

ORIGINAL ARTICLE

Human activity can influence the gut microbiota of Darwin's finches in the Galapagos Islands

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Abstract

The gut microbiota of animal hosts can be influenced by environmental factors, such as unnatural food items that are introduced by humans. Over the past 30 years, human presence has grown exponentially in the Galapagos Islands, which are home to endemic Darwin's finches. Consequently, humans have changed the environment and diet of Darwin's finches, which in turn, could affect their gut microbiota. In this study, we compared the gut microbiota of two species of Darwin's finches, small ground finches (*Geospiza fuliginosa*) and medium ground finches (*Geospiza fortis*), across sites with and without human presence, where finches prefer human-processed and natural food, respectively. We predicted that: (a) finch microbiota would differ between sites with and without humans due to differences in diet, and (b) gut microbiota of each finch species would be most similar where finches have the highest niche overlap (areas with humans) compared to the lowest niche overlap (areas without humans). We found that gut bacterial community structure differed across sites and host species. Gut bacterial diversity was most distinct between the two species at the site with human presence compared to the site without human presence, which contradicted our predictions. Within host species, medium ground finches had lower bacterial diversity at the site with human presence compared to the site without human presence and bacterial diversity of small ground finches did not differ between sites. Our results show that the gut microbiota of Darwin's finches is affected differently across sites with varying human presence.

KEYWORDS

birds, Darwin's finches, Galapagos Islands, microbiome, species interactions

1 | INTRODUCTION

Human-induced environmental changes can dramatically affect the evolutionary ecology of animals (Alberti et al., 2017; Johnson & Munshi-South, 2017). For example, the presence of humans can change food resource availability by reducing the abundance of natural food items but also by increasing the abundance of unnatural food through wild bird feeders and the disposal of human rubbish in the environment (Bosse et al., 2017; Murray, Becker, Hall, & Hernandez, 2016; Start, Bonner, Weis, & Gilbert, 2018). Such changes in food

availability can affect animal morphology, physiology (e.g., immune function, hormones and digestion), and behavioural traits (e.g., communication, predator avoidance and boldness; Atwell et al., 2012; Hendry et al., 2006; Møller, 2008; Samia et al., 2017; Sol et al., 2018; Strandin, Babayan, & Forbes, 2018; Van Donselaar, Atma, Kruyf, LaCroix, & Proppe, 2018).

The presence of humans can also have an indirect effect on animals by affecting their gut microbiota (Phillips, Berlow, & Derryberry, 2018; Teyssier et al., 2018). For example, birds at sites with human presence have lower gut bacterial diversity than birds at

sites without human presence, potentially due to the differences in land cover types in each environment (Phillips et al., 2018). In contrast, a few other studies have found that gut bacterial diversity was higher in birds at sites with human presence compared to birds at sites without human presence, which was thought to be driven primarily by differences in diet (Furst, Veit, Hahn, Dheilly, & Thorne, 2018; Teyssier et al., 2018). This explanation is corroborated by other studies that have shown a direct effect of host diet composition on their gut microbiota (Bletz et al., 2016; Carmody et al., 2015; David et al., 2014). Determining which environmental factors affect gut microbiota is important because the microbiota can influence many physiological and behavioural traits of their hosts (reviewed in Grond, Sandercock, Jumpponen, & Zeglin, 2018; Kohl, 2012); for example, increased diversity in gut bacteria can lead to increased host resistance to parasites (Dillon, Vennard, Buckling, & Charnley, 2005; Knutie, Wilkinson, Kohl, & Rohr, 2017).

Over the past 30 years, ecotourism and the permanent resident human population has grown exponentially on the Galapagos Islands of Ecuador. The Galapagos currently hosts 225,000 tourists each year and is home to ~30,000 permanent residents (Walsh & Mena, 2016). Consequently, in areas with the presence of humans (e.g., towns and areas with high human visitation rates), the natural habitat and diet of endemic species, such as Darwin's finches, have been altered (de León et al., 2018). In turn, changes in the natural and unnatural diet of Darwin's finches has affected selection on their bill size and shape (Hendry et al., 2006; de León, Podos, Gardezi, Herrel, & Hendry, 2014; de León et al., 2018) and thus might also affect their gut microbiota. Michel et al. (2018) found that the gut microbiota of Darwin's finches did not vary among species, except for the vampire finch, which feeds periodically on bird blood. However, Michel et al. (2018) focused on natural, undisturbed areas that were not influenced at all by the continued presence of humans.

