Influence of human activity on gut microbiota and immune responses of
Darwin’s finches in the Galápagos Islands

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ABSTRACT

Urbanization can influence animal traits, including immunity and gut microbiota. Over the past several decades, the Galápagos Islands have seen rapid resident human population growth and tourist activity, leading to varying levels of human activity across Islands. Consequently, diet, gut microbiota, and immunity of endemic animals, such as Darwin’s finches, may have changed. The goal of this study was to determine the effect of land use on the immune response, gut microbiota, and body measurements of Darwin’s finches in 2008, at a time of rapidly increasing human activity in the Islands. Specifically, we compared proxies of immunity (lysozyme activity, and haptoglobin, complement antibody, and natural antibody levels), gut microbiota (bacterial diversity, community structure and membership, and relative abundance of bacterial taxa), and body measurements (body mass, tarsus length, and scaled mass index) across undeveloped, agricultural, and urban areas for medium ground finches (Geospiza fortis) and small ground finches (G. fuliginosa). We found that lysozyme activity was lower and observed bacterial species richness was higher in urban areas compared to non-urban areas across both finch species. For small ground finches, relative abundances of three bacterial genera (Pseudoxanthomonas, Cloacibacterium, and Dietzia spp.) were higher in urban areas compared to non-urban areas, but this pattern was not observed in medium ground finches. Medium ground finches were smaller in undeveloped areas compared to the other two areas, but body measurements of small ground finches did not differ across areas. Our results suggest that human activity can impact immune measures and gut microbiota of Darwin’s finches.
INTRODUCTION

Human activity is rapidly changing the natural environment, which can influence avian ecology and evolution. For example, anthropogenic change can influence many avian traits, including morphology (e.g., bill size), behavior (e.g., boldness, predator avoidance, problem solving), and physiology (e.g., digestion, immunity) (Hendry et al., 2006; Møller, 2008; Atwell et al., 2012; Samia et al., 2017; Sol et al., 2018; Strandin et al., 2018). One factor that can mediate the effect of human activity on avian traits is changes in food availability. Humans can change food resource availability for birds by increasing the abundance of unnatural food through wild bird feeders, the disposal of human food waste into the environment, and by reducing the abundance of natural food items through environment changes (Murray et al., 2016; Bosse et al., 2017; Start et al., 2018). In turn, birds lacking natural food resources may be forced to invest less in maintaining body mass or physiological processes, such as immune responses, which can be energetically costly (Sheldon and Verhulst, 1996; Svensson et al., 1998; Lochmiller and Deerenberg, 2000; Demas, 2004; Sternberg et al., 2012; Cornet et al., 2014; Howick and Lazzaro, 2014).

In addition, human activity can alter avian traits through impacts on the gut microbiota, the community of symbiotic microbial species that live within the gut of their host (Grond et al., 2018). Different environments, including human-altered ones, contain different microbial communities that can colonize the gut of the host. As a result, the gut microbiota of the host can change in response to changes in land use, including urbanization (Phillips et al., 2018; Teyssier et al., 2018; Knutie et al., 2019; Berlow et al., 2021), and pollution of water and soil (Jin et al., 2017). In turn, the gut microbiota can interact with host cells to change host physiological traits, such as the immune system (reviewed in Round and Mazmanian, 2009);
increased bacterial diversity and the presence of specific bacterial taxa can be related to increased resistance to virulent parasites and pathogens. As human activity increases, along with the concern over parasite-causing diseases in birds, disentangling these relationships is important to understand how novel stressors can influence birds and help aid in their management and conservation.

