino acid research in birds has been conducted in poultry, so observational work to quantify free amino acid concentrations in wild birds is largely unexplored (see Langlois and McWilliams 2021; Hebert et al., 2002 for exceptions). By combining any amino acid concentration differences with potential differences in body measurements, we can understand how urbanization and brood parasitism may be impacting developing song sparrows.

Chapter objectives:

For the first two chapters of my dissertation, I used field studies in southwestern Virginia to quantify how arthropod communities differ between urban and rural habitats, the effect this may have on nestling song sparrow diets, and ultimately, differences in development and a nutritional endpoint. The second chapter also investigates how cowbird brood parasitism acts as an additional constraint on nestling nutrition by measuring both body condition metrics and amino acid concentrations in song sparrow nestlings. The third chapter of my dissertation is a meta-analysis focusing on brown-headed cowbirds, namely how their presence affects host clutch sizes and nest success rates across different habitat types. Additionally, I investigated how cowbird clutch sizes, a proxy of fitness, differed by habitat type. Ultimately, I found that despite reduced arthropod food availability for urban birds, there were no differences in body size and minimal differences in circulating amino acid concentrations when comparing nestlings from different habitat types and parasitism status.

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Chapter 1: Differences in urban arthropod communities may not limit the nestling diet of a generalist songbird

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Abstract

Anthropogenic land use, including urbanization, has caused population declines across diverse taxa including arthropods and songbirds. Declines in one taxa can impact other groups based on its role in a community. In particular, declines in lower trophic level taxa, such as arthropods, could have negative impacts on higher trophic level species. Here we examined how urban arthropod communities compare to rural ones and how these differences may impact song sparrow (*Melospiza melodia*) nestlings living in urban and rural habitats. We quantified differences in the abundance, biomass, and diversity of arthropod communities between replicate urban and rural sites. At the same sites, we also compared the stomach contents of nestlings because song sparrows rely upon arthropod prey during development. We found that the arthropod community in urban habitats had lower average abundance, average biomass, and Simpson's diversity compared to rural habitats. The arthropod communities also significantly differed in the relative abundance of some higher trophic level taxa, such as spiders. However, we found no difference in the total stomach content mass, nor the mass of invertebrate food items in the stomachs of urban and rural nestlings. Thus, though urban habitats had lower availability of arthropods, there was no evidence of a negative impact on the quantity of food provided to urban song sparrow nestlings.

Introduction

Anthropogenic environmental change is the leading cause of wildlife population declines worldwide (Tilman et al., 2017). Land use change or altered disturbance regimes from human activities can result in local extinctions of some species. When taxa are lost, community

diversity is reduced, which can make other species within the community more vulnerable to disturbance (Elmqvist et al., 2003). The loss of taxa is often evident when charismatic species such as higher trophic level vertebrates decline. However, the loss of lower trophic level taxa, such as arthropods, can drive the decline of these charismatic species (Tallamy and Shriver 2021). Recent studies show precipitous declines in arthropod abundance worldwide. Across 10 years of sampling, a 67% decrease in arthropod biomass was observed in Germany (Seibold et al., 2019). Over 34 years of sampling in Puerto Rico, arthropod biomass declined ten to sixty-fold (Lister and Garcia 2018). A 10-year period in Denmark saw a greater than 80% decline in arthropod abundance (Møller 2019). These declines are often inferred to result from anthropogenic causes such as climate change and pesticide use, but additional factors such as land use changes can reduce arthropod populations (Attwood et al., 2008). The consequences of such declines in arthropod communities have yet to be fully realized (McIntyre 2000; Butchart et al., 2010).

Urbanization is predicted to increase by 1.2 million km^2 from 2012 to 2030 with dramatic consequences for wildlife (Seto et al., 2012). Studies have shown that urbanization not only reduces the abundance of many arthropod taxa, but also decreases the diversity of arthropod communities (Fenoglio et al 2020; Gossner 2016). Specifically, urbanization is associated with a reduced abundance of arthropods such as spiders, ground-dwelling beetles, and caterpillars (Seress et al., 2018; Martinson and Raupp 2013; Delgado et al., 2020). Changes in the arthropod community can have consequences for higher trophic level organisms. For example, the loss of pollinators or predators can reduce ecosystem services in urban habitats (Bennett and Lovell et al., 2014; Bates et al., 2011; Dale and Frank 2018). Additionally, the loss of high trophic level arthropod taxa can increase the abundance of herbivorous arthropods with consequences for the

entire food web (McIntyre et al., 2001), in addition to the loss of ecosystem services (Losey and Vaughan 2006). This loss of arthropod abundance and diversity can also have negative consequences for vertebrate predators such as insectivorous songbirds (Møller 2019; Planillo et al., 2020; Lister and Garcia 2018).

Songbirds might be especially vulnerable to arthropod declines because many species time breeding to coincide with insect emergence, allowing them to provide nestlings with high-protein diets critical to rapid development and fledging (Gray 1993; Davis et al., 2005). Indeed, across a gradient of urbanization intensity arthropod and bird abundance are positively correlated, suggesting that urban bird populations are limited by the availability of arthropod prey (Planillo et al., 2020). Further evidence that declining arthropod abundance has negative consequences for songbirds is that lower arthropod abundance is linked to lower nestling body mass (yellow hammers, *Colaptes auratus,* Hart et al., 2006) and compromised fledging success (winchats, *Saxicola rubetra*, Britschgi et al., 2006). Understanding how variation in arthropod abundance in urban areas impacts songbirds requires determining how arthropod communities relate to nestling diet.

In this study we compared arthropod abundance, biomass, and community diversity across replicate urban (n=3) and rural (n=3) sites in Southwestern Virginia, USA. We expected to see lower arthropod abundance and biomass in urban areas. We especially expected lower abundance and biomass of high trophic level orders such as spiders (Lindeman 1942). We also expected arthropod community diversity to be lower in urban areas (Chiari et al., 2010). To begin to explore the consequences of variation in arthropod communities for predators we also compared the stomach contents of nestling song sparrows (*Melospiza melodia*) from those same sites to determine how their diet differed. We expected urban nestling stomachs to contain less

arthropod mass compared to rural nestlings. Additionally, we expected urban nestlings to have fewer higher trophic level taxa in their stomachs than rural birds.

Methods

Study Area

We sampled arthropods and song sparrow nestlings from 3 urban and 3 rural sites near Blacksburg, Virginia, a temperate location in the Appalachian Mountains. These established study sites (Goodchild et al., 2022; Lane et al., 2021; Davies et al., 2018; Davies and Sewall 2016), are characterized as the most urban or rural sites along an urbanization gradient, based on the quantification of urbanization described in Seress et al., 2014. Briefly, mean building density, number of cells with high building density, number of cells with paved surface, mean vegetation density, and number of cells with high vegetation density were calculated over 1 km^2 grids broken into 100 m x 100 m cells. (Davies and Sewall 2016). Principal component (PC) analyses were then used to select the most urban and most rural sites. Our urban sites are local university campuses, while our rural sites include an experimental farm, a riparian restoration site, and a community park. The density of song sparrow adults at our rural sites was 117.53 per km^2, while urban habitat had 95.77 adults per km^2. We found 85.06 nests per km^2 in rural habitat, and 96.3 nests per km^2 in urban habitat (likely because urban parents were more likely to have multiple nests per year; Appendix Tables A9 and A10; Lane et al., 2023).

Arthropod Sampling

We collected arthropods along transects using vacuum sampling. Four, fifty-meter transects were selected at each site, for a total of twelve rural and twelve urban transects (see Figure 1 for examples). Each transect was sampled three times per year during the spring and summer in 2020 and 2021. In 2021 these transects were sampled once at the start of each month from May-July (Bennett and Lovell 2014), but due to disruptions caused by COVID-19, sampling in 2020 occurred mid-May, early June, and early July. Starting locations for each transect were based on randomly selected song sparrow nests found in 2019. If the starting location was within 100 m of another transect, we randomly selected a different nest. Headings were then created using a random number generator, which were reselected if they met one or both of the following criteria: 1) part of the transect would be impassable (e.g., it would go through a building), or 2) more than 50% of the transect was impermeable surface (e.g., pavement). Any hazards along the transect were skipped, and this distance was added to the end of the transect. For example, a transect with 8 m of road would be 58 m long, although only 50 m would be sampled. Transects were subdivided into five 10 m long sections to prevent the vacuum from becoming blocked by debris.

We conducted vacuum sampling (Buffington and Redak 1998; Perner et al., 2005) using a Black and Decker leaf blower with the included vacuum attachment on its highest power setting (120 MPH 90 CFM 40V MAX Lithium-Ion Cordless Handheld Leaf Sweeper/Vacuum). Paint strainer bags (1 gallon) were fastened to the opening of the vacuum using an elastic band. One site was sampled per day starting at 10 am, although start times were pushed back until any visible dew evaporated, or pushed to the next day in the event of rain. Date, ambient temperature,

start time, and transect duration (to control for observer effort) were all recorded. All transects were sampled by the same observer. Each step by the observer was accompanied by a 180° sweep of the vacuum, keeping the tip against the substrate. Samples were stored at -20°C within three hours of collection until processing.

To quantify arthropods, samples were removed from their bags, and arthropods were manually separated from any debris (grass, mulch, trash) and then sorted into order (Araneae, Diptera, Lepidoptera, Coleoptera, Isopoda, Orthoptera, Hymenoptera, Hemiptera, and 'other'). Each order was then individually counted and weighed (Fisher Science Education ALF64 balance) to the nearest ten-thousandth to determine wet mass.

Stomach Contents Assessment

In the summer of 2022, we collected 20 song sparrow nestlings from rural nests and 40 nestlings from urban nests from the same sites from which we sampled arthropods. Briefly, we located nests using behavioral observation and systematic searching (Lane et al., 2023), and collected nestlings between 5 and 10 days old (average of 6.9 days old). Two nestlings were collected from each nest when possible (16 rural and 30 urban nestlings); in some nests, only a single nestling was collected (1 rural, 7 urban nestlings), and others had three individuals collected (3 rural and 3 urban nestlings). Within 5 minutes of parents visiting the nest with food, nestlings were euthanized via a lethal dose of inhaled isoflurane and stored at 4°C for approximately 24 hours before we dissected out the proventriculus and gizzard and stored their contents in Eppendorf tubes at -80°C. We then massed stomach contents to the nearest ten-thousandth of a gram and invertebrate food items were separated from debris and vegetation using a dissecting microscope (Leica MZ7). We sorted invertebrates into taxonomic order

(Araneae, Diptera, Lepidoptera, Coleoptera, Hymenoptera, Hemiptera, Gastropoda, and 'unidentifiable') and counted individuals conservatively (e.g., eight separate spider legs, a cephalothorax, and an abdomen would be counted as a single spider, while eight cephalothoraxes would be counted as eight individuals). Invertebrates outside of the listed orders and parts that could only be identified as invertebrates due to damage were categorized as 'unidentifiable.' We measured the total wet mass of all invertebrate food items to the nearest ten-thousandths of a gram.

Data Analysis

Arthropod analysis

We conducted all analysis using Program R (R version 4.2.2; R Core Team 2023). We analyzed the arthropod data with linear mixed effects models (LMMs) using the 'lme4' package (version 1.1.31; Bates et al., 2014). In each model, site ID and/or transect ID were the random effects, while habitat type, Julian date, and year were fixed effects. We ran two separate LMM's to test for habitat differences in average arthropod abundance and average arthropod biomass, respectively. The residuals from initial models were not normally distributed, so we applied a square root transformation to both datasets. Additionally, we ran multiple LMMs to test for habitat differences in the biomass of specific taxa, and generalized linear mixed models (GLMMs) to test for habitat differences in the abundance of specific taxa. Models assumptions were checked using the 'performance' package (version 0.10.5; Lüdecke et al., 2021).

We calculated Simpson's diversity of each site based on vacuum sampling using the package 'phyloseq' (version 1.42.0; McMurdie and Holmes 2013). Simpson's diversity between habitat types was then compared using Kruskal-Wallis chi square testing. We performed PERMANOVA testing of Bray-Curtis matrices using the function 'adonis2' from the package

'vegan' (version 2.6-4) to determine differences in relative abundance by habitat type with site as a fixed effect (Dixon 2003). Each PERMANOVA had 999 permutations.

Stomach content analysis

We used three separate models to test for habitat differences in (1) average nestling stomach content mass, (2) average invertebrate food item mass, and (3) average abundance of all invertebrate food items. Nestling age was included as a fixed variable, and nest of origin was included as a random effect to account for possible correlations among nestlings from the same nest. Differences in stomach content mass (square root transformed) and invertebrate food mass (square root transformed) were tested using LMMs, while abundance was tested using a negative binomial generalized linear model (GLM).

We additionally tested if individual invertebrate orders differed in average abundance across habitats using negative binomial GLMs. Finally, we tested for habitat differences in the presence of invertebrate orders in nestling stomach contents using multiple generalized linear mixed-effects models (GLMMs) with a binomial distribution to indicate the presence or absence of a given order. Once again we included nest origin as a random effect in these models.

Results

Arthropod Community Results

Rural and urban arthropod communities differed significantly in abundance, biomass, and diversity. We found that average arthropod abundance was higher in rural habitats than urban

(Figure 2) (SE = 0.16, df = 22, t value = -5.415, Pr(>|t|) <0.001). There was no effect of Julian date or year on the average arthropod abundance. Rural habitats also had significantly higher average biomass (Figure 2) (SE = 0.103, t value = -4.298, Pr(>|t|) = 0.013) than urban habitats. There was a significant positive effect of Julian date (SE = <0.001, df = 4, t value = 3.80, Pr(>|t|) <0.001), but no effect of year.

When we compared specific orders across habitat types we found that abundance and biomass were significantly higher in rural habitats for Araneae, Coleoptera, Lepidoptera, Orthoptera, and Hemiptera. The exceptions to this trend were Diptera, Hymenoptera, and 'Others,' which did not differ significantly in abundance between habitat types, and Isopoda, which did not differ significantly in biomass or abundance between habitat types (Table S1 in Appendix S1).

Simpson's diversity at the taxonomic level of order was significantly higher in rural habitats compared to urban ($p < 0.001$). We also found relative abundance of arthropods differed significantly between habitat types ($R^2 = 0.22$, $F = 40.97$, $Pr(>F) = 0.001$) and among sites ($R^2 = 0.055$, $F = 2.60$, $Pr(>F) = 0.002$).

Stomach Content Results

We found no significant difference in total stomach content mass (SE = 0.054, t value = 0.261, Pr(>|t|)=0.796), or invertebrate food item mass (SE = 0.03, t value = 0.987, (Pr(>|t|) = 0.332) between urban and rural song sparrow nestlings. However, the abundance of invertebrate food items, and the abundance of specific invertebrate orders differed by habitat. Specifically,

urban nestlings had a higher average abundance of invertebrate food items in their stomachs compared to rural nestlings (SE = 0.256, z value= 2.127, Pr(>|z| = 0.033). However, rural nestlings had a greater abundance of Araneae (SE = 0.586, z value = -2.953, Pr(>|z|) = 0.003) and Lepidoptera (SE = 0.508, z value = -1.975, Pr(>|z|) = 0.048), while urban nestlings had a higher abundance of Coleoptera (SE = 0.171, z value = 3.19, Pr(>|z|) = 0.001), Hymenoptera (SE = 0.505, z value = 2.93, Pr(>|z|) = 0.003), and Gastropoda (SE = 0.558, z value = 2.129, Pr(>|z|) = 0.033). There were no significant differences between urban and rural nestlings in the abundance of Hemiptera (SE = 0.835, z value = 0.483, Pr(>|z|) = 0.629) or 'unidentifiable' arthropods (SE = 0.247, z value = 1.043, Pr(>|z|) = 0.230), (Table S2 in Appendix S1). Finally, we found no significant differences in the presence or absence of individual invertebrate orders by habitat type (Table S3 in Appendix S1).

Discussion

Urbanization often lowers the abundance and biomass of some taxa, which can reduce community diversity (Fenoglio et al., 2020). Here we examined how urbanization impacts the arthropod community and the diet of nestling song sparrows, which rely on arthropods, by comparing replicate urban and rural sites in Southwestern Virginia. We found lower average abundance, biomass, and diversity of arthropods in urban habitats. Despite this lower biomass and abundance of arthropod prey in urban areas, we found no difference in stomach content mass between urban and rural nestlings. Thus, in our study system we found no evidence that developing songbirds are negatively impacted by lower available arthropod abundance or biomass because urban birds may compensate by feeding their young similar quantities of different prey items. Thus, the effects of urbanization we measured on arthropod abundance and

biomass may not be of sufficient magnitude to negatively impact nestling song sparrows, especially because they are generalists and capable of shifting their diet in urban areas to cope with lower availability of arthropod prey.

Lower arthropod abundance and biomass in urban areas

We found that urban habitats had lower average arthropod abundance and average biomass than rural habitats (Figure 2). Of note, the biomass and abundance of Araneae, Coleoptera, Lepidoptera, Orthoptera, and Hemiptera were all lower in urban habitats compared to rural. Lower abundance and biomass of arthropods could result from differences in resource availability, or disturbance. Urban areas often have fragmented habitat and less greenspace (Liu et al., 2016), which can reduce available resources for arthropods and limit which arthropod taxa are present (McIntyre 2000; Philpott et al., 2014; Shochat et al., 2004). In contrast, rural communities often have access to extensive green space and are typically not as limited by resource availability, except for some desert habitats (Faeth et al., 2005; Miles et al., 2019). Urban habitats are also often characterized by more frequent human disturbance, such as lawn mowing or pesticide use, which has been shown to reduce arthropod abundance (Sattler et al 2010; Siviter et al., 2023) or, in some cases, to increase the prevalence of herbivorous arthropod species while reducing the prevalence of higher trophic level arthropod predators (Raupp et al., 2010; Szczepaniec et al., 2011). Our findings therefore contribute to growing evidence that urbanization negatively impacts arthropod abundance and biomass. Our finding of lower abundance and biomass of Araneae is of particular interest because lower abundance of such predator taxa suggests there is less prey available to support arthropods at higher trophic levels

(Shochat et al., 2004). Additionally, a lower abundance of high trophic level arthropods could, in turn, limit resources for vertebrate predators. High trophic level arthropods are often higher in protein, which could be especially important to vertebrate predators (Kohl et al., 2015). This is true for songbirds, as many species rely upon higher trophic level arthropods, such as Araneae, and other preferred orders such as Lepidoptera, to feed their young (Seress et al., 2018; Kaspari and Joern 1993; Cowie and Hinsley 1988). Loss of high trophic level arthropod prey has detrimental effects on songbirds ranging from reduced breeding success to worsened offspring body condition and reduced population sizes (Illera and Diaz 2006; Grames et al., 2023; Planillo et al., 2020). Though prior studies in our system have found higher nest success in urban areas due to reduced nest predation (Lane et al., 2023), it is not yet clear if altered arthropod abundance could impact nestling condition and future survival and reproduction.