The goal of this study was to compare the gut microbiota of two species of Darwin's finches (small ground finches [*Geospiza fuliginosa*] and medium ground finches [*Geospiza fortis*]) across three sites, each with varying exposure to the presence of humans. We focused on localities that were studied by de León et al. (2018) because the authors showed that Darwin's finches, such as small and medium ground finches, in areas with human presence preferentially consumed human-based food (i.e., crisps, biscuits and rice) over their natural foods salt bush (*Cryptocarpus pyriformis*), smooth-stemmed tournefortia (*Tournefortia psilostachya*) and thorn shrub (*Scutia spicata*); in contrast, finches in areas without human presence did not forage on human-based food when given the option. Because diet can influence gut microbiota, we hypothesized that measures of the gut bacterial communities, such as diversity, relative abundance of taxa, and community structure and membership, would differ across a gradient of human disturbance. We did not have an a priori prediction of the directional change in the gut bacterial diversity because recent studies have found both positive and negative effects of human presence on gut bacterial diversity of birds (Furst et al., 2018; Phillips et al., 2018; Teyssier et al., 2018). Because niche overlap (e.g., diet overlap) of finches is highest in areas with human

presence compared to areas without humans (de León et al., 2018), we predicted that gut microbiota would be more distinct between finch species in the sites without human presence than sites with humans. However, we acknowledge that we might also find no difference in the gut microbiota between species, as found by Michel et al. (2018).

We also examined whether site influences the morphology, such as bill size and body mass, of small and medium ground finches. We predicted that body mass would be higher in areas with humans because the finches forage more on processed food from humans in this site as compared to the site without humans (de León et al., 2018), and eating "junk food" can cause body mass gain in other species (Bayol, Farrington, & Stickland, 2007; French, Story, & Jeffery, 2001; Hill & Peters, 1998). We hypothesized that finch body mass would correlate with gut bacterial diversity because previous studies have found a relationship between the two factors (Backhed et al., 2004; Ley et al., 2005). We also predicted that bill size would vary across localities because the bill size of a Darwin's finch population can change in a relative short period of time (e.g., years) in response to changes in natural and unnatural food availability (Hendry et al., 2006; de León et al., 2014, 2018).

2 | METHODS

2.1 | Field site and study system

We studied breeding female small ground finches (*Geospiza fuliginosa*) and medium ground finches (*Geospiza fortis*) from March 5 to 14, 2016 on the eastern coast of Santa Cruz Island, Galapagos Islands, Ecuador. Field sites were in a low-elevation, arid zone within 2 km of each other but varied in the level of human presence (de León et al., 2018). During the breeding season, the territory size of a pair of Darwin's finches is less than 250 m² (Price, 1984), and therefore the focal females were unlikely to forage at more than one of our sites. At the first sampling site, El Garrapatero ("no human presence," 0°41.200'S, 90°13.311'W), humans are rare (except for researchers) and thus human food is rare and finches feed primarily on salt bush (*Cryptocarpus pyriformis*), smooth-stemmed tournefortia (*Tournefortia psilostachya*) and thorn bush (*Scutia spicata*) (de León et al., 2014, 2018). At the second site, the parking lot ("intermediate," 0°41.400'S, 90°13.354'W), transient humans are present throughout the day but primarily only to walk from their vehicle to the beach. Human food is not found often in the parking lot (S.A. Knutie and K.M. Gotanda pers. obs.), probably because the consumption of food by humans is typically done at the beach. The third site, the beach ("human presence," 0 41.621'S, 90°13.266'W), does not support a permanent human population but about five to 20 tourists visit the beach each day (and sometimes many more; de León et al., 2018). Humans often have picnics and consume food at the beach and finches recognize human food, such as crisps, biscuits and rice, as a resource at this site (de León et al., 2018). These sites are located ~10 km from the nearest human settlements (Bella Vista and Puerto Ayora). Within the Galapagos archipelago, Santa Cruz hosts the largest resident