The Galápagos Islands of Ecuador are relatively pristine but are facing increasing human activity, which allows for the study of host-associated microbiota and immune systems in response to urbanization. Over the past 20 years, ecotourism and the permanent resident human population has grown rapidly on the Islands (Walsh and Mena 2016). For example, from 2007 to 2017, the number of permanent human residents increased from approximately 20,000 to 30,000 people and tourist numbers increased from 140,000 to 225,000 people (Watkins and Cruz, 2007; Walsh and Mena, 2016). Consequently, in areas with a human presence (e.g., towns and popular tourist sites), the natural habitat and diet of endemic species have been altered with the introduction of agricultural plants and human processed food (de León et al., 2018). Studies have shown that changes in habitat type can affect gut microbiota and metrics of immunity of Darwin’s finches (Zylberberg et al., 2013; Michel et al., 2018; Knutie et al., 2019; Loo et al., 2019). However, all studies on the gut microbiota of Darwin’s finches have occurred since 2016 and so we know little about how earlier years of human activity has influenced microbiota in Darwin’s finches. Furthermore, the effect of human-influenced land use on the interaction between gut microbiota and immunity has received little attention. A better understanding of human impacts on avian immune systems could be especially important because introduced parasites, such as avian pox and Philornis downsi, have been wreaking havoc on the survival of birds in the islands (Wikelski et al., 2004; Fessl et al., 2010; Koop et al., 2016).
The goal of this study was to determine the effect of land use on the immune response, gut microbiota, and body measurements of Darwin’s finches in 2008. Specifically, we characterized the gut microbiota (bacterial diversity, community structure and membership, and relative abundance of bacterial taxa), immune response (lysozyme activity, and haptoglobin, complement antibody, and natural antibody levels), and body measurements (body mass, tarsus length, and scaled mass index) in small ground finches (*Geospiza fuliginosa*) and medium ground finches (*G. fortis*) across three different habitat types in 2008 on Santa Cruz Island in the Galápagos. Specifically, we caught finches in three different areas that varied in human activity and presence including an area with: 1) little human activity and no permanent human population (hereon, undeveloped), 2) developed land for agricultural activities but only a small permanent human population (hereon, agricultural), and 3) developed land for a substantial, permanent human population (hereon, urban).

Previous studies have shown that the immune response of Darwin’s finches can vary temporally across land use types. For example, Zylberberg et al. (2013) found that finches living in agricultural and undeveloped areas had different immune responses across years, whereas finches in urban areas showed no change across years. One explanation is that food availability changed across years but not in all habitats. Variation in food availability could either directly affect the immune response or the gut microbiota, which in turn would affect the immune response. However, Zylberberg et al. (2013) did not examine the effect of land use on the immune response within each year. Thus, for the present study, we examined the impact of land use on four metrics of immunity: lysozyme activity, and complement antibody, natural antibody, and haptoglobin (using PIT54 acute phase proteins) levels. We chose these four aspects of the innate immune system because each plays an integral and complementary role in the first line of
defense against novel pathogens (as previously discussed in Zylberberg et al., 2013). In short, the
complement system of proteins activates the lysis of foreign cells, enhances antibody activity,
and directly destroys viruses (Hirsch, 1982; Murphy et al., 2017). Natural antibodies bind novel
pathogens, facilitate phagocytosis, and promote cell lysis (Casali and Schettino, 1996; Caroll and
Prodeus, 1998). The PIT54 protein minimizes self-damage during inflammation and stimulates
the white blood cell response upon pathogen exposure (Wicher and Fries, 2006; Quaye, 2008).
Lysozymes lyse gram positive bacteria (Millet et al., 2007). Together, these measures afford a
broad view of the innate immune system by providing information on both inducible and
constitutive components of innate immunity.

We hypothesized that variation in land use would influence the immune response and gut
microbiota of finches. Specifically, we predicted that finches in areas with lower human activity
( agricultural and undeveloped areas) will have more similar immune responses and gut
microbiota than finches living in areas with higher human activity (urban areas). Furthermore,
because studies have shown that the metrics of the immune system can relate to changes in the
gut microbiota community (Round and Mazmanian, 2009), we hypothesize that bacterial
diversity will relate to immune measures in the finches. Finally, because food availability and
thus diet differs across sites, we predict that body size (mass and tarsus length) and condition
(scaled mass index; Peig and Green, 2009), will differ across land use type, as found in Knutie et
al. (2019). Overall, our study will provide insight into the effect of human activity on the gut
microbiota and immune proxies of island birds, which could have conservation implications for
Darwin’s finches (Ohmer et al., 2021).

METHODS
Field site

We conducted this study on Santa Cruz Island, Galápagos, Ecuador during the breeding season in February 2008. To examine the effect of land use patterns on microbiota, immune response, and body size of finches, we sampled small ground finches and medium ground finches at six sites, spanning three different land use types (urban, agricultural, and undeveloped). We selected the study sites to represent the range of vegetation types and precipitation present in each land use and elevation category. The urban site was in Puerto Ayora (population < 20,000 in 2007; (Watkins and Cruz, 2007), in the lowland arid zone. Three sites were located within the agricultural zone of the island, which contains a combination of small farms, fruit plantations and cattle ranches. One agricultural site was in the lowlands and two in the highlands. Two sites were in undeveloped areas, representing both arid lowland and moist highland zones. The Galápagos is well known for extreme fluctuations in precipitation as a result of the El Niño weather pattern; however, 2008 was neither particularly wet nor particularly dry on average (Zylberberg et al., 2013).