Arthropod diversity differs by habitat

Our urban arthropod community has lower Simpson's diversity than our rural community. This is consistent with a recent meta-analysis concluding that urban areas have less diverse arthropod communities than rural ones (Fenoglio et al., 2020), although there are often taxon specific exceptions (Jones et al., 2012; Chatelain et al., 2023). A limitation to our approach is that we sorted arthropods by order, which underestimates diversity that could exist within orders. However, other studies characterizing arthropods by family or genus also found lower arthropod diversity in urban habitats (Bang and Faeth 2011; Chatelain et al., 2023). Lower community diversity is associated with lower community resilience due to less redundancy of functional niches (Elmqvist et al., 2003; Mori et al., 2013). This in turn has the potential for

effects throughout the entire community from producers to vertebrate predators, as arthropod diversity often influences the success of other taxa (Bowler et al., 2019; Bennett and Gratton 2013).

We also found differences between urban and rural arthropod communities based on the relative abundances of taxa present (Figure 3), which is not surprising given the other documented differences. Differences in relative abundance could be driven by factors outside of the scope of this project such as microsite diversity, producer biomass, or interspecies interactions (Philpott et al., 2014; Greenberg and McGrane 1996; Perner et al., 2005; Miles et al., 2019). These differences in diversity suggest that the urban arthropod community could be at greater risk of further disturbance (Tilman 1996), which could in turn put vertebrate predators in urban areas at greater risk of population decline.

Nestling stomach content, but not mass of food items, differs by habitat

There were no significant differences in urban and rural nestling stomach content mass, nor was there a difference in the mass of invertebrate food items (Fig. 4). Urban nestlings did have a significantly higher average abundance of invertebrate prey items in their stomachs, suggesting that more items were required to reach stomach mass equal to that of rural birds. The finding that the mass of stomach contents did not differ between habitat types suggests that song sparrow parents provide similar amounts of food to their offspring in both habitats, though the types of arthropods may differ. Prior studies of nestling diets have reported that songbirds rely upon spiders as a rich source of protein (Razeng and Watson 2015; Ramsay and Houston 2003; Wiesenborn 2011). We found that the stomachs of rural nestlings had significantly more spiders and caterpillars than those of urban nestlings (Appendix S1 Table S3). The stomachs of urban

nestlings contained significantly more beetles, however, also have high concentrations of protein (Ramsay and Houston 2003). Urban nestlings also had a greater average number of ants in their stomachs than rural birds. Though we did not quantify Gastropods in the arthropod community, we found that urban nestlings did have a higher average number of Gastropods in their stomachs than rural nestlings (Appendix S1 Table S3). Thus, though we found lower abundance and biomass of arthropods in urban habitats, our analysis of song sparrow nestling stomach contents shows that birds in both habitats have equal masses of invertebrate prey. It is important to note that, though songbird nestlings rely upon high-protein invertebrate foods during development (Birkhead et al., 1999), song sparrows are a generalist species that may more readily adjust their diet to different invertebrate taxa (Stofberg et al., 2019). Studies of specialist species may yield very different findings, and the consequences of any adjustments in the diet of urban nestlings for reproduction and survival remain unknown.

Conclusion

Urbanization threatens a wide variety of wildlife. Understanding the consequences of urbanization for wildlife requires research across levels of biological organization from individual condition and fitness to impacts on population size and community structure. Measuring the impacts of urbanization on lower trophic level taxa, such as arthropods is crucial, as the loss of lower trophic level organisms can have consequences for both their predators and entire communities (Tallamy and Shriver 2021). However, changes in lower trophic levels may need to reach a tipping point before consequences are seen for higher trophic level predators. In this study, we did not find any differences in the mass of stomach contents of nestling song

sparrows despite a lower abundance of arthropods in urban habitats relative to rural ones. Though we found no evidence of negative consequences of altered arthropod communities for the diet of song sparrow nestlings, future work on diverse predator species and community dynamics is critical to understanding the consequences of urbanization across trophic levels.

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Figures

Figure 1: Examples of 50 m transects shown in yellow in both urban (left) and rural (right) habitats. Satellite imagery obtained from Google Earth (version 10.38.0.0).

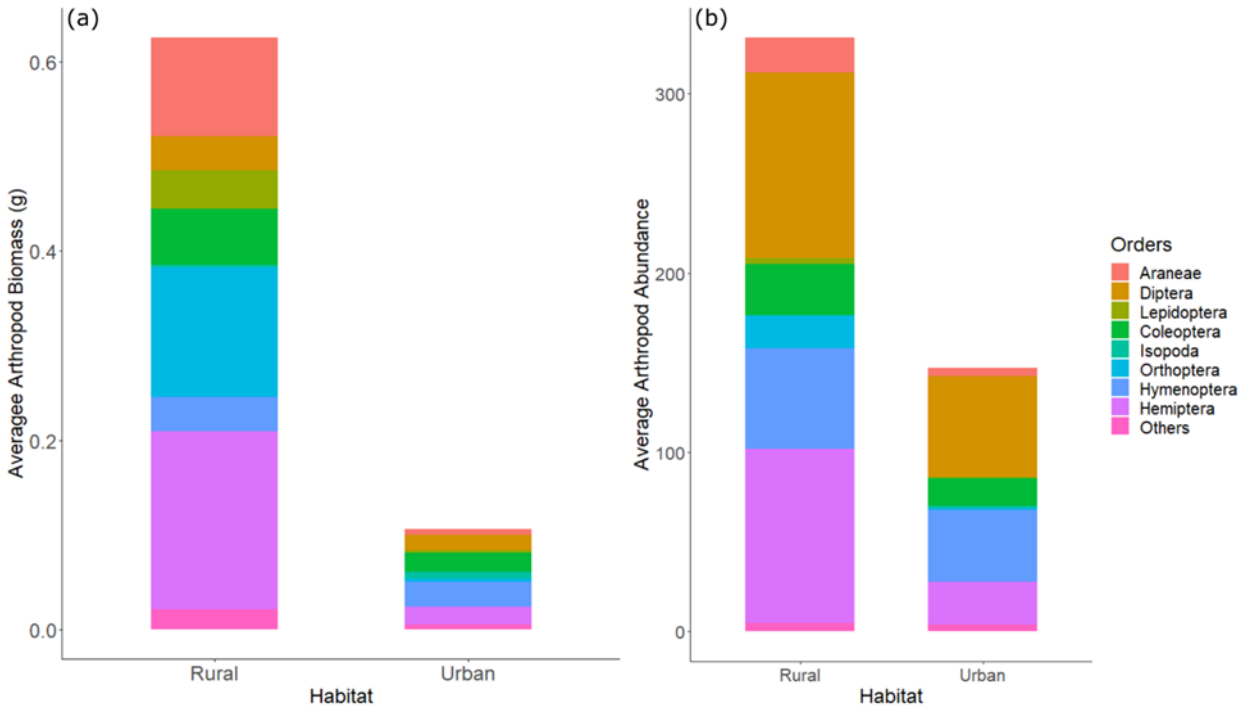

Figure 2: Average arthropod biomass in grams (a) and abundance quantified as the average number of arthropods per transect (b) was lower in rural habitats than urban habitats. Stacked bars indicate the biomass and abundance of each taxa.

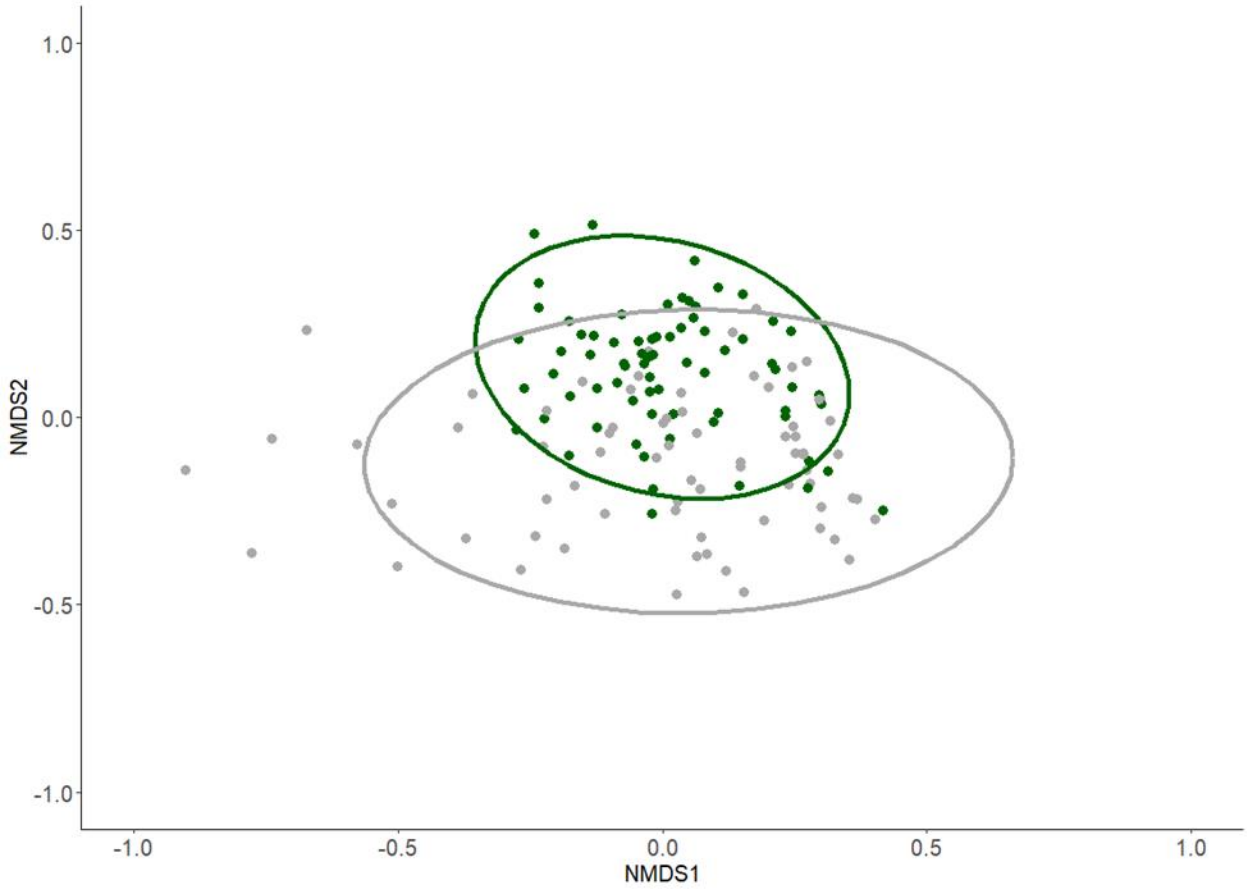

Figure 3: Non-metric Multidimensional Scaling (NMDS) plot of rural (green) and urban (gray) arthropod communities. The composition of arthropod communities differed between habitat types as measured through a Bray-Curtis dissimilarity matrix, which describes differences in the relative abundances of taxa within the community.

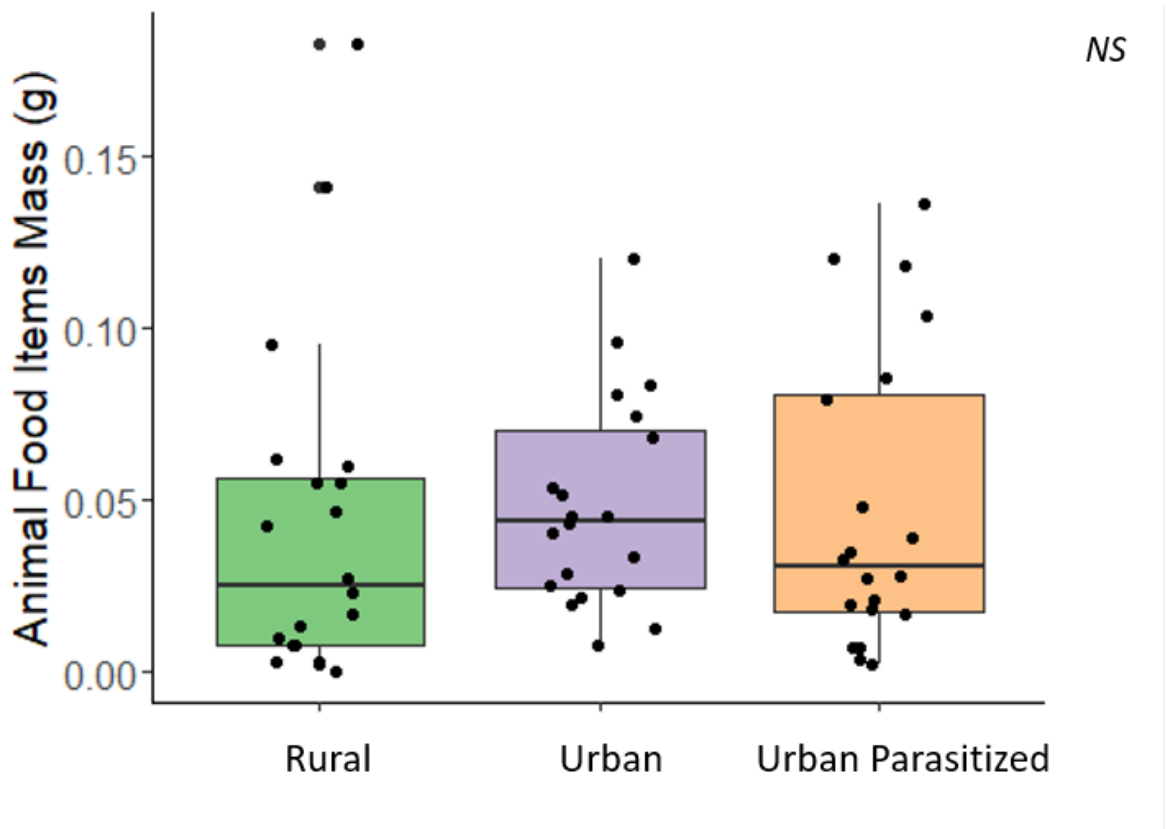

Figure 4: Average mass of invertebrate food items in stomachs across our three nestling groups. Median is shown for each nestling group. We found no significant differences in average mass across groups.

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Appendix

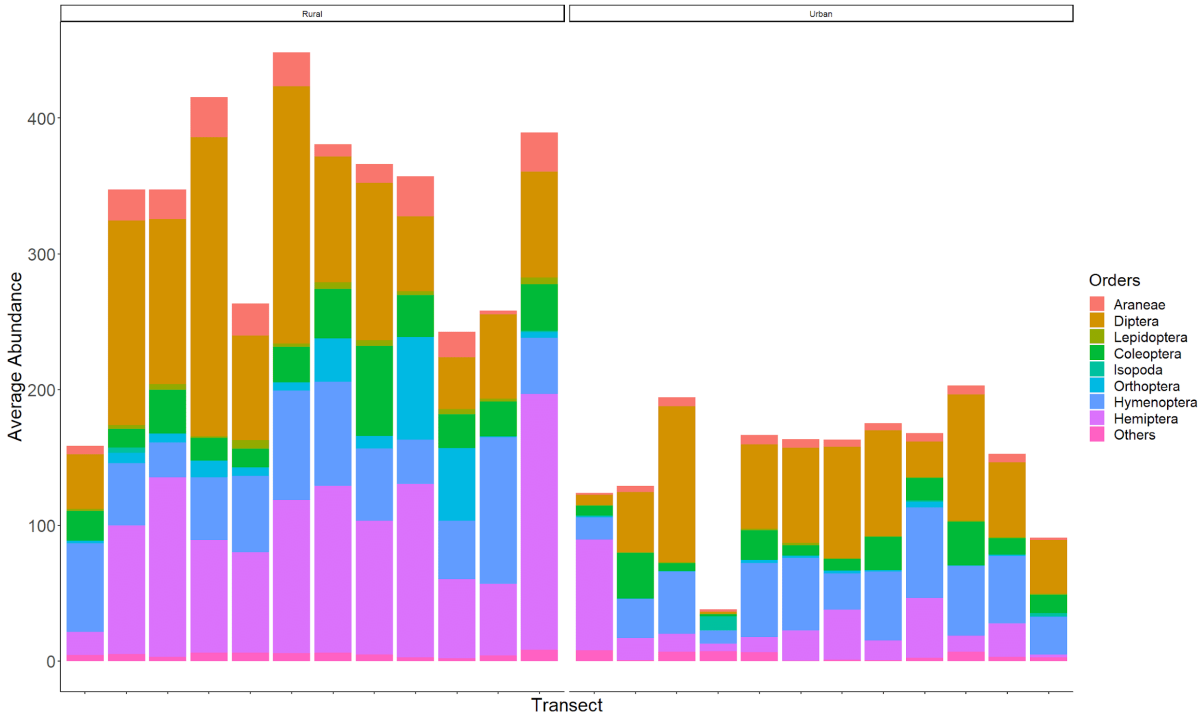

Figure A1: Arthropod Abundance by Transect. Average arthropod abundance for each transect divided into rural and urban habitat. Each transect was replicated a total of 6 times across 2020 and 2021

