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Characterizing the Gut Microbiome Diversity of Urban and Non-Urban Dark-eyed Juncos (*Junco Hyemalis*)

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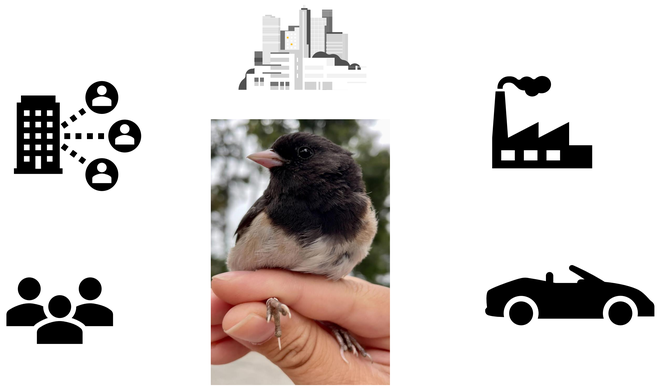
Abstract

Urbanization — the process by which areas with greater concentrations of human activities and development are formed — results in environmental changes and subsequent alterations to available resources. Consequently, gut microbial community composition of urban organisms are expected to be affected. Our study examined whether urbanization could affect gut microbiome diversity of dark-eyed juncos. Using iNEXT, alpha diversity metrics were calculated based on MiniOn-sequenced DNA cloacal swab reads of urban and montane junco populations. Urban juncos had higher Shannon diversity index values compared to montane juncos based on the Welch two-sample t-test (t=2.8850, df=11, P=0.0148). Our results suggest that urban-associated resources increase microbial diversity—possibly due to increases in plasticity of junco gut microbiomes correlated with urbanization-based changes in environment, resources and microbial sources. By studying how increases in microbial diversity alter mutualistic relationships between gut microbiomes and their hosts, the regulation of organism adaptation to increasing urbanization could be further understood.

Background

- The **gut microbiome** refers to the diverse community of microorganisms found within an animal's digestive tract. Often forming mutualistically beneficial relationships with their hosts, many gut microbiomes regulate functions essential for host survival including nutritional uptake, detoxification and immune defense.
- Avian gut microbiomes are plastic and can change with **urbanization** (Murray et al., 2020; Teyssier et al., 2020). However, the exact effects and nature of this relationship remains unknown.
- We aim to answer the **question:** How is the gut microbiome of organisms impacted as a result of urbanization?
- Dark-eyed juncos (*Junco hyemalis*) are small migratory songbirds originating from North American forests. Studied extensively across several urban and non-urban sites within Southern California, they are an effective means through which the effects of urbanization on organism gut microbiomes can be studied.
- Understanding the effects of processes like urbanization on gut microbiota of organisms can provide valuable insight into the regulation of organism adaptation and survival within changing environments.



Dark-eyed Junco
(Photo Credit: Lillian Ma, Yeh Lab)

Methods

Gut Microbiome Sample Collection
- Cloacal swabs were gathered from urban UCLA dark-eyed junco and non-urban montane Angeles National Forest (ANF) dark-eyed junco populations

Classification of Gut Microbiome
- MiniON sequencing was used to sequence DNA reads from the cloacal swabs
- DNA reads were taxonomically classified

Analysis of Gut Microbiome Diversity
- iNEXT alpha diversity metrics (Species Richness, Shannon diversity & Simpson diversity) were calculated for urban junco DNA reads (n=8) and non-urban junco DNA reads (n= 6)

Results

- Urban dark-eyed juncos had greater gut microbiome diversity compared to montane dark-eyed juncos. The mean Shannon diversity index value was 1.73 for montane juncos and 1.78 for urban juncos.
- Results of the Welch two-sample t-test performed on Shannon diversity index measurements between urban and montane dark-eyed juncos (t=2.8850, df=11, P=0.0148) indicated that the differences in gut microbiome diversity were statistically significant.