Sample collection

To obtain immune function and microbiota data, we caught birds in mist nets that were actively watched. Birds were removed from the mist net and processed as quickly as possible; in the event that multiple birds entered the mist net, they were removed and kept in cloth bags until they were banded and a blood sample collected. Body mass (g) and tarsus length (mm) were measured, and a scaled mass index (a metric of body condition) was calculated based on the methods from Peig and Green (2009). We banded each bird and collected blood samples using heparinized microcapillary tubes. For birds that defecated during handling, the fecal sample was
immediately collected and placed in formalin until used for the fecal bacterial DNA extraction at the University of Connecticut in 2019. Blood samples were kept on ice (for 4-6 hours) until they were centrifuged to separate the red blood cells from the plasma. Plasma was then frozen at -20°C.

**Immune measures**

We used a hemolysis-hemagglutination assay to measure levels of natural antibodies (lysis activity) and complement antibodies (agglutination activity) (Matson et al., 2005). We used a commercial kit (from Tri-delta Diagnostics Inc., Morris plains, NJ, USA) to determine the plasma concentration of PIT54 acute phase protein (haptoglobin levels) following Millet et al. (2007). We used the lyso-plate assay described in Millet et al. (2007) to measure levels of lysozyme activity in plasma samples. Each of these assays was carried out with previously described protocols (Zylberberg et al., 2014).

**Bacterial DNA extraction and sequencing**

Before starting the extraction from the finch feces, samples were centrifuged for 10 minutes at 10,000 rpm at 4°C and the supernatant (i.e., the formaldehyde) was then removed. Nanopure water (500 uL) was added, and the sample was vortexed and centrifuged again for 5 minutes at 4°C at 10,000 rpm. The supernatant was removed and the step was repeated. Nanopure water (200 uL) was then added to the sample and the sample was vortexed. Total DNA was extracted from finch feces using a Qiagen PowerFecal DNA Isolation Kit. The protocol listed in the kit was followed with the exception of the heating step; samples were heated for 30 minutes at 65°C. DNA extracts were then sent to the University of Connecticut Microbial Analysis.
Resources and Services for sequencing with an Illumina MiSeq platform and v2 2x250 base pair kit (Illumina, Inc.). We also amplified a laboratory blank to control for kit contamination and had no detectable product. Bacterial inventories were conducted by amplifying the V4 region of the 16S rRNA gene using primers 515F and 806R (Caporaso et al., 2012) and with Illumina adapters and dual indices (Kozich et al., 2013). Raw sequences were demultiplexed with onboard bcl2fastq and then processed in Mothur v.1.42.3 (Schloss et al., 2009) according to the standard MiSeq protocol (Kozich et al., 2013). Briefly, forward and reverse sequences were merged. All sequences with any ambiguities, that did not align to the correct region, or that did not meet length expectations, were removed. Sequences were aligned to the Silva nr_v128 alignment (Quast et al., 2013). Chimeric reads were also removed using UCHIME (Edgar et al., 2011). Non-bacterial sequences that are classified as chloroplasts, mitochondria, or unknown (i.e., did not classify to the level of kingdom) were removed. Sequences were grouped into operational taxonomic units (OTUs) based on a 97% similarity level and identification of the OTUs was done using the Ribosomal Database Project Bayesian classifier (Wang et al., 2007) against the Silva nr_v128 taxonomy database. Alpha and beta diversity statistics were calculated by averaging 1,000 random subsampling of 4,000 reads per sample. We calculated observed species richness (sobs) and evenness, Shannon diversity index, and Simpson diversity index. Sobs describes the number of observed species and evenness describes the distribution of abundance across the species. The Shannon diversity index and Simpson index are estimators of species richness and species evenness but species richness is weighted more for Shannon and species evenness is weighted more for Simpson. The resulting data sets included a total of 1,827,365 sequences and an average of 25,032 reads per sample (min: 4,079, max: 121,506).
Statistical analyses

Generalized linear models (GLMs) were used to determine the effect of land use on bacterial diversity metrics (sobs, Shannon Index, Simpson Index, and evenness), immune metrics (lysozyme activity, and haptoglobin, complement antibody, and natural antibody levels), and body size and condition (body mass, tarsus length, and scaled mass index). Shapiro-Wilks tests were used to determine whether the variables met normality; if the variables did not meet normality, they were log transformed for the analyses. Analyses were conducted using the glm function within the lme4 package (Bates et al., 2015). Probability values were calculated using log-likelihood ratio tests using the Anova function in the car package (Fox and Weisberg, 2019).