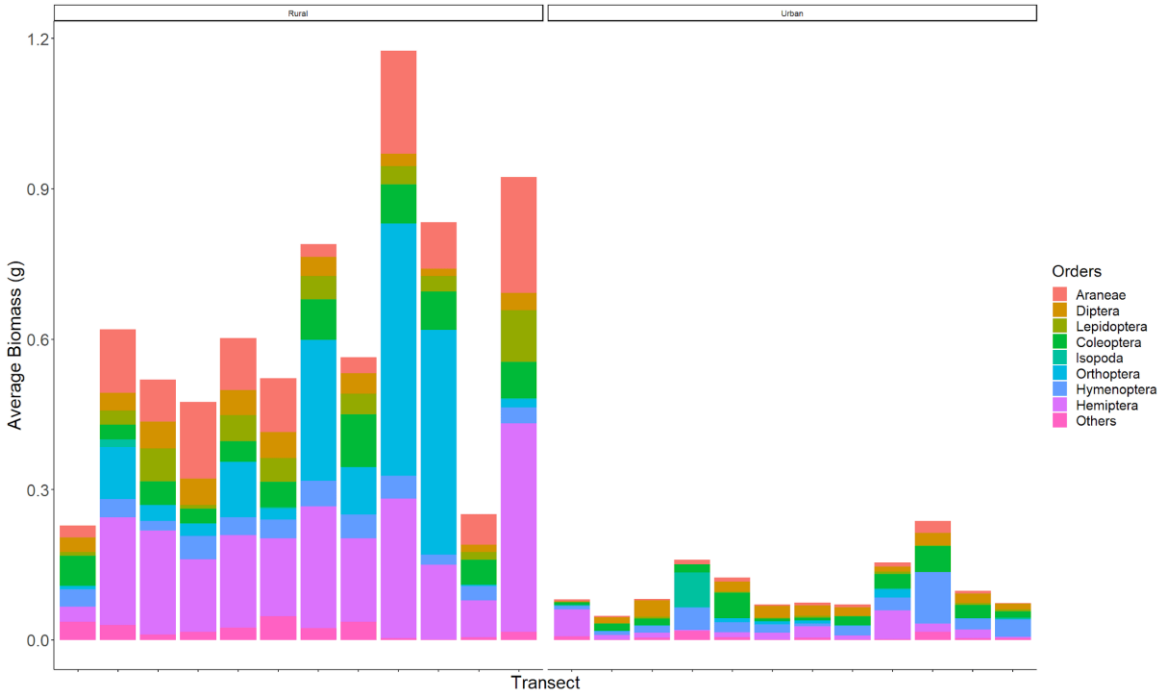

Figure A2: Arthropod Biomass by Transect. Average arthropod biomass for each transect divided into rural and urban habitats. Each transect was replicated a total of 6 times across 2020 and 2021

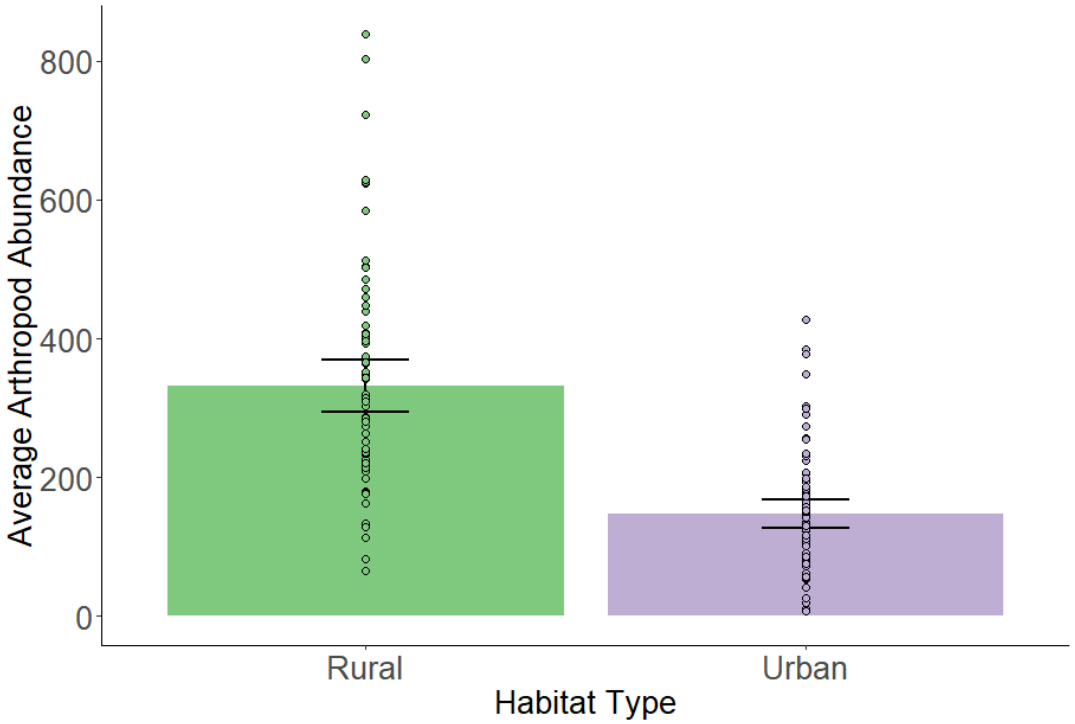

Figure A3: Average Arthropod Abundance by Habitat Type. Average arthropod abundance of all arthropods by habitat type featuring 95% confidence interval whiskers. Each point represents the number of arthropods found on a transect.

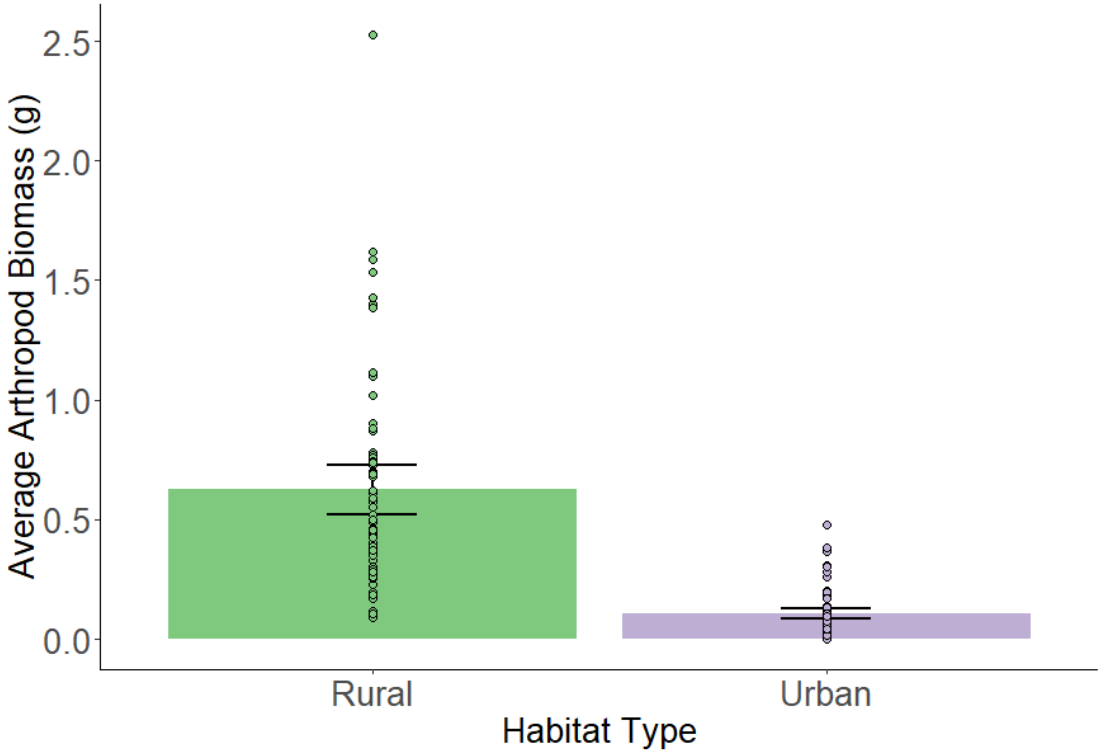

Figure A4: Average Arthropod Biomass by Habitat Type. Average arthropod biomass of all arthropods by habitat type featuring 95% confidence interval whiskers. Each point represents the mass of arthropods found on a transect.

Table A1: Individual Order Model Outputs for Abundance and Biomass. Differences between habitat type in arthropod abundance (left) and biomass (right) by order via generalized linear mixed effects and linear mixed effects models respectively. Negative z or t values indicate urban nestlings had either lower abundance or biomass of a given order compared to rural birds.

	Abundance			**Biomass**		
Order	SE	Z Value	Abundance (Pr(>|z|))	SE	T value	Mass (Pr(>|t|))
Araneae	0.241	-7.425	**1.13e-13***	0.010	-7.254	**2.88e-07***
Diptera	0.250	-1.551	0.12096	0.018	-3.722	**0.00118***
Lepidoptera	0.375	-5.128	**2.94e-07***	0.018	-7.132	**3.75e-07***
Coleoptera	0.254	-4.161	**3.17e-05***	0.019	-5.967	**5.27e-06**
Isopoda	0.938	1.517	0.1293	0.018	0.258	0.799
Orthoptera	0.453	-6.323	**2.57e-10***	0.551	-3.566	**0.00173***
Hymenoptera	0.200	-0.895	0.3706	0.018	-2.146	**0.0432***
Hemiptera	0.272	-0.934	**1.25e-07***	0.040	-7.306	**2.57e-07***
Others	0.330	0.700	0.48415	<0.001	3.496	**0.001***

Table A2: Invertebrate Abundance in Stomachs by Habitat. The difference in abundance of various orders in stomachs by nestling habitat type. Values are urban nestlings in relation to rural (negative z value indicates urban birds have fewer of a given order). Values following order names are total individuals identified across all 60 nestling stomachs.

Order	SE	Z Value	Pr(>|z|)
Araneaea (19)	0.586	-2.953	**0.00315***
Lepidoptera (33)	0.508	-1.975	**0.0483***
Coleoptera (208)	0.171	3.19	**0.00143***
Hymenoptera (199)	0.5051	2.93	**0.00339***
Hemiptera (20)	0.835	0.483	0.629
Unidentifiable Arthropods (122)	0.247	1.043	0.29677
Gastropoda (51)	0.558	2.129	**0.0332***

Table A3: Presence/Absence of Each Invertebrate Order Found in Stomachs. The presence of each order within individuals stomachs by habitat type. No significant results were found.

Order	SE	Z Value	Pr(>|z|)
Araneaea	1.021	-1.783	0.0746
Lepidoptera	1.179	-1.138	0.255
Coleoptera	0.958	1.262	0.20679
Hymenoptera	0.793	1.734	0.0828
Hemiptera	2.153	-0.365	0.7148
Unidentifiable Arthropods	0.930	-0.358	0.720
Gastropoda	0.606	1.648	0.0993

Table A4: Total Arthropod Abundance by Habitat Type

Response Variable:	Arthropod abundance	
Random effect	Variance	SD
Transect ID	3.275	1.810
Residual	12.753	3.571
Number of Obs: 144	Groups:	Transect.ID, 24

Fixed Effects	Estimate	SE	Df	t value	P value
Intercept	14.323	2.338	136.027	6.125	**<0.001**
Habitat Type: Urban	-6.122	0.949	22.010	-6.452	**<0.001**
Julian Day-of-year	0.021	0.014	120.118	1.512	0.133
Year	-0.451	0.607	118.125	-0.743	0.459

Table A5: Total Arthropod Biomass by Habitat Type

Response Variable:	Arthropod biomass		
Random effect	Variance	SD	
Transect ID	0.002	0.049	
Site ID	0.014	0.120	
Residual	0.025	0.157	
Number of Obs: 144	Groups:	Transect.ID, 24	Site.ID, 6

Fixed Effects	Estimate	SE	Df	t value	P value
Intercept	0.830	0.559	0.018	1.540	0.127
Habitat Type: Urban	-0.441	0.103	4.000	-4.298	**0.013**
Julian Day-of-year	0.002	0.006	0.018	3.804	**<0.001**
Year	-0.041	0.027	0.018	-1.534	0.127

Table A6: Total Invertebrate Abundance in Nestling Stomachs

Response Variable: Invertebrate abundance

Fixed Effects	Estimate	SE	z value	P value
Intercept	2.355	0.410	5.743	**<0.001**
Habitat Type: Urban	0.564	0.196	2.882	**0.004**
Nestling Age	-0.055	0.055	-0.995	0.320

Chapter 2: Neither urbanization nor brood parasitism correlate with free amino acid concentrations in nestling song sparrows

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ABSTRACT

Unifying the fields of urban ecology and ecophysiology will inform understanding of how changes in land use, such as urbanization, can have impacts across levels of biological organization from molecules, to individual organisms, to the community. Energy transfer between levels of biological organization is a key mechanism linking components of food webs and could ultimately link the effects of urbanization on ecosystems and communities to impacts on the condition and fitness of individual organisms. The effects of anthropogenic-induced changes across levels of biological organization may be most evident in species and individuals facing higher nutritional demands, such as developing animals in periods of rapid growth. For example, songbirds in urban areas both have access to less arthropod prey and experience increased brood parasitism rates compared to birds in rural habitats. Such reduced prey and higher brood parasitism could result in restricted nutrition, especially for nestlings. Limited protein may be of particular importance due to the role of amino acids in tissue formation. To explore the possibility that the effects of urbanization at the community level could alter individual body condition and health through nutritional dynamics, we compared plasma amino acid concentrations in nestling song sparrows (*Melospiza melodia*) from urban and rural sites. We took body measurements and collected nestling plasma samples from three categories of nests: rural, urban without brood parasites, and urban with brood parasites. We then used liquid chromatography-mass spectrometry to determine concentrations of free essential (EAA) and non-essential (NEAA) amino acids. We found no differences in concentrations for EAAs among groups, but did find that gamma-aminobutyric acid, an important neurotransmitter, was lower in urban parasitized nestlings. We found no differences in body condition measures among groups,

indicating that lower arthropod availability and increased brood parasitism in urban areas did not have any significant effects on nestling body size or amino acid profiles.

1. Introduction

Anthropogenic environmental change has impacts across levels of biological organization. For example, the effects of urbanization have been documented on biological processes ranging from ecosystem services to cellular processes (Isaksson 2015; Rosenberg et al., 2022; Goodchild et al., 2022). Fully understanding the consequences of rapid environmental change, such as urbanization, therefore requires studies across levels of biological organization. Ecophysiologists have examined the effects of urbanization on the physiology and condition of animals in modified habitats (Iglesias-Carrasco et al., 2020; Heiss et al., 2009; Isaksson 2020; Meillere et al., 2015; Chatelain et al., 2021; Partecke et al., 2006). A next step in this work is to link studies of physiology with ecological research examining the impacts of urbanization on higher levels of biological organization such as species interactions and community dynamics (Fenoglio 2020; Faeth et al., 2005; Bang et al., 2012). Unifying ecophysiology and urban ecology to examine how individual physiology is impacted by higher-level processes will improve predictions about the effects of anthropogenic change. For example, species interactions, such as predator-prey (Fischer et al., 2012; Planillo et al., 2020; Bennett and Lovell 2014) and parasite-host dynamics (Werner and Nunn 2020; Dautel and Kahl 1999; Fenoglio et al., 2017) are impacted by urbanization. Both of these relationships have clear implications for individual condition and survival, as insufficient prey or excessive parasitism can compromise condition and fitness. This may be especially true for individuals within a population that have the greatest energetic demands, such as developing animals. Though most studies of urbanization

focus on adult animals, developing young might be most vulnerable to ecological conditions, and their physiology is therefore more likely to reflect disruptions at higher levels of biological organization.

The effects of environmental conditions on developing songbirds have been studied extensively because nestlings go through a rapid period of post-hatching growth, relying upon their parents for food and thermoregulation (Monaghan 2007; Gil et al., 2019; Wingfield et al., 2017; Cockburn 2006; Schmidt et al., 2012). Limited nutrition or exposure to other stressors during this critical period can compromise nestling survival and have life-long effects on condition and fitness (Martin 1987; Ricklefs 1979; MacDonald et al., 2006; Searcy et al., 2004; Nowicki et al., 2002). In addition to adequate calories, nestling songbirds require adequate amino acids to build protein, which is critical to tissue formation and growth (Chandel 2021; Wu et al., 2012). Deficits of essential amino acids, the group of amino acids that cannot be synthesized and must be acquired through diet, can result in consequences such as reduced body size, deformities, and death (Almquist 1952; Baker 2009; Alagawany et al., 2021; Murphy et al., 1993; Atasoglu et al., 2003). Though the impacts of protein deficits and other aspects of 'developmental stress' on songbirds have been studied in both field and lab settings, relatively few studies have examined nutritional stress in the context of anthropogenic change (though see Harvey et al., 2021; Heiss et al., 2009; Seress et al., 2018).

Two ecological conditions in urban areas raise the possibility that urbanization limits the availability of essential amino acids, and thus could compromise the physiology and condition of urban nestlings. First, there are fewer arthropod prey available in urban areas and arthropods are a critical source of protein (Fenoglio 2020; Chatelain et al., 2023; Simpson et al., 2015; Razeng and Watson 2015). Second, some urban areas have higher densities of brood parasites, such as

brown-headed cowbirds (*Molothrus ater*), which are birds that lay their eggs in the nests of other species to be raised by host parents (Rodewald 2009; Chace et al., 2003). The presence of a brood parasite in a nest can limit food and induce nutritional stress through competition (Payne 1977; Ladin et al., 2015) in a manner similar to sibling competition (Boonekamp et al., 2014; Bourgeon et al., 2011). Host nestlings that survive the presence of a brood parasite are generally smaller and less developed when they fledge (Dearborn et al., 1998; Lorenzana and Sealy 1999). Previous ecophysiology studies found that nestlings in urban areas have reduced body size (Heiss et al., 2009; Liker et al., 2008), reduced fledging success (Chatelain et al., 2021; Bailly et al., 2016), and even have smaller brain regions associated with song learning (Potvin et al., 2016). All of these physiological consequences could be linked to urbanization altering higher levels of biological organization including prey availability and increased pressure from brood parasites. Further, reduced arthropod prey and brood parasitism could negatively impact nestling growth in urban habitats through restricted availability of amino acids.