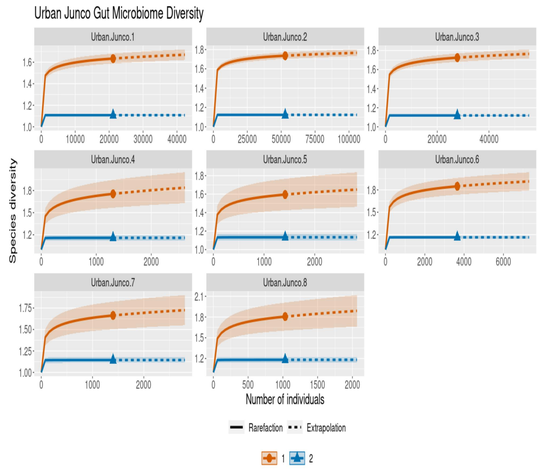


Figure 1: Shannon diversity (orange) and Simpson diversity (blue) alpha diversity graphs for urban dark-eyed juncos. Each graph represents the relative Shannon and Simpson diversity for a single junco.

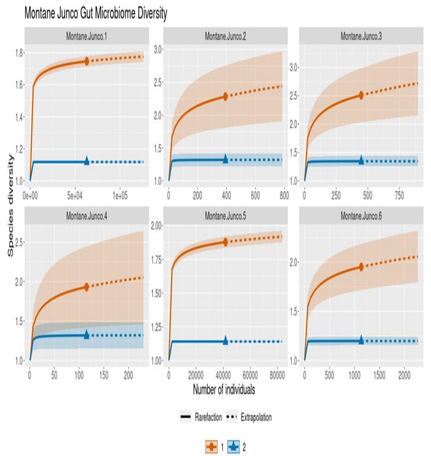


Figure 2: Shannon diversity (orange) and Simpson diversity (blue) alpha diversity graphs for montane dark-eyed juncos. Each graph represents the relative Shannon and Simpson diversity for a single junco.

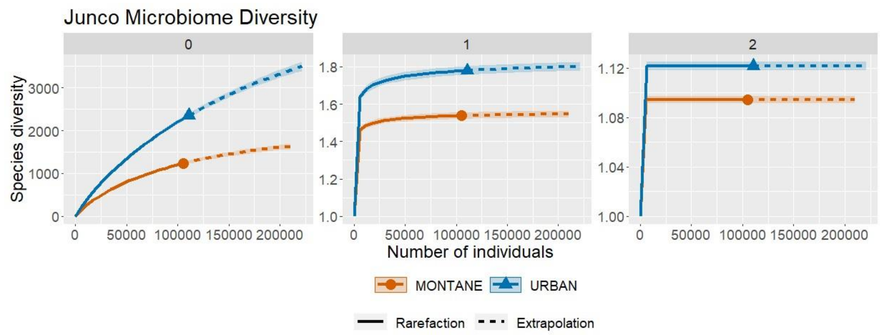


Figure 3: Species richness (0), Shannon diversity (1) and Simpson diversity (2) alpha diversity graphs for montane (orange) and urban (blue) dark-eyed juncos.

Discussion & Conclusion

Effects of Urbanization on Gut Microbiome Diversity

Statistically significant differences found between gut microbiome diversity of urban and montane dark-eyed juncos indicate that urbanization likely increases gut microbiome diversity

Potential Explanations for Observed Urbanization Effects on the Gut Microbiome

- Junco gut microbiomes are responding to increases in newer varieties of microbial sources with urbanization-associated changes in environment such as increases in impervious surfaces (Maraci et al., 2022).
- Prior to urbanization, junco diets primarily consisted of seeds and insects. More diverse sources of food associated with urbanization such as human food waste correlate with increases in junco microbial gut diversity.

Future Studies/Considerations

- While our findings are supported by some studies of urban avian populations (Obrochta et al., 2022), other studies contrastingly find decreased microbial diversity associated with urban birds (Teyssier et al, 2018).
- To better understand the exact impacts of urbanization on organism gut microbiome and its diversity, future studies can examine how specific elements of urbanization — such as increases in impervious surfaces or urban-derived food sources — individually and in combination with each other affect the gut microbiome.

Impacts and Significance

- Studying plasticity of gut microbiomes to factors such as urbanization can have possible positive implications for understanding how organisms adapt to changes in their environment and consequently, regulate their health and survival.

Acknowledgements

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