Analyses were conducted in R (2021, version 4.0.4) and RStudio (2021, version 1.4.1103).

The effects of location and species on bacterial community dynamics were examined using the Bray-Curtis dissimilarity and Jaccard similarity matrices. The matrices were created using the ‘vegdist’ function in the vegan package in R (Oksanen et al., 2019). The ‘adonis2’ function in the vegan package was used to perform PERMANOVA to assess the differences in bacterial community composition and membership between different species and locations (Oksanen et al., 2019). Both Bray-Curtis and Jaccard are dissimilarity matrices, with Bray-Curtis taking into account the relative abundances of shared taxa (community structure) while Jaccard only considers the presence or absence of such taxa (community membership). Principal Coordinates Analyses (PCoA), where the distances among the samples are converted onto a graph, were done to compare and visualize differences between groups.

Relative abundances (arcsine square root transformed; (Shchipkova et al., 2010; Kumar et al., 2012) of bacterial phyla and genera were compared between species and location groups. Data were manipulated using packages tidyr, reshape2, and plyr in R (Wickham, 2007, 2011;
Wickham and Henry, 2019) and ANOVAs were run in the car package in R (Fox and Weisberg, 2019); false discovery rate (FDR) tests were used to control for multiple analyses. A Tukey post-hoc test was done to determine which land use types differed from each other. All figures were created in Prism (2020, version 9).

RESULTS

Effect of land use and finch species on the gut microbiota

Bacterial diversity, as measured by sobs, differed among land use types for finches (Table S1; Fig. 1A). Birds in the urban area had higher sobs values than birds in agricultural and undeveloped areas (Table S2; \( P < 0.05 \)). However, there was no effect of species or an interaction between species and land use type on sobs values (Table S1). Land use, bird species, and their interaction did not affect Shannon Index, Shannon evenness, and the Simpson Index (Tables S1-S2).

Bacterial community structure \( (F = 1.33, P = 0.04) \) and membership \( (F = 1.19, P = 0.045) \) differed between urban areas compared to the other land use types for medium ground finches (Fig. 2A-B). However, bacterial community structure \( (F = 0.71, P = 0.98) \) and membership \( (F = 0.83, P = 0.98) \) did not differ significantly among land uses in small ground finches (Fig. 2A-B).

For small ground finches, relative abundances of several genera, including genera *Pseudoxanthomonas*, *Cloacibacterium*, and *Dietzia*, were higher in urban areas compared to the other two areas (Table S3). Relative abundances of phyla did not differ among land use types for small or medium ground finches \( (P > 0.05) \). Relative abundances of genera did not differ among land use types for medium ground finches either \( (P > 0.05) \).
Effect of land use and finch species on the immune response

Lysozyme activity differed among land use types for the medium and small ground finches (Fig. 1B; Table S4). Overall, urban finches had statistically lower lysozyme levels than finches in the agricultural and undeveloped areas, but this effect was more pronounced in medium ground finches (Fig. 1B; Table S5). Haptoglobin, complement antibody, and natural antibody levels did not differ significantly among land use types in either medium or small ground finches (Table S4-S5). Immune metrics did not correlate significantly with the bacterial diversity metrics ($R^2 < 0.20$ for all pairs).

Effect of land use and finch species on body size and mass

Body mass differed between small and medium ground finches (Fig. 3; Table S6-S7). For small ground finches, body mass did not differ significantly among land use types, but for medium ground finches, individuals in the undeveloped area had lower body mass than individuals in agricultural and urban areas (Fig. 3; Table S7). Tarsus length and scaled mass index differed significantly between species but not among land use types (Table S6-S7).