The availability of amino acids and the prevalence of specific amino acids could be important for nestling growth, and deficits of nearly any amino acid can compromise nestling condition. The essential amino acids (Table S1) and especially lysine, methionine, and arginine are important for muscle development and growth (Tesseraud et al., 2000; Langlois et al., 2018; Chen et al., 2013; Klasing 1998). Other amino acids are not considered essential because they can be synthesized, but supplementation suggests they have benefits for early development. For example, taurine is suspected to be important to the early development of wild blue tits (*Cyanistes caeruleus*) because they are known to feed their young a large number of taurine-rich spiders around days 5-6 of development (Ramsay and Houston 2003; Surai et al., 2020). Additionally, glycine functions as a precursor to many proteins, particularly creatine (Hans et al.,

1955), and is essential during development in birds because young fail to endogenously produce enough glycine to meet metabolic demands (Siegert and Rodehutscord 2019; Li and Wu 2018). Animals also benefit from non-essential amino acids obtained through diet as they can supplement endogenous synthesis to meet physiological demands (Wu et al., 2012). Thus, very generally for developing birds, deficits of essential amino acids can be catastrophic, limited availability of any amino acid has the potential to cause problems, and higher amino acid concentrations are generally better. Collectively, the finding that urbanization is associated with reduced arthropod prey, increased brood parasitism, and poor nestling growth and survival raises the hypothesis that the impacts of urbanization could be mediated by changes in species interactions that have consequences for nestling physiology, and amino acid availability could be a mechanism that could mediates this flow of effects across levels of biological organization.

While the majority of work on amino acids in birds has focused on laboratory work, there has been some research into how environmental conditions can alter the amino acids of wild birds. For example, Hebert et al., (2002) found that herring gulls (*Larus argentatus*) had varied levels of circulating amino acids based upon which lake they were nesting at, and furthermore females with higher amino acid concentrations had increased body condition and number of young. Differences in amino acids found in preferred species of arthropod prey for *Parid* birds has also been quantified by Ramsay and Houston (2003), who also investigated how day of year changed amino acid concentrations. They also highlighted that taurine is particularly implicated in nestling growth around days 5 and 6 based on food selection by parents. Langlois and McWilliams (2021) used wild caught songbirds with different diets (*Catharus guttatus* and *Zonotrichia albicollis*) to investigate the dietary requirements of lysine, methionine, and arginine, and showed that birds would shift their diet if deficient in these essential amino acids.

Our song sparrow study system, which spans replicate urban and rural sites (Davies and Sewall 2016), presents an opportunity to investigate how community-level differences impact nestling physiology. We previously found that urban habitats have far less arthropod abundance and biomass (VanDiest et al., in review), that urban areas have a much higher rate of brood parasitism by brown-headed cowbirds, and that rural nestlings are larger than urban nestlings from parasitized nests just before fledge (Lane et al., 2023). However, this prior work (VanDiest et al., in review) also suggested that while nestling diets differed in content, all nestling groups received similar mass of invertebrates in their diets. We hypothesized that these differences in arthropod availability and species interactions could impact nestling growth through the availability of amino acids critical to growth and condition. Like other species of songbirds, adult song sparrows seasonally shift from a largely granivorous diet to feed their young a diet containing approximately half arthropods (Judd 1901; Martin et al., 1951). Song sparrows are vulnerable to early nutritional stress if food is limited; experimental nutritional restriction in early development reduces the body size of song sparrow nestlings (Searcy et al 2004). Additionally, brood parasitism can generate competition for the food parents deliver to their young and cause additional nutritional limitations (Ladin et al., 2015; Lorenzana and Sealy 1999).

To understand how community dynamics could impact the physiology and condition of nestlings we compared free amino acid concentrations in plasma collected from song sparrow nestlings in rural nests without brood parasites, urban nests without brood parasites, and urban nests with brood parasites across our 3 urban and 3 rural study sites in Southwestern Virginia (rural nests are rarely parasitized so could not be included in comparisons; Lane et al., 2023). Free amino acids are individual amino acids not bound in a protein molecule and are generated

through the breakdown of both dietary and cellular proteins or through *de novo* synthesis in cells, so they reflect both endogenous and dietary availability (Chandel 2021). Because song sparrow nestlings in urban habitats that have a brood parasite in their nest have the lowest body mass (Lane et al., 2023), we predicted that these nestlings would also have the lowest concentrations of circulating amino acids in plasma, while rural nestlings would have the highest concentrations, despite finding similar diets between the three nestling groups (VanDiest et al., in review). Further, based on prior findings in our system, and because amino acids are vital to nestling growth and body condition, we predicted that concentrations of amino acids would correlate positively with body size measurements.

2. METHODS

2.1 Field Site Selection

We collected blood samples from 45 song sparrow nestlings across 3 urban and 3 rural sites in Southwest Virginia from 2020-2022. Our study sites (VanDiest et al., in review; Lane et al., 2023; Goodchild et al., 2022; Lane et al., 2021; Davies et al., 2018; Davies and Sewall 2016), were determined to be the most urban or rural sites near Blacksburg, Virginia that also had significant song sparrow populations using methods described in Seress et al. 2014. An urbanization gradient was calculated using 1 km^2 grids broken into 100 m x 100 m cells across many potential field sites (Davies and Sewall 2016). The scores from these cells were then used to create summary land use measures per site: mean building density score, number of cells with high building density, number of cells with paved areas, and number of cells with high greenspace density. Principal component analysis (PCA) was then used to select the most urban and most rural sites based on an urbanization index calculated from the first principal

component, resulting in size matched sites representing the most urban and rural locations. Our urban sites are local university campuses of varying size (1.05 km^2, 0.51 km^2, and 0.33 km^2), while our rural sites include an experimental farm (0.80 km^2), a community park (0.49 km^2), and a riparian restoration site (0.25 km^2).

2.2 Sample Collection

We located nests using behavioral observation and systematic searching from mid-April through early July (Lane et al., 2023). Our sample size included 13 rural nestlings from 10 nests, 15 urban nestlings from 15 nests without brood parasites, and 15 urban nestlings that experienced brown-headed cowbird brood parasitism from 14 nests. Rural nests rarely experienced brood parasitism in our system, and therefore brood parasitized rural nests were excluded as a nestling group. Two rural nestlings were dropped from analysis due to age differences, resulting in a sample size of 13 for this group. We took measurements of nestling body mass, tarsus length, wing chord, and pin feather length and took blood samples via venipuncture of the brachial vein with a 26 gauge needle when they were 9 days old, with the exceptions of 3 nestlings that were 8 days old (2 urban with cowbird parasite and 1 rural nestling), and 3 urban nestlings without parasites that were 10 days old. We performed all blood collection between 0655 and 1232 from May 10th to July 25th across our 3 year study.

2.3 Sample Preparation

We diluted nestling plasma 1:10 with liquid chromatography-mass spectrometry (LCMS) grade methanol, and after incubating at -20°C we further diluted the mixture 1:10 with our mobile phase. We used Sigma-Aldrich amino acid standards (A9906) to establish which amino acids we could detect in our plasma samples (See Table S1 for upper and lower detection points

of the standard curve), and used Cambridge Isotope Laboratories Canonical Amino Acid Mix (MSK-CAA-1) as our internal standards. We used a Raptor Polar X (2.7uM, 100mm x 2.1mm [cat.#9311A12]) column maintained at 35°C, and a Raptor Polar EXP guard column cartridge (2.7uM, 5mm x 2.1mm [cat.#9311A0252]), before quantifying free amino acids in samples using a Shimadzu mass spectrometer. We ran all analytes in positive mode.

2.4 Data Analysis

Data Processing was performed with Lab Solutions V software. We checked peaks and retention times to ensure that the program was correctly identifying analytes in each sample. Alpha-aminoadipic acid, cystine, homocysteine, hydroxylysine, 3-methylhistidine, beta-alanine, beta-aminoisobutyric acid, and anserine were included in the standards mixture but were not at detectable levels (<0.5 uM) in any of the nestling samples, and were thus excluded from analysis. We conducted analyses on a remaining panel of 27 amino acids and their derivatives listed in Table S1. For samples with values below the lower detection limit, we set their values to one-half of the lower detection limit for the given amino acid (see Table S1 for each AA's upper and lower detection limits). We ran the averaged value of two samples apiece for one rural and one urban nestling in order to control for intra-sample variation.

All statistics were carried out in Program R (version number 4.3.2). We used generalized linear models (GLMs) with a 'gamma' distribution specified to test if amino acid concentrations differed among the nestling groups. Gamma was selected due to the right skew, positive value nature of the dataset (Smyth 1989). We made models for each amino acid (27 separate models) as a response variable, with nestling group (rural, urban, and parasitized urban) and year as a fixed effect. Serine, citrulline, and tryptophan were run as linear models because they were

determined to be normally distributed using Shapiro tests. We ran gamma generalized linear models on body mass, tarsus length, wing chord, and pin feather length to determine if these traits differed among rural nestlings, urban nestlings, and urban nestlings from parasitized nests. For each model, the response variable was the morphometric measurement, while nestling group and year were included as fixed effects. Age did not change the significance of any differences between nestling groups and was dropped from final analysis.

Amino acids can be interrelated because many of them can be synthesized from one another. Therefore, to determine if nestlings from different habitats and with different brood parasite status differed in their amino acid panels, we also ran a principal component analysis (PCA) including all essential amino acids and taurine. We then used the resulting first principal component (PC1) as the response variable in a linear model with nestling group and year as fixed effects. We also used PC1 as a fixed effect in four gamma generalized linear models for measures of body size. Models featured mass, tarsus length, wing chord, or pin length as the respective response variables, with PC1 and year as our fixed effects. Finally, we tested to see if any amino acids predicted a morphometric endpoint when all nestlings were pooled across nestling group by using a set of gamma GLMs. Body mass, tarsus length, wing chord, and pin feather length were each given their own set of 27 models, one for each analyte. Each of these sets featured the morphometric as the response variable, and one of the 27 analytes and year as fixed effects.

3. RESULTS

3.1 Nestling Morphometrics

We found no significant differences in body mass, tarsus length, wing chord, or pin feather length between nestlings from brood parasitized urban nests, urban nests without brood parasites, and rural nests. Body mass: (Urban: SE = 0.004, t value = 1.372, Pr(>|t|) = 0.178; Urban with BHCO: SE = 0.004, t value = 1.331, Pr(>|t|) = 0.191), tarsus length: (Urban: SE = 0.001, t value = 1.606 Pr(>|t|) = 0.117, Urban with BHCO: SE = 0.001, t value = 1.410 Pr(>|t|) = 0.292), wing chord: (Urban: SE = 0.002, t value = 0.728, Pr(>|t|) = 0.471, Urban BHCO: SE = 0.002, t value = 0.964, Pr(>|t|) = 0.342), and pin feather length: (Urban: SE < 0.001, t value = -0.005, Pr(>|t|) = 0.996; Urban BHCO: SE < 0.001, t value = 1.569, Pr(>|t|) = 0.126).

3.2 Nestling Amino Acid Concentrations

There were no significant differences among nestling groups in the composite measure of essential amino acid concentrations (PC1; Figure 2; (Urban: SE = 1.034, t value = -0.264, Pr(>|t|) = 0.793; Urban BHCO: SE = 1.034, t value = 0.533, Pr(>|t|) = 0.597). When tested individually, none of the free essential amino acids significantly differed among nestling groups (Figure 1). Out of all non-essential amino acids, only gamma-aminobutyric acid (GABA), (SE = 0.392, t value = 2.429, Pr(>|t|) = 0.02) differed among nestlings from different groups; urban nestlings with brood parasites in their nests had lower GABA concentrations. While GABA concentrations had low values across all nestling groups, each group's average was above the lower detection limit of the mass spectrometer. Two non-essential amino acids and an amino acid derivative also showed trends that were not significant (p value of <0.1). There was a trend for non-parasitized urban nestlings to have higher concentrations of carnosine (SE = 0.041, t value= -1.880 Pr(>|t|)

= 0.068), for both parasitized and non-brood parasitized urban nestlings to have higher hydroxyproline (Urban: SE = 0.028, t value= -1.960 Pr(>|t|) = 0.057; Urban brood parasitized: SE = 0.027, t value= -1.697 Pr(>|t|) = 0.098), and for urban parasitized nestlings to have higher aspartic acid (SE = 0.023, t value= -1.706 Pr(>|t|) = 0.096). Year was not a significant factor in any amino acid models except for ornithine, for which values decreased across years of the study (SE = 0.092, t value = 2.152, Pr(>|t|) = 0.038). The average concentration for each amino acid for nestlings in each group is reported in Table 1, which also includes detection limits for all amino acids we investigated. We found amino acid concentrations were associated with a morphometric endpoint when nestlings from all groups were pooled for cystathionine, threonine, alanine, carnosine, sarcosine, histidine, glycine, and taurine (see Table B1 for full details).

4. DISCUSSION

Compromised nutrition, including restricted amino acids, can reduce the body size of nestling songbirds (Heiss et al., 2009; Ricklefs 1979; Searcy et al., 2004), presenting an opportunity to examine the relationships amongst urbanization, species interactions, and individual physiology and condition. Specifically, in our study system of song sparrows, urban sites have lower arthropod biomass and abundance (VanDiest in review) suggesting limited prey availability, and also higher rates of brood parasitism by brown-headed cowbirds (Lane et al., 2023). We hypothesized that urbanization-related changes in prey availability and brood parasitism would have negative consequences for the body size and condition of individual nestlings in urban habitats, and that amino acids are one of the mechanisms that could highlight the effects of urbanization at the community level to impacts on individuals. Based on prior findings in our system (Lane et al., 2023) we specifically predicted that urban nestlings in nests

with brown-headed cowbird chicks would have lower body size and condition and lower concentrations of amino acids in their plasma.

Contrary to our predictions, we found no differences among nestlings from urban nests with and without brown-headed cowbirds and rural nests in body size measurements, plasma amino acid concentrations, or our composite metric (PC1) of amino acids (Fig. 2). The only amino acid that differed among nestling groups was gamma-aminobutyric acid (GABA), which was lower in parasitized urban nestlings than non-parasitized urban or rural nestlings (Fig. 1). We conclude that all nestlings sampled in our study were above a threshold of amino acids that supported their growth and survival.

One amino acid, GABA, was lower in urban parasitized nestlings, but it was not associated with any measures of nestling body size or condition. GABA is a neurotransmitter and not involved in tissue formation. However, future work in this system interrogating other aspects of growth, such as brain development, may be warranted, as GABA functions as an inhibitory neurotransmitter (McCormick 1989), is associated with stress (Jie et al., 2018), and can increase body size in poultry by downregulating leptin and ghrelin production, which can limit feeding behaviors (El-Naggar et al., 2019). Plasma GABA has also been found to be lower in humans with PTSD, (Trousselard et al., 2016). Despite being a neurotransmitter, GABA has been shown to also be produced in the pancreas (Boonstra et al., 2015), and can even pass the blood brain barrier in small amounts, meaning the circulating GABA we detected may have origins in both CNS and the periphery.

The absence of morphological differences among nestling groups suggests that nutritional requirements are likely met despite lower arthropod availability and higher brood parasitism in urban habitats. Parents likely compensate for these challenges by providing adequate food to

nestlings across ecological conditions in our study system (Lane et al., 2023), though other studies of nestlings [great tits (*Parus major*), blue tits (*Cyanistes caeruleus*), house sparrows (*Passer domesticus*), and American crows (*Corvus brachyrhynchos*)] in urban habitats have found negative impacts on growth, body size (Seress et al., 2018; Corsini et al., 2021; Seress et al., 2012), and protein concentration in the case of American crows (Heiss et al., 2009).

Though the nestlings we studied did not show evidence of free amino acid deficits in plasma, it may be worth considering non-significant variation given the importance of amino acids for growth and physiological function. Two non-essential amino acids showed trends of p values below 0.1 between nestling groups: hydroxyproline and aspartic acid. Hydroxyproline trended higher in urban birds, especially in non-brood-parasitized urban nestlings compared to rural nestlings. Hydroxyproline can aid the development of birds (Wu et al., 2011), at least in part because it is a component of collagen (Li and Wu 2018). A trend of higher hydroxyproline suggests that urban nestlings have access to appropriate levels of this important tissue-forming amino acid. Carnosine, a derivative of histidine and b-alanine, tended to be higher in urban non-parasitized nestlings. Carnosine supplementation increases body mass, and specifically the size of the pectoralis in poultry (Kopec et al., 2020), and is also a 'neuroprotectant' anti-oxidant (Reddy et al., 2005). Again, this finding is consistent with urban nestlings having adequate amino acid reserves. In a previous comparison of adult urban and rural song sparrows we did not find differences in two measures of oxidative stress, glutathione (total GSH, free GSH and GSH:GSSG), and d-ROMs (Goodchild et al., 2022), but this trend suggests it might be worth measuring oxidative stress in urban and rural nestlings. We also found a trend for urban nestlings with brood parasites to have higher aspartic acid, which is involved in tissue formation and can

function as a neurotransmitter (D'Aniello 2007), but there is no clear biological relevance to this trend.

Survivorship bias should be taken into consideration when interpreting our results, as we sampled song sparrow nestlings just before they fledged from the nest but were unable to sample nestlings that died. Anecdotally, song sparrow nestlings in nests with brown-headed cowbirds are more like to go missing before fledging and parasitized nests fledge fewer song sparrow nestlings than non-parasitized nests suggesting that attrition does occur (Lane et al., 2023). Our failure to detect differences in amino acids other than GABA among nestling groups cannot rule out that younger song sparrow nestlings, or nestlings that did not survive, differed in plasma amino acid concentrations at a given time. In fact, our findings suggest that free amino acid concentrations may only be depleted in plasma when an animal is experiencing protein deficits, making comparisons of plasma amino acids potentially relevant when nestlings are found to die of starvation or fledge at lower body mass and condition. We conclude that nestlings in our system that survive and fledge maintain relatively standard concentrations of amino acids in plasma. This is perhaps unsurprising based on prior work in our system finding that nestlings were receiving similar amounts of invertebrate foods regardless of nestling group (VanDiest et al., in review).

5. Conclusions

The growing fields of urban ecology and urban ecophysiology will both be advanced by research that can explain how the impacts of urbanization affect multiple levels of biological organization. We predicted that amino acids, nutritional elements critical to the rapid growth of developing songbirds, were positioned to serve as a mechanistic link between community

dynamics and individual condition. Though we failed to find an effect of urbanization on plasma amino acid concentrations in our study system of urban brood parasitized, urban non-brood parasitized, and rural song sparrow nestlings, there may be value in examining amino acids in other systems when growth deficits are evident. We conclude that nestling urban song sparrows are not nutritionally restricted even when brood parasites are present, and thus it is not surprising that they do not show deficits in free amino acid levels. Rather, these findings join growing evidence that many species of songbirds do well in urban areas despite the prevalence of novel stressors.

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Figures

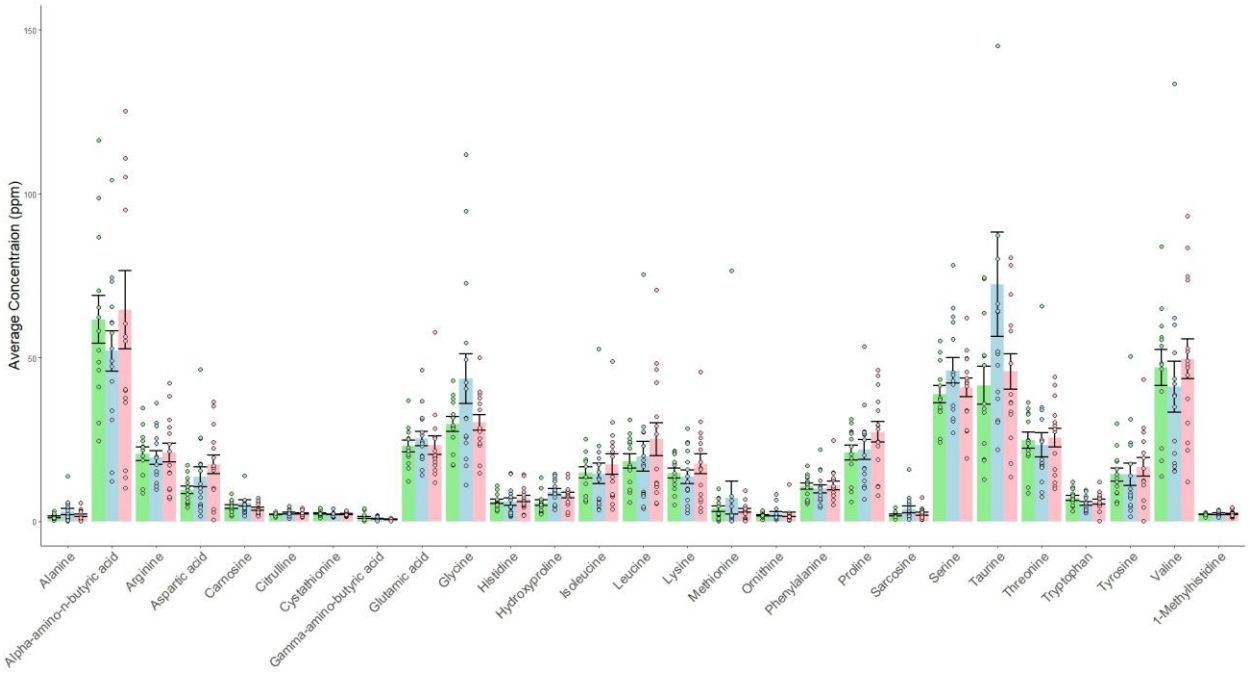

Figure 1: Mean concentration of circulating free amino acids and analytes for song sparrow nestlings across three nestling groups with standard error shown. Green points and bars represent rural nestlings (n=13), blue represents urban non-brood-parasitized nestlings (n=15), and pink represents urban brood parasitized nestlings (n=15). Only gamma-aminobutyric acid (GABA) differed between nestling groups (significantly lower in urban parasitized nestlings).

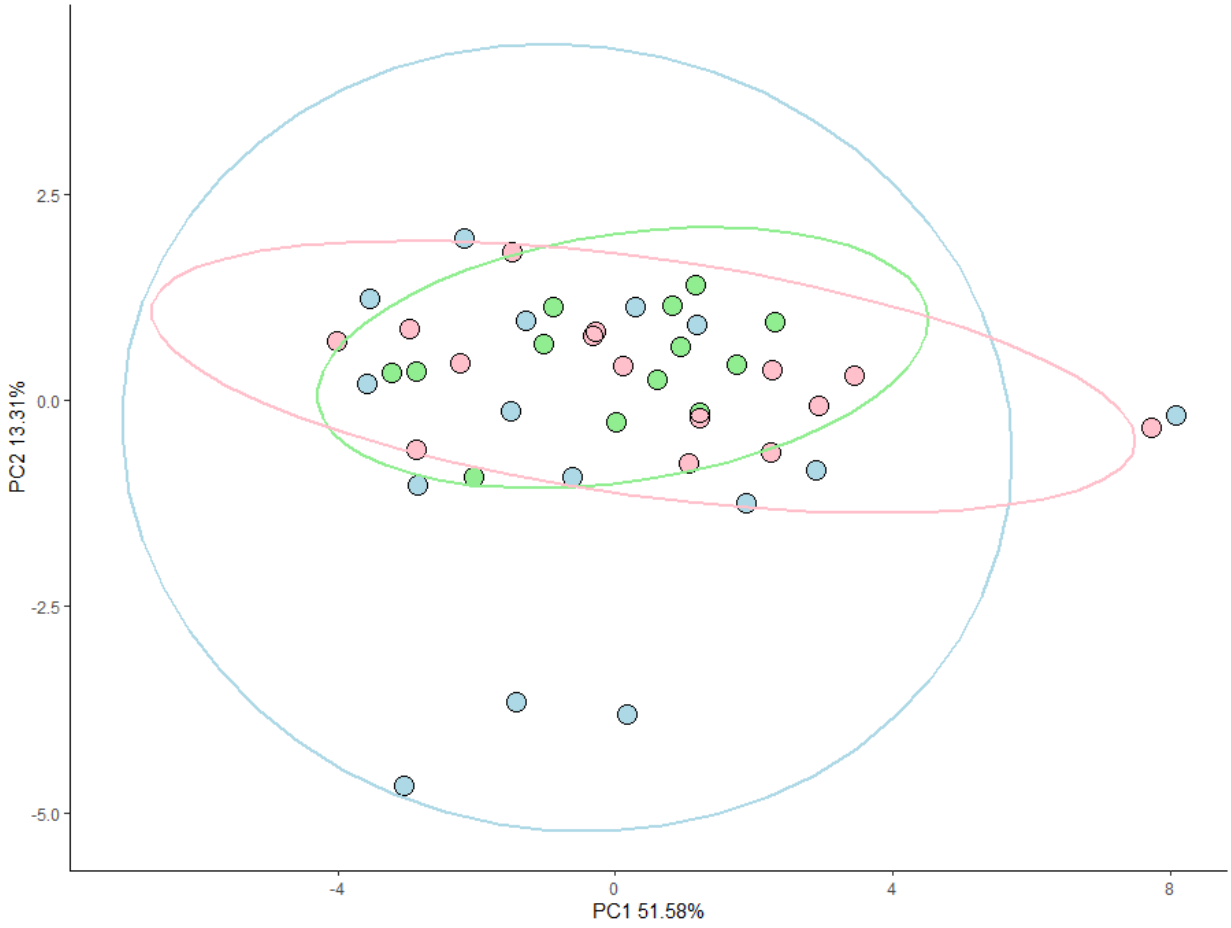

Figure 2: Score plot of essential amino acids and taurine by nestling group, with ellipses encompassing 95% of the dataset. Points that are closer to one another signify that birds had similar free plasma amino acid profiles. Green represents rural nestlings (n=13), blue represents urban nestlings (n=15), and pink represents urban brood parasitized nestlings. Principal component one explains 51.58% of the variation in this principal component analysis, while principal component two explained 13.31% of the variation. PC1 did not significantly differ by nestling group.

TABLES

Amino acid	Rural avg (ppm)	Urban avg (ppm)	Urban BHCO avg (ppm)	Lower detection limit (µM)	Upper detection limit (µM)
Essential Amino Acids					
Lysine	14.751	13.661	17.639	0.5	100
Methionine	3.892	7.246	3.376	0.5	100
Arginine	20.592	19.548	21.002	0.5	100
Isoleucine	14.982	14.641	17.418	0.5	100
Leucine	18.440	19.841	25.094	0.5	100
Proline	21.070	21.967	27.344	0.5	100
Serine	38.834	46.072	40.943	0.5	100
Threonine	24.769	23.306	25.576	0.5	100
Valine	47.000	41.040	49.602	0.5	100
Histidine	6.060	6.099	6.940	0.5	100
Tryptophan	7.180	5.498	6.089	0.5	100
Phenylalanine	10.848	9.940	11.046	5	100
Other Amino Acids					
Glycine	29.778	43.504	30.209	5	100
Taurine	41.515	58.453	45.796	5	100
Alanine	1.414	2.972	1.929	0.5	100
Tyrosine	14.303	14.399	16.659	0.5	100
Carnosine	4.520	5.611	3.892	0.5	100

Amino acid	Rural avg (ppm)	Urban avg (ppm)	Urban BHCO avg (ppm)	Lower detection limit (µM)	Upper detection limit (µM)
Hydroxyproline	5.690	9.026	7.987	0.5	100
Citrulline	2.178	2.532	2.508	0.5	100
Cystathionine	2.425	2.175	2.236	0.5	100
Glutamic Acid	23.007	25.304	23.197	0.5	100
Sarcosine	1.938	3.726	2.374	0.5	100
1-Methylhistidine	2.162	2.338	2.282	0.5	100
Ornithine	1.912	2.346	2.173	1	100
α-Amino-n-butyric acid	61.640	52.048	64.619	5	100
*γ- Aminobutyric acid	1.178	0.876	0.559*	0.5	100
Aspartic acid	9.664	13.684	17.401	1	100

Table 1: The average concentrations of all analytes by our three nestling groups (n = 15 for both urban groups and n = 13 for rural). Additionally includes upper and lower detection limits used in LCMS. Note all analytes that were undetectable (Alpha-aminoadipic acid, cystine, homocysteine, hydroxylysine, 3-methylhistidine, beta-alanine, beta-aminoisobutyric acid, and anserine) were dropped from analysis for being below the lower detection limit threshold (<0.5 µM).

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Appendix B: Chapter 2 Supplementary Materials

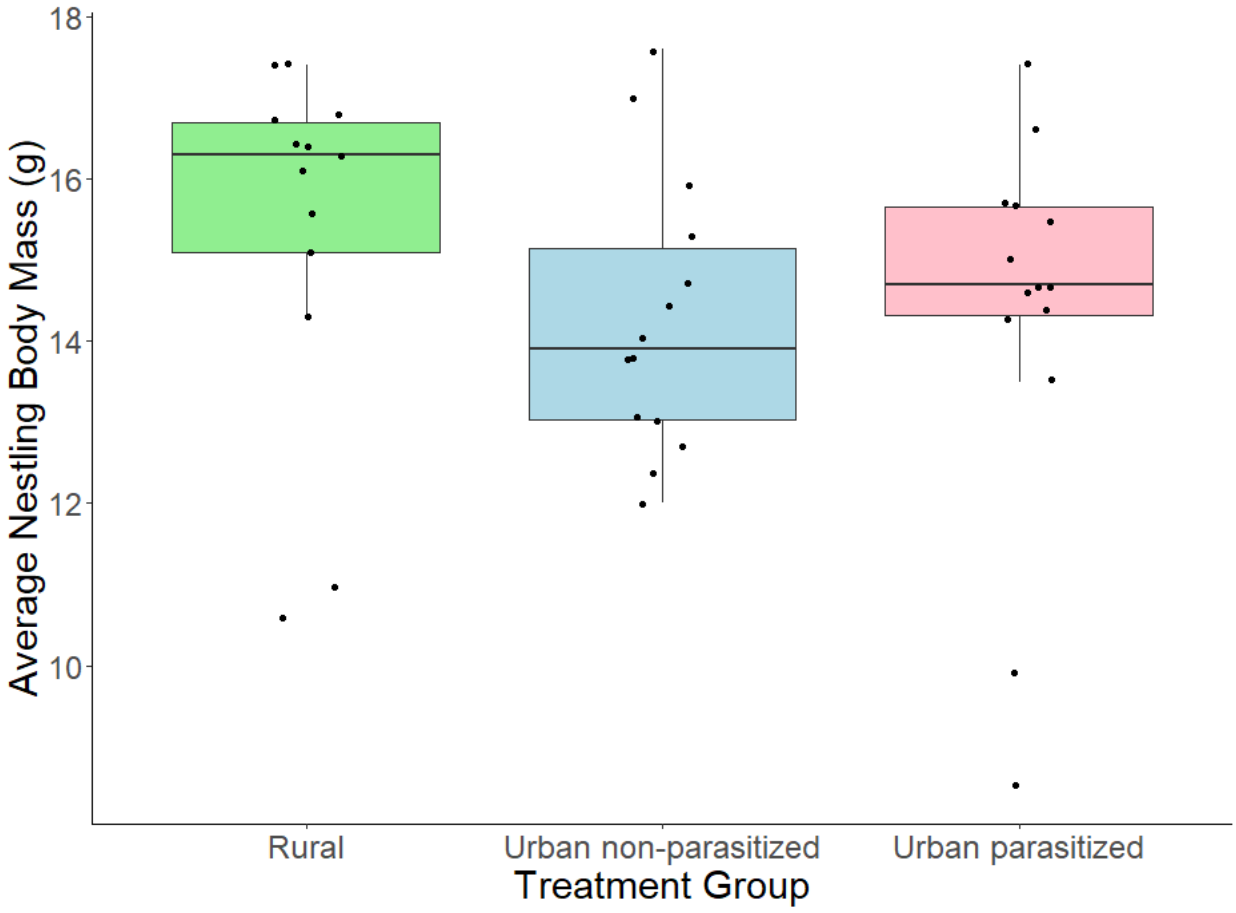

Figure B1: Nestling body mass by treatment group with medians shown. No significant differences by treatment group were found. Rural n = 13, urban non-parasitized n = 15, urban parasitized n =15.

	Relationship	Estimate	SE	T Value	Pr(>|t|)
MASS					
Cystathionine	Positive	-0.006	0.002	-2.454	0.019
Threonine	Positive	<-0.001	< 0.001	-2.159	0.037
TARSUS					
Alanine	Negative	<0.001	< 0.001	2.108	0.042
Carnosine	Negative	<0.001	< 0.001	2.25	0.03
Sarcosine	Negative	< 0.001	< 0.001	2.25	0.03
Histidine	Negative	<0.001	< 0.001	2.661	0.011
WING CHORD					
Alanine	Negative	0.001	< 0.001	3.195	0.003
Glycine	Negative	<0.001	< 0.001	3.249	0.003
Sarcosine	Negative	0.001	< 0.001	3.288	0.002
Histidine	Negative	<0.001	< 0.001	3.679	<0.001
Taurine	Negative	<0.001	< 0.001	2.439	0.02

Table B1: Relationship between song sparrow nestling amino acid concentrations and mass, tarsus length, and wing chord. All models are separate Gamma GLMS, meaning t value directionality is inverse (negative t values indicate a positive relationship between the amino acid concentration and morphometric size). Note that no amino acid concentrations pooled across nestlings were predictive of pin feather length.

Chapter 3: A meta-analysis of brown-headed cowbird (*Molothrus ater*) effects on the nesting success of their hosts

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Keywords: Brood parasitism, nest success, meta-analysis, clutch size, habitat type

Abstract

Brood parasitism is a life history strategy where an animal offloads parental care requirements for their young onto hosts, often to the detriment of host young. In the United States, the brown-headed cowbird (*Molothrus ater*) is the most ubiquitous heterospecific avian brood parasite, parasitizing 144 bird species. Prior reviews have been conducted on the effects of brown-headed cowbirds on host species nesting success, but additional work across more varied habitats has occurred since the past reviews were performed. To better understand the effects of brown-headed cowbirds on host nesting success we performed a meta-analysis on 29 studies conducted from 1976-2020. Each study focused on brown-headed cowbirds and their hosts in the United States and had to contain extractable nest success data for hosts. We found that the average clutch size of host species parasitized by brown-headed cowbirds was smaller than non-parasitized host species clutch sizes, but that parasitized nests were actually less likely to fail than non-parasitized nests across species. Additionally, we found no differences in mean number of cowbird eggs per host nest across habitat types. These findings highlight the complex relationship between brood parasites and their hosts, which has often been over-simplified. Future work focused on under-represented habitat types such as urban areas is needed to better understand cowbird and host species interactions that may change in response to rapid anthropogenic environmental change.