DISCUSSION

Our study examined the effect of land use on the gut microbiota, immune metrics, and body measurements of two Darwin’s finch species in 2008, at the start of a rapid increase in human activity in the Galápagos Islands. Studies of samples collected in 2016 have found significant impacts of human activity on metrics of the gut microbiota and body measurements of Darwin’s finches. Based on data collected eight years earlier, we found marginal effects of urbanization on the observed species richness (sobs) across bird species and a few bacterial
genera (*Pseudoxanthomonas*, *Cloacibacterium*, and *Dietzia*) in small ground finches. Similarly, we found few effects of land use on immune metrics and body measurements, with urbanization affecting lysozyme levels across bird species and body mass in medium ground finches. Furthermore, bacterial diversity did not correlate with any of the immune measures. Although humans have had a permanent presence in the Galápagos for decades, our results suggest that initially, increased human activity (starting ~2007) had marginal effects on the finches.

Lysozyme activity, but not the other immune metrics, was influenced by land use type; urban birds had lower lysozyme activity than non-urban birds. Lysozymes are part of the innate immune system, which are transferred from mother to egg whites. During the breeding season, lysozyme activity declines in females during pre-laying and egg laying (Saino et al. 2002). Urban Darwin’s finches differ in the length and timing of their breeding season compared to non-urban birds (Harvey et al. 2021). Samples were collected from female finches in February, which is early in the breeding season. Therefore, timing of breeding could be responsible for differences in lysozyme activity in urban vs. non-urban birds. Furthermore, although lysozyme is an antimicrobial enzyme, we found no relationship between lysozyme activity and bacterial diversity. This lack of relationship is likely because the lysozymes quantified in our study were in the plasma, rather in the gut mucosal tissue. Overall, because lysozyme activity differs across land uses in 2008, early in urbanization, future studies could focus on how lysozyme activity has changed in finches since then, especially in response to emerging diseases (Wikelski et al. 2004).

Observed species richness (sobs) was higher in urban areas compared to non-urban areas. However, the other alpha diversity metrics that consider species evenness did not differ across habitats. Other studies that have found higher observed species richness and Shannon index in urban birds indicate that this could be caused by man-made landscapes and a higher grass and
tree cover (Phillips et al., 2018; Berlow et al., 2021). However, there have also been studies demonstrating less bacterial diversity in species living in an urban habitat when compared to species living in a non-urban habitat (Barelli et al., 2015; Teyssier et al., 2018, 2020; Knutie et al., 2019). A less diverse gut community in urban areas could be caused by habitat disturbances or differences in diets between urban and non-urban populations (Sonnenburg et al., 2016; Teyssier et al., 2018, 2020; Knutie, 2020). The time of sampling should also be considered, as Teyssier et al. (2018) found that birds in a non-urban habitat exhibited less microbiome diversity during the winter months, while microbiome diversity in birds in an urban habitat did not differ across seasons. As the birds included in this study were sampled at the start of the rainy/breeding season, it is possible that a seasonal effect contributed to the difference seen between birds from non-urban and urban habitats. Overall, our results contribute to the growing body of literature demonstrating that there are many factors to consider when studying the relationship between urbanization and gut microbiome diversity.

Relative abundances of several genera, including Pseudoxanthomonas (phylum Proteobacteria), Cloacibacterium (phylum Bacteroidetes), and Dietzia (phylum Actinobacteria), were higher in urban areas than non-urban areas in small ground finches in 2008. Studies have not specifically documented differences in these genera across land use types but have found higher relative abundances of family Weeksellaceae (includes Cloacibacterium spp.) in urban areas compared to non-urban areas (Berlow et al., 2021). Interestingly, small ground finches in 2016 only showed increases in the relative abundance of Steroidobacter spp. in the presence of humans (Knutie et al., 2019). Although we found no effect of human activity on bacterial phyla in 2008 finches, 2016 finches in areas with higher human activity had higher relative abundances of phyla Chlamydiae and Cyanobacteria than in other areas.
One explanation for differences in bacterial taxa between 2008 and 2016 finches is that the gut microbiota of finches has changed over eight years coinciding with a change in the finches’ environment. Davidson et al., (2020) found that relative abundances of phyla Proteobacteria, Bacteroidetes, Actinobacteria in great tits (Parus major) were higher in urban areas compared to rural areas and/or when tits were fed an insect-based diet compared to a seed-based diet. Recent studies have found that urban small and medium ground finches tend to prefer human food diets (e.g., chips and cooked rice) rather than their natural diet (e.g., seeds) which could explain our results (de León et al., 2018). Alternatively, urban contaminants could be responsible for changes in the gut bacterial taxa observed in the finches. Pseudoxanthomonas spp. have been found in oil-polluted water bodies and soil because the bacterial taxa can metabolize and degrade the contaminant (Young et al., 2007). Although human activity in the Islands did not rapidly increase until approximately 2007, sources of human-driven pollution were present in towns such as Puerto Ayora. For example, the major presence of vehicles began in the 2000s with the completion of major paved roads. Although contaminants can affect the abundance of bacterial taxa, medium ground finches were likely exposed to the same environmental contaminants as the small ground finches but, nonetheless, did not show differences in bacterial taxa across land uses. However, since small and medium ground finches do eat different natural diets, perhaps the natural diet of the small ground finches shifted in response to urbanization over time, while the medium ground finches did not.

Body mass differed across land-use types for medium ground finches, with non-urban birds having lower body mass than those in urban areas. These results were also found by McNew et al. (2017) and can potentially be explained by differences in food availability across sites; human activity can affect food availability and preference for wildlife. For example, the
diet of non-urban birds includes mostly natural foods, such as seeds, fruits, and insects, while urban birds can prefer human processed food (Murray et al., 2016; de León et al., 2018; Phillips et al., 2018). A human-based diet can affect body morphometrics of animals, including increased body mass (Banks and Dickman, 2000; Bayol et al., 2007; Wilcoxen et al., 2015). In contrast, we found no effect of land use on the body mass of small ground finches. Small ground finches have smaller bill sizes (length, width, depth) and therefore different diets than medium ground finches (Abbott et al., 1977). One explanation is that perhaps medium ground finches are better able to exploit the urban diet than small ground finches. Interestingly, small ground finches in areas with more human activity in 2016 were larger than in areas with no human activity, which might be related to the increase in human activity over time. Since museum specimens and long-term field data exist, a future study could determine the effect of human activity on different finch species in urban and non-urban areas across islands.

CONCLUSION

Our study suggests that immunity, gut microbiota, and body mass of two species of Darwin’s finches vary across land use prior to the rapid increase in human activity in Galápagos Islands. These results suggest that earlier human activity can affect the ecology of birds. Over the past several decades, Darwin's finches have faced increasing challenges from invasive parasites (Wikelski et al., 2004; B. Fessl et al., 2010; Koop et al., 2016; Knutie, 2018) and predators (Gotanda, 2021), anthropogenic debris (Theodosopoulos and Gotanda, 2018; Harvey et al., 2021), and dynamic annual changes in natural and novel food availability (Grant and Grant, 1995; de León et al., 2018), which can all affect the physiology and gut microbiota of animals. Given our results and the novel challenges facing the Galápagos Islands, the iconic Darwin’s
finch system has exciting potential for future ecoimmunology and microbiome research in a changing world (Ohmer et al., 2021).

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Authors’ contributions

MZ and SAK conceived the study, MZ collected the samples and field data, MZ and AA conducted the laboratory work, AA conducted the bioinformatics, JNB, AA, and SAK did the data analyses. All authors wrote, revised, and approved the manuscript.

Data accessibility
Data are available at FigShare (doi: available upon acceptance) and sequences have been uploaded to GenBank (BioProject accession number: available upon acceptance).

Conflict of Interest

The authors declare that they have no conflict of interest.

LITERATURE CITED


Gotanda KM (n.d.) Human influences on antipredator behaviour in Darwin’s finches.


Figure Legends

Fig 1. The effect of land use type (undeveloped, agricultural [Ag], urban) on mean (± SE) sobs bacterial diversity (A) and lysozyme activity (B). Each point represents an individual.

Fig. 2. The effect of land use type on bacterial community structure and membership for medium ground finches (A: structure, B: membership) and small ground finches (C: structure, D: membership).

Fig. 3. The effect of land use type (undeveloped, agricultural [Ag], urban) and finch species (small ground finch [SGF], and medium ground finch [MGF]) on body mass.
Fig. 1.
Fig. 2.

![Diagram showing PCoA plots for different land use categories.](image-url)
Fig. 3.

![Graph showing body mass (g) of medium and small ground finches across different environments (Un-developed, Ag, Urban).](image-url)