1. Introduction

Brood parasites are birds from multiple taxa that offload all parental care requirements to other parents: they lay their eggs in the nests of host birds, whereupon the hosts raise the brood parasite young alongside their own, often to the detriment of their own brood (Payne 1977). The

heterospecific version of this life history trait evolved independently in cuckoos, indigobirds, honeyguides, and cowbirds, which occupy a wide variety of habitat types across multiple continents, ultimately accounting for approximately one percent of all bird species (Hamilton and Orians 1965). This addition to the nest often results in the death of host eggs via ejection from the nest, or targeted attacks on host eggs and young by the brood parasite (Payne 1977). Indirect effects of brood parasitic young on host offspring includes reduced fledging success and increased likelihood of a nest failing (Lorenzana and Sealy 1999; Payne 1977; Payne 1998). Additionally, the presence of brood parasites can lead to host nestlings being smaller at fledge compared to non-parasitized nestlings (Lane et al., 2023; Marvil and Cruz 1989; Dearborn 1998). Lower body mass in parasitized nests is often due to the simple fact that brood parasites are typically larger than their hosts, making it difficult for host nestlings to compete with their larger nest-mates for food.

Much of the work on brood parasitism in the 'old world' focuses on cuckoos, a family where brood parasitism occurs in approximately one-third of the species (Winfree 1999), but the cowbirds of North America have also been of great interest, as they parasitize a wide variety of species across a large geographic range with diverse habitat types. The brown-headed cowbird (*Molothrus ater*) in particular has been the model species for investigating the effects of brood parasitism in North America, as it lives throughout the entire US, southern Canada, and northern Mexico (Mayfield 1965). Brown-headed cowbirds, (cowbirds from here on) are documented as having successfully parasitized 144 species, and have attempted to parasitize an additional 76 species (Friedman 1963). Cowbirds are native to North America, but their negative effects on host fitness have made cowbirds a target of conservation action, as their removal can aid in the

recovery of populations of threatened and endangered species when coupled with increasing available habitat for those species of concern (Peer et al., 2020; Chace et al., 2005).

Cowbirds can reduce host nest survival and compromise host growth through direct and indirect effects. Cowbirds generally lay a single egg in each host nest, and a single female averages 11 eggs per breeding season (Payne 1965). When multiple cowbird nestlings are in a single nest the success of the cowbird and host nestlings decline but, paradoxically, too few host nestlings also reduce overall nest success for the cowbird and hosts because the host parents may reduce care for all young or abandon the nest during incubation (Payne 1977). To optimize the ratio of host nestlings to cowbird nestlings, female cowbirds may puncture or eject host eggs to increase the likelihood of their own young fledging (Fiorini et al., 2009). In some cases even cowbird young will eject host young (Dearborn 1996). Arcese et al., (1996) found that adult cowbirds will even destroy song sparrow (*Melospiza melodia*) nests that are too far along to successfully parasitize so that they have a better chance at parasitizing the following re-nesting attempt. Other effects on hosts are indirect, such as parents feeding less nutritious food to host young when compared to non-parasitized young of the same species (Ladin et al., 2015). Cowbirds have even been found to alter host songbird life history; species that are more likely to be parasitized spend less time in the nest as nestlings, grow faster, and fledge at lower body mass compared to species that are less likely to be parasitized (Remeš 2006). There are also cases where non-parasitized nests have been found to fail more frequently than nests parasitized by cowbirds, either due to cowbirds selecting nests that are less likely to fail for other reasons (e.g. predation), or due to direct punishment on potential host nests (e.g. adult cowbirds destroying host eggs) for hosts removing cowbird eggs (Avilés et al, 2006; Hoover and Robinson 2007).

However, past literature reviews found that, overall, brood parasitized nests are more likely to fail (Lorenzana and Sealy 1999; Payne 1977).

Investigating what habitat types cowbirds prefer across multiple spatial scales can give insight into which species are most at risk of brood parasitism, and where cowbirds are most successful. Cowbirds prefer fragmented and edge habitats over contiguous forests (Morrison and Hahn 2002). Cowbirds in fragmented habitats will settle where they have access to anthropogenic food sources, potentially increasing cowbird success near modified habitats (Chace et al., 2005; Morrison and Hahn 2002). Many of these heavily modified habitats, such as urban areas, agricultural land, and heavily fragmented forests, already bring their own suite of stressors for native birds to overcome, and brood parasites could be an additional stressor (Seress et al., 2018; Phillips et al., 2018; Planillo et al., 2020). Cowbirds can be more likely to parasitize nests the closer hosts are to urban habitats (Rodewald 2009; Chace et al, 2002; Lane et al., 2023), though the opposite patterns has also been observed (Buxton et al., 2018). Urban areas often have their own set of stressors such as increased disturbance, artificial light at night, fragmented habitat, and altered species interactions which can result in compromised physiology and fitness for birds (Seress et al., 2018; Toledo et al., 2016; Heiss et al., 2009; Corsini et al., 2020; Salmón et al., 2018; Meillère et al., 2015), and these additional effects added to the pressures of brood parasitism may result in urban hosts having lower fitness than hosts in other habitat types.

We performed a meta-analysis to determine how cowbird brood parasitism affects host clutch size and nest failure rates, and how cowbird clutch size may vary by habitat type. Our analysis builds on prior reviews (Payne 1977; Payne 1998; Lorenzana and Sealy 1999) because considerable research on cowbirds has occurred in the last 25 years. We collected and analyzed data on brood parasite papers with available host nesting success data in the United States from

1976 to 2020. We predicted that parasitized host nests would have smaller clutch sizes of host offspring and higher nest failure rates than non-parasitized hosts due to the additional resources necessary to raise cowbird young. Additionally, we documented which habitat types were most represented to determine if particular habitat types are understudied in the context of host nesting success.

2. Methods

2.1 Dataset Assembly

2.1.1 Literature Search

Our procedure was guided by the Transparent Reporting of Systematic Reviews and Meta Analysis (PRISMA) method shown in Supplemental Figure C1 (Knobloch et al., 2007). We gathered papers through Web of Science with search terms relating to brood parasitism of brown-headed cowbirds and their hosts. The search terms used were (Nest Parasitism OR Brood Parasitism OR cowbird*) AND (songbird* OR passerine*) AND (population*), which resulted in 541 studies from 1976 to 2020. All authors reviewed the results and filtered through these studies by screening abstracts to check for relevant information. The papers that passed the abstract screening were then closely examined for data to include in the analysis. Deciding factors such as brown-headed cowbird brood parasitism as opposed to other brood parasites, study location (only North American studies were utilized in this meta-analysis), and presence of nest success data were used in this screening. A second screening of studies determined by the availability of extractable data, such as nest failure incidence and number of parasitized vs non parasitized nests was then performed. Ultimately, 29 papers had the relevant data for our meta-analysis (Table 3).

2.1.2 Dataset Construction

Each of these 30 studies contained multiple types of data, which included the years the study was conducted, habitat type, host species, mean host clutch and brood sizes, mean cowbird clutch size, lifestage of the data (e.g. egg, nestling, fledgling), total nests surveyed in a study, and number of nest failures in a study. For every unique datapoint (e.g. the mean value of the non-parasitized host clutch sizes for vesper sparrows) a unique row was created. Nest failure rates were calculated for each data point by dividing the number of failed nests by the total number of nests reported. Every data point was assigned a 'habitat type' of either agricultural, forest, grassland, shrubland, riparian, or urban based on study site descriptions. 'Agricultural' in this case refers to heavily cattle grazed lands, while 'riparian' includes riparian sites, but some wetlands as well. Unfortunately, we did not have enough studies to break down habitat type into more specific classifications. Each study was given a 'study ID' to control for having multiple data points per study.

2.2 Data Analysis

All analysis was completed in Program R (version 4.3.1; R Core Team 2023). We used the lme4 package (Bates et al., 2014) to perform linear mixed effects models. To investigate the effects of brood parasitism on mean host clutch size, we ran four linear mixed effects models. First, for all host nests pooled across life stages, mean host clutch size was the response variable, brood parasitism status the fixed effect, and study ID was our random effect (n = 228). We then repeated this model three times with dataframes that contained only the egg stage (n = 132), nestling stage (n = 30), and juvenile stage (n = 66), respectively.

To determine the effect of brood parasitism on nest failure rate we ran a linear mixed effect model on pooled life stages where nest failure rate, the number of failed nests out of all nests surveyed in a study, was the response variable, parasitism status was our fixed effect, and study ID was the random effect (n = 44). We could not run separate models for each life stage because of too few data points.

Finally, to find how the average number of cowbird eggs in host nests might differ by habitat type we created a linear mixed effects model with mean number of cowbird eggs pooled across life stages as the response variable (n = 77), habitat type as the fixed effect, and study ID as our random effect (see Table 1 for the sample sizes of each habitat type). We could not run separate models for nestling or juvenile life stages because of too few data points. However, the egg stage model, where the average number of cowbird eggs in host nests was the response variable, habitat type as the fixed effect, and study ID as our random effect, had enough power for the model to converge. Models assumptions were checked using the 'performance' package (Lüdecke et al., 2021).

3. Results

3.1 Meta-analysis results

Brood-parasitized host nests had smaller average host clutch sizes than non-parasitized hosts when all habitats were pooled together (Fig. 1). This was true for pooled life stages, as well as the egg, nestling, and juvenile stages individually (see Table 2 for model results). Nest failure rates for hosts were lower in parasitized nests than non-parasitized nests (Fig. 2) when all life stages were pooled (β = -16.399, SE = 3.82, df = 36.668, t value = -4.29, Pr(>|t|) <0.001). Brown-headed cowbirds had no significant differences in number of cowbird eggs per host nest

by habitat type (Fig. 3) when all life stages were pooled (when compared to 'Agricultural,' Forest: β = -0.434, SE = 0.823, df = 6.443, t value = -0.527, Pr(>|t|) = 0.616, Grassland: β = -0.875 , SE = 0.777, df = 5.825, t value = -1.127, Pr(>|t|) = 0.304, Riparian: β = -0.701, SE = 0.809, df = 5.976, t value = -0.867, Pr(>|t|) = 0.419, Urban: β = -0.700, SE = 1.051, df = 7.677, t value = -0.666, Pr(>|t|) = 0.525), nor in the egg stage (Forest: β = -0.437 , SE = 0.814, df = 6.393, t value = -0.532, Pr(>|t|) = 0.6130, Grassland: β = -0.739, SE = 0.771, df = 5.841, t value = -0.958, Pr(>|t|) = 0.376, Riparian: β = -0.699, SE = 0.800, df = 5.941, t value = -0.873, Pr(>|t|) = 0.416).

4. Discussion

Our meta-analysis of brown-headed cowbird host success from 1976-2020 in the US revealed a few key findings. First, as predicted we found a decrease in average host clutch size for brood-parasitized nests compared to non-parasitized nests. Second, we found that the average number of brown-headed cowbird eggs per host nest did not significantly differ by habitat type. Finally, we discovered that non-parasitized nests actually had higher rates of nest failure compared to parasitized nests, a finding that contradicted our prediction that brood parasitism negatively impacted nest success.

Previous reviews reported that host clutch sizes were smaller when experiencing brown-headed cowbird brood parasitism (Payne 1977; Lorenzana and Sealy 1999). We also found lower mean clutch sizes when all life stages were pooled (Fig. 1), as well as when the egg, nestling, and juvenile stages were examined individually. Host parents have a much more difficult time feeding their own young on top of the cowbirds, often leading to the death of their own young before fledge, or will have some of their own eggs directly destroyed by cowbird parents (Payne

1998). Either case ultimately results in decreased fitness for the host parents, while the costs they incur as parents, such as decreased body condition, remain very similar. This decrease in mean clutch size has direct fitness effects on the host parents, as they are fledging fewer young per clutch, and therefore per breeding season, than parents whose nests avoid both brood parasitism and predation throughout the breeding season.

Despite cowbirds having known preferences for habitat type, particularly for fragmented, anthropogenic habitat (Chace et al, 2002; Chace et al., 2005), the average number of cowbird eggs per host nest (1.59 cowbird eggs) did not differ between habitats. This is somewhat surprising because, while cowbirds typically lay a single egg per nest, when cowbird density is high, host nests can receive eggs from multiple cowbirds (Petit 1996). Further work quantifying parasitism rates and number of cowbird eggs per host nest by habitat type could have conservation implications for imperiled hosts (Chace et al., 2005; May and Robinson 1985).

Despite prior reviews on how cowbirds affect their hosts discovering that parasitized nests would be more likely to fail, we found the opposite: non-parasitized nests had higher failure rates. While these reviews have found that nests are generally more likely to fail when parasitized (Payne 1977; Lorenzana and Sealy 1999), there are multiple studies that have found parasitized nests are actually less likely to fail. For example, in sites with high densities of shiny cowbirds, mockingbird nests were found to have higher egg survivorship when parasitized (Gloag et al., 2011). Hosts can also be punished when they eject brood parasite eggs from nests; brood parasites will return to destroy the host eggs (Hoover and Robinson 2007). This scenario is known as ‘the mafia hypothesis,’ and can result in non-parasitized nests being more likely to fail than parasitized ones. We have no evidence that this is what happened to the hosts in our dataset, as there were not enough systematic observations to quantify how nests were failing. Other work

suggests that brown-headed cowbirds are less likely to parasitize nests that will eventually be predated (Avilés et al, 2006), and are therefore associated with lower rates of nest failure. Taken together with the finding of decreased clutch size in parasitized nests, brown-headed cowbirds may parasitize nests that are less likely to fail, but a non-parasitized nest is likely to fledge more young if it survives. However, since this work partially contradicts prior reviews, these findings should be regarded with healthy skepticism, as only using papers that have host nest success and clutch size information available may have affected these results. Further work to better evaluate all of the non-lethal effects of brood parasitism on host nestlings requires additional work in wild systems.

While brown-headed cowbirds and their hosts have been studied across a wide variety of habitat types, some, particularly urban habitats, are understudied (Table 1). Brood parasites do not respond to urbanization in a uniform way. There have been reports of both increases and decreases in parasitism rates (Morrison and Hahn 2002; Chace et al., 2002; Rodewald 2009), (Zhang et al., 2023), as well as reports that find no differences between urban and non-urban parasitism rates (Islam Nahid et al., 2020). However, brown-headed cowbirds specifically appear to prefer disturbed or urban habitats, and their hosts experience increased rates of parasitism closer to urban areas (Morrison and Hahn 2002; Chace et al., 2002; Rodewald 2009), though this is not universally true (Buxton et al., 2018). Further work investigating if cowbirds prefer parasitizing hosts in urban areas, and if these urban hosts see any differences in nesting success compared to conspecifics in rural habitat types, would add to our knowledge of cowbird habitat preference, and further our understanding of what hosts have to contend with in urban environments.

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Figures

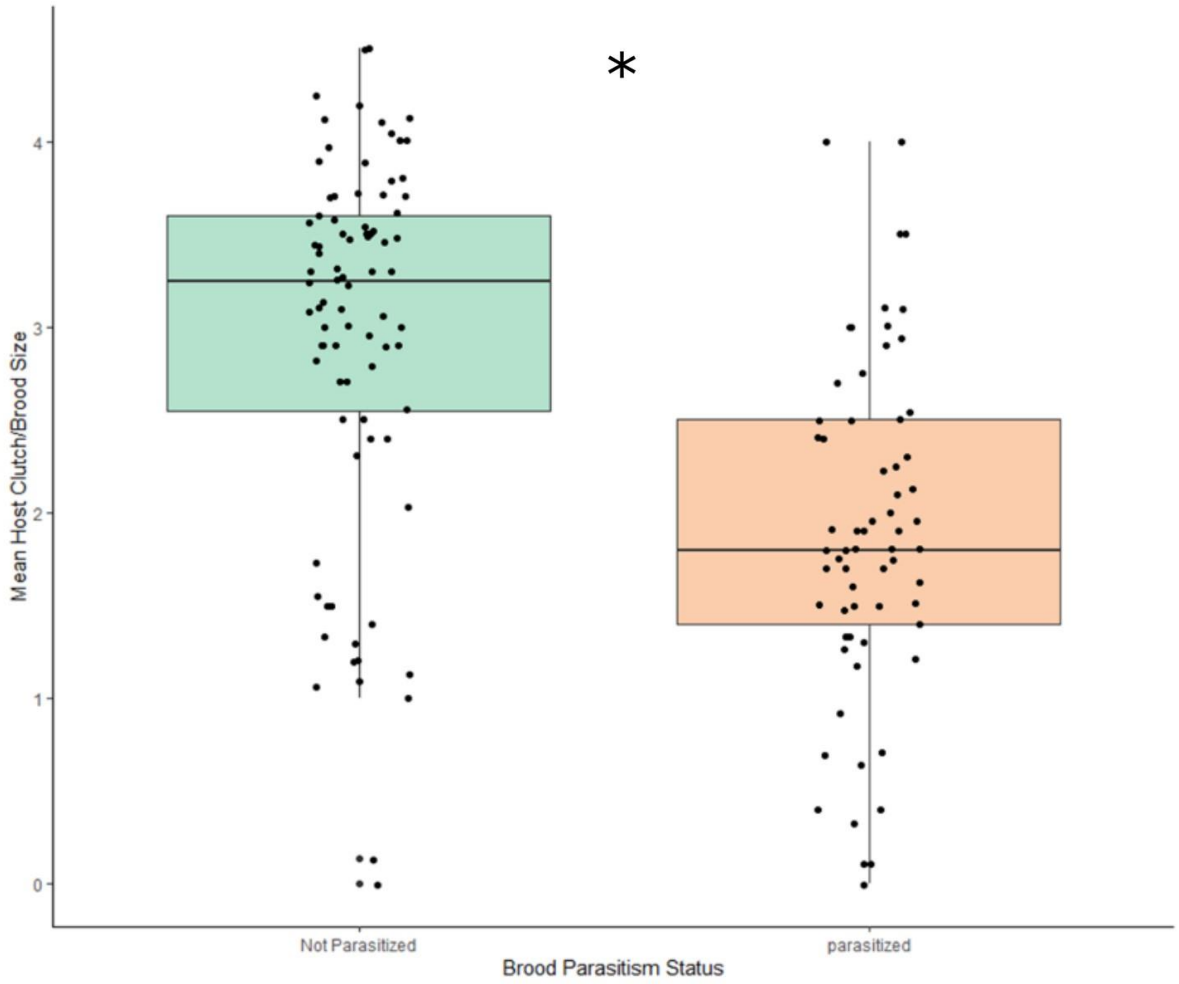

Figure 1: Combined stages (egg/nestling/juvenile) average clutch/brood size by brood parasitism status. Mean values were found to be significantly higher in non-brood-parasitized nests.

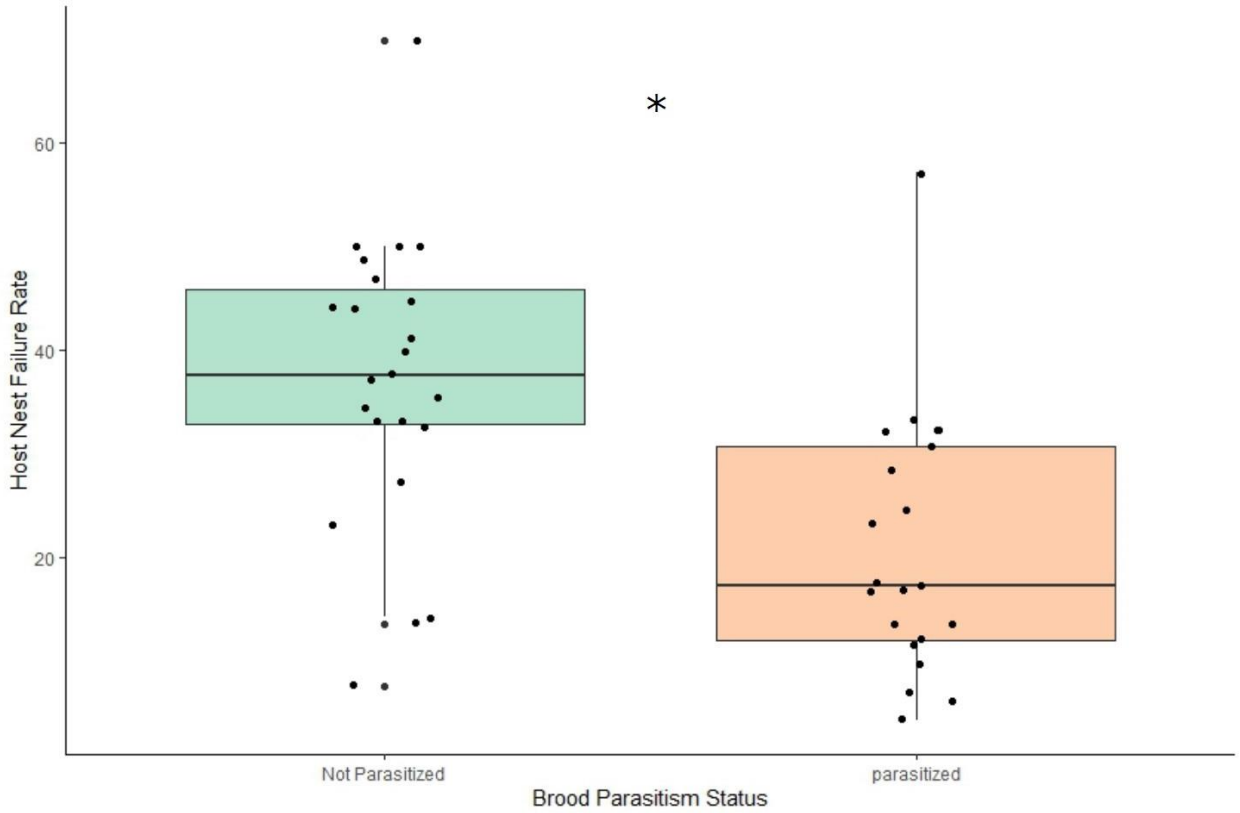

Figure 2: Nest success by brood parasitism status. Parasitized nests were found to be more successful than non-parasitized nests when all host bird life stages were pooled, as well as for individual life stages.

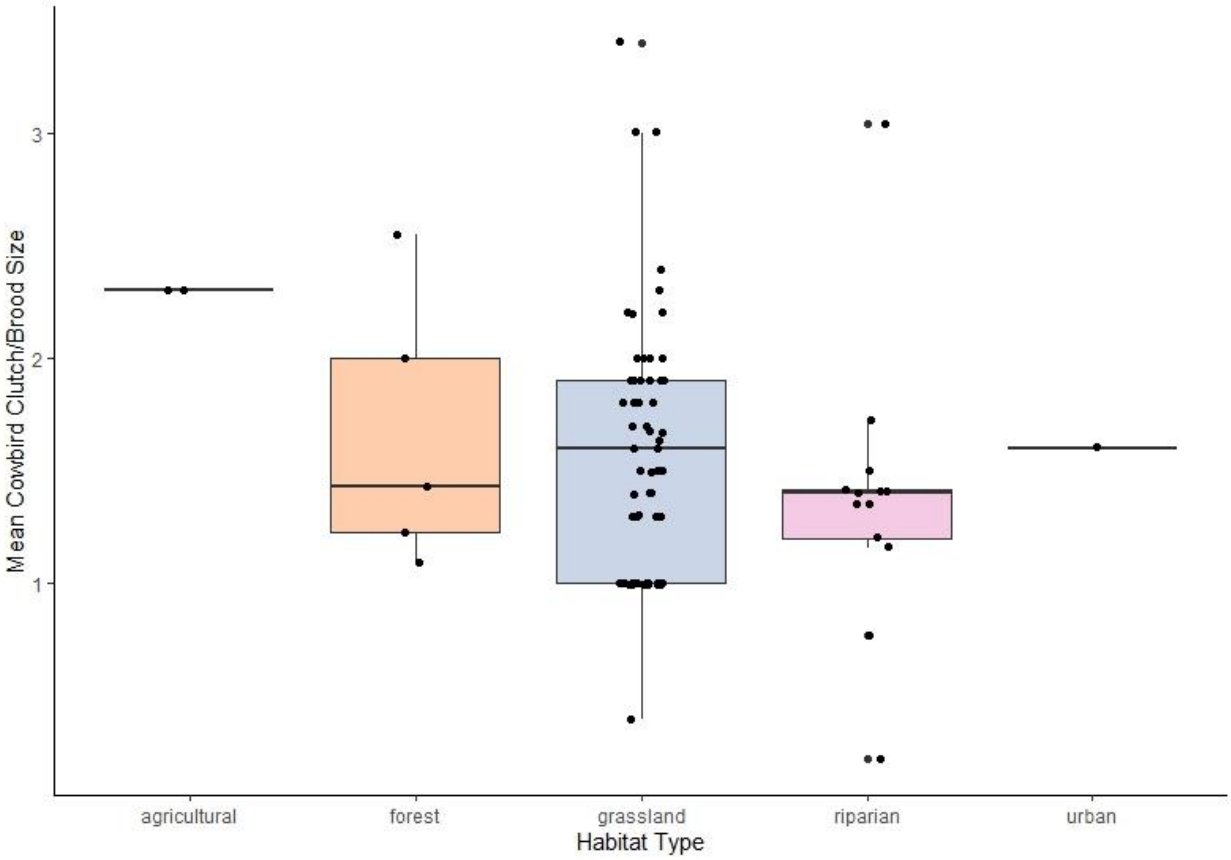

Figure 3: Average clutch size of brown-headed cowbirds per host nest in various habitat types. ‘Note that while cowbirds were found to parasitize nests in ‘shrubland,’ habitat, no data on cowbird clutch size was available for ‘shrubland’ habitat.

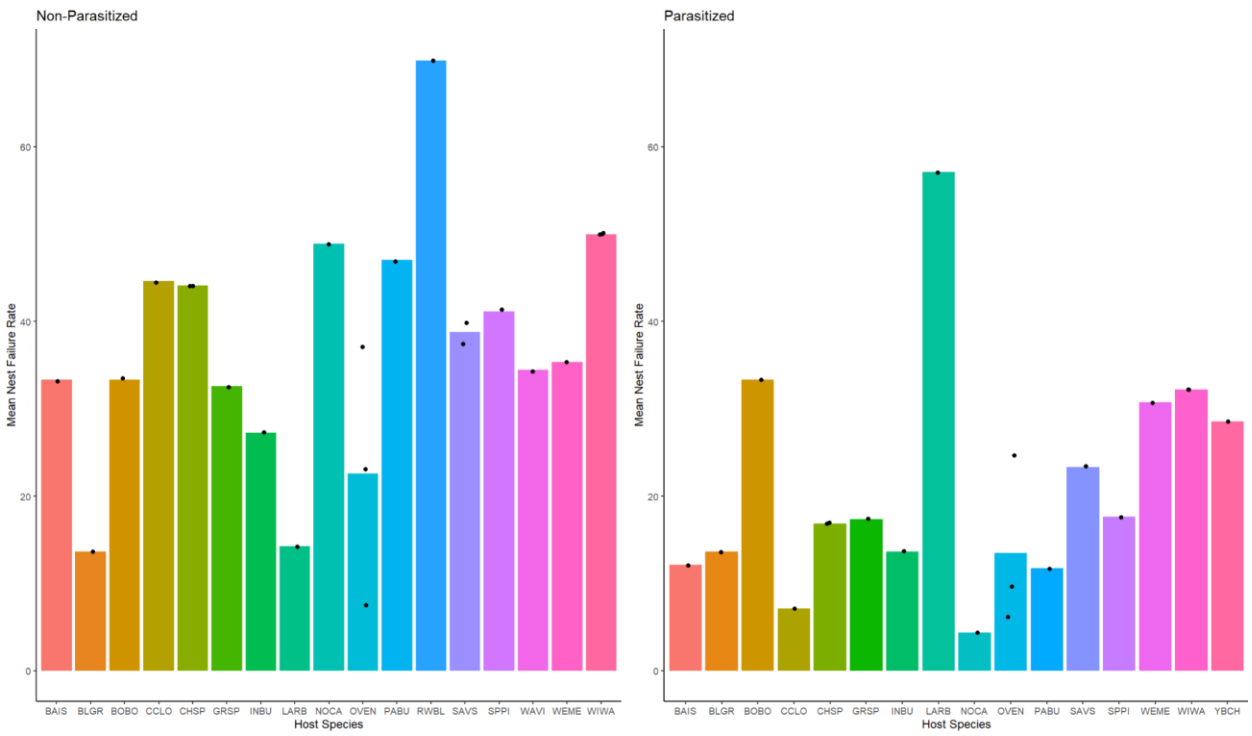

Figure 4: Visualization of mean nest failure rate for host species by parasitism status. Note that some species are only represented in either non-parasitized (RWBL and WAVI) or parasitized (YBCH) nests. Sample sizes per species were too low for meaningful analysis (sample size per species is indicated by the number of datapoints shown per column). Please see Figure 2 for mean nest failure rate by parasitism status when species are pooled. Species codes and their common names are as follows: BAIS (Baird's sparrow), BLGR (blue grosbeak), BOBO (bobolink), CCLO (chestnut-collared longspur), CHSP (chipping sparrow), GRSP, (grasshopper sparrow), INBU (indigo bunting), LARB (lark bunting), NOCA (northern cardinal), OVEN (oven bird), PABU (painted bunting), RWBL (red-winged blackbird), SAVS (savannah sparrow), SPPI (Sprague's pipit), WAVI (warbling vireo), WEME (western meadowlark), WIWA (Wilson's warbler), YBCH (yellow-breasted chat).

Tables

Habitat Type	Number of Studies	Host NP avg clutch size	Host P avg clutch size	Cowbird avg clutch size
Agricultural	3	2.662	1.633	*2.3*
Grassland	10	2.765	1.947	1.607
Forest	9	3.128	1.830	1.66
Shrubland	2	3.441	1.750	NA
Riparian	5	2.517	1.498	1.382
Urban	1	3.950	2.550	*1.6*

Table 1: Number of studies that had data usable for the meta-analysis by habitat type and the mean clutch size values for non-parasitized hosts (NP), parasitized hosts (P), and cowbirds by habitat type. Mean values should not be compared between habitat types due to differences in species studied in each, but rather how mean values between non-parasitized and parasitized nests changes in each habitat. Note that for the cowbird column both ‘Agricultural’ and ‘Urban’ only have a single data point and are therefore not actual mean values.

Life Stage	β value	Standard Error	Degrees of freedom	t value	Pr(>|t|)
Pooled	-0.909	0.156	143.049	-5.824	**<0.001**
Egg	-23.361	5.265	25.162	-4.437	**<0.001**
Nestling	-1.291	0.178	21.02	-7.238	**<0.001**
Juvenile	-0.680	0.285	54.398	-2.388	**0.0210**

Table 2: Model results for the average host clutch size for multiple life stages comparing non-parasitized to parasitized. All life stages had significantly smaller average clutch sizes when parasitized.

Year	Journal	Author	Title
2019	Acta Oecologica	Kelly, J.K., Suckow, N.M., Ward, M.P.	*Preferential settling at sites with higher conspecific density does not protect yellow warblers (Setophaga petechia) from brood parasitism*
2017	The Royal Society	Bernath-Plaisted, J. Nenninger, H. Koper, N.	*Conventional oil and natural gas infrastructure increases brown-headed cowbird (Molothrus ater) relative abundance and parasitism in mixed-grass prairie*
2017	Journal of Field Ornithology	Hargrove, L., Unitt, P.	*Poor reproductive success of Gray Vireos in a declining California population*
2016	Biological Conservation	Walker, L.E., Marzluff, J.M., Cimprich, D.A.	*Source-sink population dynamics driven by a brood parasite: A case study of an endangered songbird, the black-capped vireo*
2016	Wilson Journal of Ornithology	Jenkins, J.M.A., Faaborg J	*Potential effects of brown-headed cowbirds (Molothrus ater) on host postfledging dispersal and survival*
2014	Wilson Journal of Ornithology	Ludlow, S.M., Brigham, R.M., Davis S.K.	*Nesting ecology of grassland songbirds: effects of predation, parasitism, and weather*
2012	PLoS One	Cox, W.A., Thompson III., Root, B., Faaborg, J.	*Declining Brown-Headed Cowbird (Molothrus ater) Populations Are Associated with Landscape-Specific Reductions in Brood Parasitism and Increases in Songbird Productivity*
2012	Journal of Wildlife Management	Jacobs, R.B., Thompson III, F.R., Koford, R.R., La Sorte, F.A., Woodward, H.D., J.A., Fitzgerald	*Habitat and landscape effects on abundance of Missouri's grassland birds*
2008	The Auk	Sandercock, B.K., Hewett, E.L., Kosciuch, K.L.	*Effects of experimental cowbird removals on brood parasitism and nest predation in a grassland songbird*
2008	Ecological Applications	Kosciuch, K.L., Sandercock, B.K	*Cowbrid removals unexpectedly increase productivity of a brood parasite and the cowbird host*
2007	The Auk	Mattsson, B.J., Cooper, R.J.	*Which life-history components determine breeding productivity for individual songbirds? A case study of the Louisiana waterthrush (Seiurus motacilla)*

Year	Journal	Author	Title
2007	The Auk	Podolsky, A.L., Simons, T.R., Collazo, J.A.	*Modeling population growth of the ovenbird (Seiurus aurocapilla) in the southern appalachians*
2007	Canadian Field-Naturalist	Igl, L.D., Johnson, D.H.	*Brown-headed cowbird, molothrus ater, parasitism and abundance in the northern great plains*
2007	Ecological Applications	Vander Haegen, W. M.	*Fragmentation by agriculture influences reproductive success of birds in shrubsteppe landscape*
2007	PNAS	Hoover, J.P., Robinson, S.K.	*Retaliatory mafia behavior by a parasitic cowbird favors host acceptance of parasitic eggs*
2005	Journal of Field Ornithology	Small, S.L.	*Mortality factors and predators of spotted towhee nests in the Sacramento Valley, California*
2004	Wilson Bulletin	Michaud, J.C., Gardall. T., Nur, N., Girman, D.J.	*Effects of nest predation and brood parasitism on population viability of Wilson's warblers in coastal California*
2002	Ecology	Smith, J.N.M., Taitt, M.J., Zanette, L.	*Removing brown-headed cowbirds increases seasonal fecundity and population growth in song sparrows*
2002	Journal of Field Ornithology	Whitehead, M.A., Schweitzer, S.H., Post, W.	*Cowbird/host interactions in a southeastern old-field: a recent contact area?*
2002	The Auk	Hersek, M.J., Frankel, M.A, Cigliano, J.A., Wasserman, F.E.	*Brown-headed cowbird parasitism of ovenbirds in suburban forest fragments*
2001	The Condor	Ortega, C.P., Ortega, J.C.	*Effects of brown-headed cowbirds on the nesting success of chipping sparrows in southwest Colorado*

Year	Journal	Author	Title
2000	Cooper Ornithological Society	Whitehead, M.A, Schweitzer, S.H., Post, W.	*Impact of brood parasitism on nest survival parameters and seasonal fecundity of six songbird species in southeastern old-field habitat*
2000	Natural Areas Journal	King, D.I., Griffin, C.R., Champlin, P.J., Champlin, T.B.	*An evaluation of the use of The Nature Conservancy vegetation classification for mapping bird distribution at Chincoteague National Wildlife Refuge*
2000	University of Texas Press	Smith, J.N.M., Cook, T.L., Rothstein, S.I., Robinson, S.K., Sealy, S.G.	*Ecology and management of cowbirds and their hosts: studies in the conservation of north american passerine birds*
2000	The Condor	Burhans, D.E., Thompson, F.R., Faaborg, J.	*Costs of parasitism incurred by two songbird species and their quality as cowbird hosts*
2000	The Auk	Ward, D., Smith, J.N.M.	*Brown-headed cowbird parasitism results in a sink population in warbling vireos*
1978	The Wilson Bulletin	Nolan, V., Thompson, C.F.	*Egg Volume as a predictor of hatchling weight in the brown-headed cowbird*
2013	The Auk	Hill, J.M., Diefenbach, D.R.	*Experimental removal of woody vegetation does not Increase nesting success or fledgling production in two grasslandsparrows (Ammodramus) in Pennsylvania*
2009	Journal of Field Ornithology	Svagelj., W.S., Fernandez, G.J., Mermoz,M.E.	*Effects of nest-site characteristics and parental activity on cowbird parasitism and nest predation in brown-and-yellow marshbirds*

Table 3: Studies with extractable data that were used to perform this meta-analysis.

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Appendix C: Chapter 3 Supplementary Materials

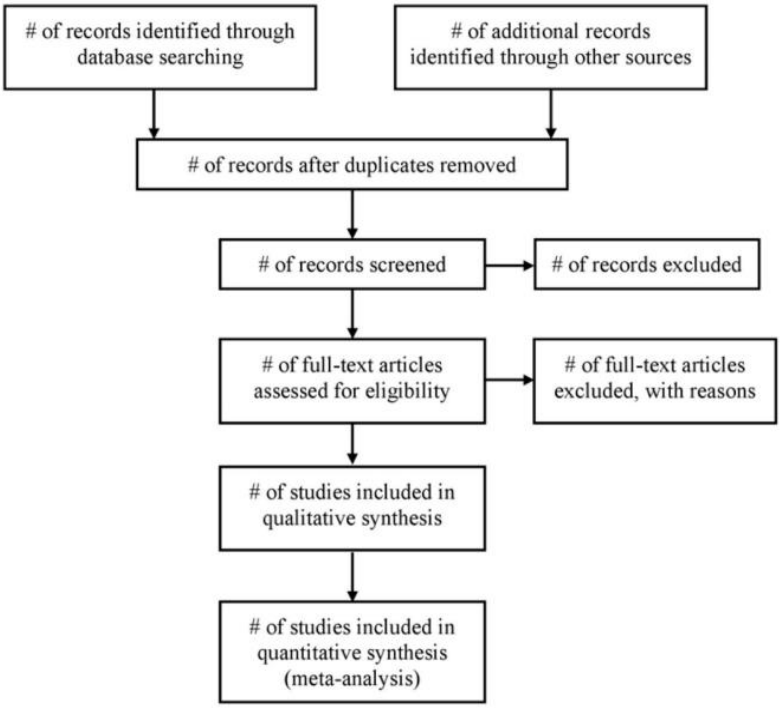

Figure C1: The PRISMA diagram for conducting meta-analysis that we followed.

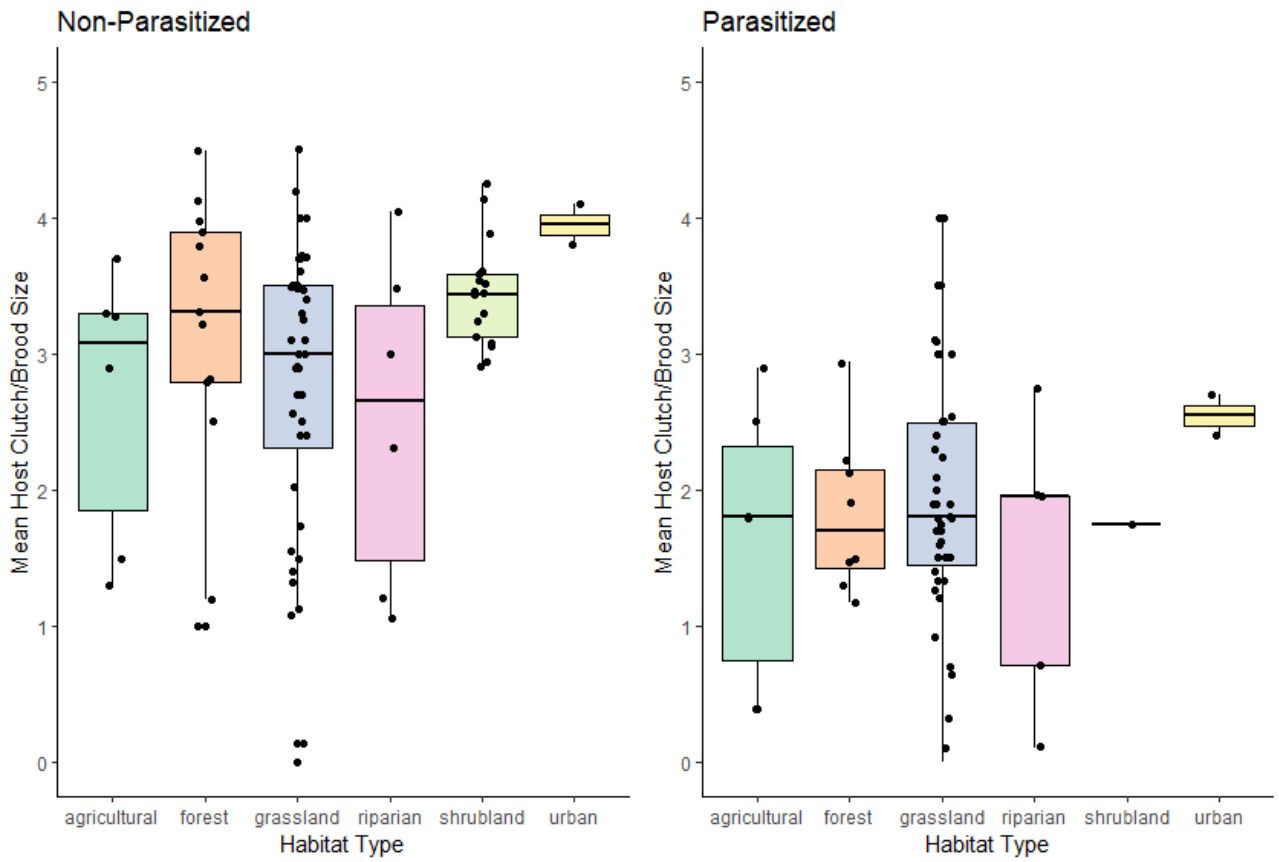

Figure C2: How parasitism status affects mean clutch/brood size for hosts, with habitat type shown as well. Mean values should not be compared between habitat types due to differences in species studied in each, but rather how mean values between non-parasitized and parasitized nests changes in each habitat.

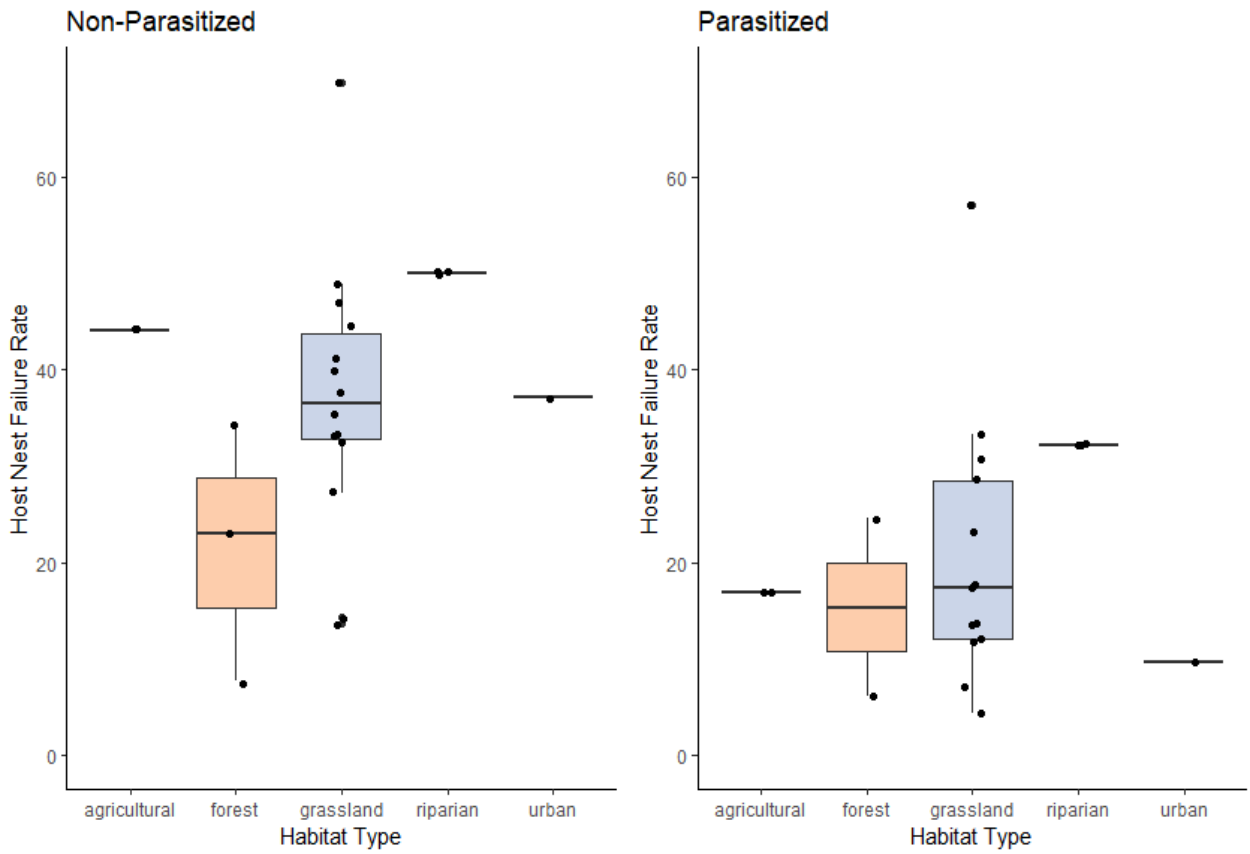

Figure C3: Difference in nest failure rate by parasitism status by habitat type. All nest failure rate 'zeros' dropped. Surprisingly, parasitized nests had significantly lower nest failure rates than non-parasitized ones.

Dissertation Conclusion

This work synthesizes some of the effects that urbanization has on a local songbird by quantifying the arthropod community song sparrows rely upon, the diets of their nestlings, and ultimately evaluates differences in nutritional endpoints and nestling development between urban and rural nestlings. Broadly speaking, my research has shown that despite potential restrictions on nutrition for developing urban birds, they appear to be avoiding any consequences at the individual level. This adds to the growing literature regarding animals that continue to exist in urban areas, showing that in many cases there are no significant costs to urban living for those taxa that are not excluded in the first place (Goodchild et al., 2022; Lane et al., 2023; Iglesias-Carrasco et al., 2020). Additionally, this work expands upon our understanding of how a common brood parasite in North America, the brown-headed cowbird, affects host species, and how the number of cowbird eggs per host nest might differ by habitat. There are many questions generated during the course of this dissertation that I could not answer, some of which I will lay out in the rest of my conclusion as suggested areas for future research.

The effect of urbanization on arthropod communities and the diet of nestling song sparrows

While there has been great work showcasing the general negative trends for biomass, abundance, and diversity of urban arthropod communities compared to rural or native habitats (Fenoglio et al., 2020; Chatelain et al., 2023), there were studies finding no differences or the opposite effect as well. Therefore, I could not assume that there was a difference in the arthropod communities between our own urban and rural sites. I wanted to quantify what nestlings were being fed, and having knowledge of the difference in arthropod communities by habitat types,

and especially the difference in potential arthropod prey items, would allow us to compare what was available on the landscape to what the nestlings were ultimately being fed. I confirmed that in the two years I sampled our urban arthropod communities had lower abundance, biomass, and decreased Simpson's diversity compared to rural arthropod communities. These differences in abundance and biomass were especially pronounced in Araneae (spiders), an order that is particularly protein-rich and known to be favored by song sparrows for feeding their young (Tompa 1971). Other preferred taxa, such as Lepidoptera (caterpillars) also had higher biomass and abundance in rural habitats compared to urban. Our work focused on a broad range of arthropod taxa, but by only looking at order I are missing some of the nuance that other studies have captured by looking into more precise taxonomic groups (Shochat et al., 2004; Chatelain et al., 2023; Bennett and Lovell 2014). Reviews such as Fenoglio et al., (2020) also point out that most studies regarding urbanization and arthropod communities are from North America and Europe, which is effectively ignoring the places where the most rapid urbanization is taking place, as well as over-representing temperate ecosystems when attempting to understand how urbanization alters arthropod communities.

Despite the drastic differences by habitat in arthropod biomass and abundance (see Figure 2 in chapter 1), the nestling diets of invertebrate food items differed much less than expected. Nestlings did differ in what they were eating and in average abundance by habitat type, but crucially not in the average biomass of all arthropod food items, meaning the mass of food they were consuming was statistically the same in urban and rural habitats. Rural birds were found to have more spiders in their diet, while urban birds had greater numbers of arthropods on average, but this was mostly driven by ants, which are much less nutrient rich than spiders or caterpillars (Razeng and Watson 2015). I quantified the abundance and biomass of invertebrate food items in

nestling stomachs, but I was only able to identify samples to the level of order due to issues with the quality of the arthropod specimens. Additional work on a similar generalist species that persists in both urban and rural habitats that could utilize DNA metabarcoding would let researchers quantify how the presence and absence of different prey species and anthropogenic food sources may differ in nestling diets between urban and rural habitats (Shutt et al., 2021; Jarrett et al., 2020). Current metabarcoding methods will not provide good resolution of variation in the amount of different prey taxa consumed, but emerging metabarcoding methods may make it possible to resolve both presence of taxa and quantities of those taxa for diet analysis in the near future.

Neither urbanization nor brood parasitism appear to affect tissue forming free amino acid concentrations of song sparrows

Prior work has evaluated how some urban bird species differ from rural counterparts across protein (Heiss et al., 2009; Machovsky-Capuska et al., 2016), calcium (Heiss et al., 2009), fats (Andersson et al., 2015; Toledo et al., 2016), and macronutrients generally (Coogan et al., 2018), but there is a lack of information on how urbanization alters amino acid concentrations for wild songbirds and how it might affect developing young. Studies have quantified amino acid concentrations in arthropod prey (Ramsay and Houston 2003), and have looked into a few essential amino acid requirements in adult songbirds (Langlois and McWilliams 2021), but to my knowledge, no quantification of an amino acid panel in nestling wild songbirds has been performed, including in the context of urbanization and brood parasitism. Despite our predictions that concentrations would be stratified from highest concentrations in rural to lowest in parasitized urban nestlings, I found that there were no differences in concentrations between

urban nestlings without brood parasites, urban nestlings with brood parasites, and rural nestlings in essential amino acids. In fact, only a single amino acid out of the twenty-seven analyzed, GABA, differed. While I am more interested in tissue-forming amino acids as they are directly coupled with growth, GABA, which functions as a neurotransmitter, has been implicated in increasing body mass and weight gain in poultry by reducing the production of leptin and Ghrelin, which slow feeding behaviors, and has been associated with nutritional stress in birds (McCormick 1989; El-Naggar et al., 2019; Jie et al., 2018). Further work connecting nutrient restriction in development to neurological endpoints can give deeper insight into how physiology and behavior can be altered by land use changes.

Another crucial aspect of chapter 2 is understanding the effects of cowbird parasitism on developing young. Studies investigating the effects of brood parasites on their hosts tend to focus on fledging success rates or body condition measurements for nestlings (Lorenzana and Sealy 1999). I wanted to investigate how brood parasitism might impact availability of amino acids for host nestlings, and if there were any measurable differences in body condition between nestlings from parasitized urban, non-parasitized urban, and rural nests. I found no evidence of differences in multiple measurements of body condition by nestling group, nor of any differences in tissue-forming amino acids. This, especially when taken alongside the findings of chapter 1, supports the conclusion that urban song sparrows are not being compromised in any meaningful way despite lower arthropod abundance and biomass at urban sites. However, this aspect of my dissertation focused on the level of the individual, whereas the effects of cowbirds and urbanization may be having an effect on the population of host species, so it is important to clarify that I am unable to address any population or community level effects for song sparrows

in the scope of this dissertation. Further work should investigate how the environment and brood parasitism can interact to potentially restrict nutrient availability.

A meta-analysis of brown-headed cowbirds (*Molothrus ater*) effects on the nesting success of their hosts

Cowbirds have great conservation implications for their hosts, and therefore their effects on a variety of hosts have been well studied (Chace et al., 2005). However, cowbird impacts on nesting success and fitness proxies for urban hosts had not been synthesized. The meta-analysis I conducted in Chapter 3 provides insights into how cowbird clutch size varies across habitat types, and the effects cowbirds have on host clutch size and nest success rates, but I was unable to address how urbanization affected these host metrics due to a lack of extractable data. I was, however, able to determine that cowbird brood parasitism significantly lowered host average clutch size, a conclusion that is well supported by past reviews (Lorenzana and Sealy 1999; Payne 1977). Our final notable finding, that brood-parasitized nests are actually more likely to succeed than non-parasitized nests, contradicts these prior reviews. However, it is supported by studies that have found parasitized host nests to fail less often than non-parasitized ones, whether due to brood parasites damaging host species eggs if they remove cowbird eggs (Hoover and Robinson 2007), or if cowbirds are less likely to lay eggs in nests that become depredated (Avilés et al, 2006). Despite the counter-intuitive nature of this finding, parasitized host nests are not necessarily more likely to fail, in fact our evidence suggests that they are more likely to succeed than non-parasitized nests, though this does not take into account the number of nestlings successfully fledge. Based on my findings of mean clutch size being smaller in

parasitized nests, I would expect there to be fewer nestlings fledging from parasitized nests, even if the nest was more likely to survive.

There are studies that interrogate multiple effects of cowbirds on hosts in urban habitats, particularly parasitism rates by habitat type (Rodewald 2009, Chase et al., 2002), but more studies are needed to better understand if the directionality of these relationships, namely higher parasitism rates in urban habitats, holds. While grasslands are a preferred habitat for brown-headed cowbirds, further studies across more varied habitat types would allow for a greater understanding of how brood parasitism and habitat type might interact to impact hosts, but also give deeper insights into how the different ecology of a habitat might impact cowbird success. Additionally, I encourage the authors of future studies to make their nest success data not only available but easily accessible in order to aid future reviews, whether in this field or any other.

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