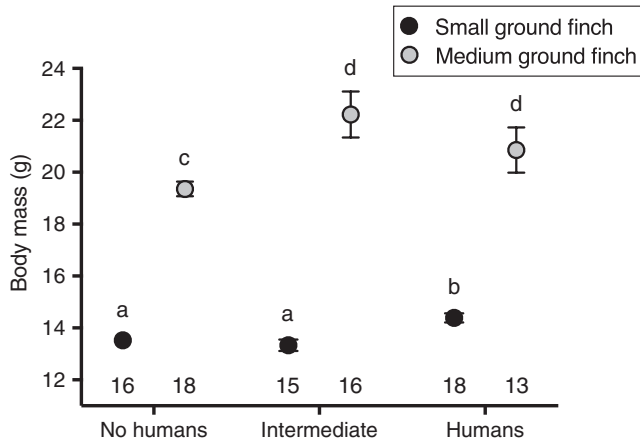


FIGURE 1 Body mass of female small and medium ground finches across sites. Letters indicate whether there is a significant difference (different letters; $p < 0.05$) or no significant difference (same letters; $p > 0.05$) between and within species across sites. Numbers represent the sample size

human population (~12,000 people) and the most tourists annually (~218,000 people; Parque Nacional Galápagos, 2016).

Female small and medium ground finches were captured with mist-nets between 06.00 and 10.00 hr. We focused on females only as opposed to both sexes because we wanted to achieve a sufficient sample size for each location and the gut microbiota can differ between sexes, especially in response to diet (Bolnick et al., 2014). Because the plumage of females and young males is similar (Grant, 1999), only individuals with a partial or full brood patch were sampled. Briefly, finches were removed from the net and placed individually in a paper bag that was lined with a sterile plastic tray and grate for 3–5 min, following the protocol from Knutie and Gotanda (2018). Once the finch defecated, the bird was removed from the bag and banded with a numbered aluminium band. Bill length, width and height (± 0.02 mm) were measured with stainless steel digital calipers and mass (± 0.1 g) was measured using an Ohaus CS200-100 portable compact scale. The faecal sample was then moved from the tray to a tube, placed on ice in the field for up to 6 hr, and then stored in a -20°C freezer until the bacterial DNA was extracted from the sample. Although studies show that the bacterial community in avian faeces does not always represent the entire digesta of the host (e.g., in the caecum; Stanley, Geier, Chen, Hughes, & Moore, 2015; Wilkinson et al., 2016), faecal samples are generally representative of the bacterial community in the large intestines (Videvall, Strandh, Engelbrecht, Cloete, & Cornwallis, 2018; Wilkinson et al., 2016) and are used when hosts cannot be euthanized (Kohl, 2017).

2.2 | Bacterial DNA extraction and sequencing

Total DNA was extracted from finch faeces using a MoBio PowerFecal DNA Isolation Kit. DNA extractions were then sent to the University of Connecticut Microbial Analysis, Resources and Services for sequencing with an Illumina MiSeq platform and version 2.2 \times 250-bp kit (Illumina). We also amplified a laboratory blank to

control for kit contamination and, after six rounds of amplification, 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, Westcott, Baxter, Highlander, & Schloss, 2013). Raw sequences were demultiplexed with onboard bcl2fastq and then processed in MOTHUR version 1.39.5 (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_v119 alignment (Quast et al., 2013). Chimeric reads were also removed using UCHIME (Edgar, Haas, Clemente, Quince, & Knight, 2011). Nonbacterial sequences that 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, Garrity, Tiedje, & Cole, 2007) against the SILVA nr_v119 taxonomy database. Alpha and beta diversity statistics were calculated by averaging 1,000 random subsamplings of 7,000 reads per sample. We calculated Shannon and Simpson diversity indices, which are measures of diversity that take into account the number of species present and their relative abundances, but the Simpson index gives more weight to common taxa. The resulting data sets included 4,372,901 sequences and an average of 48,053 reads per sample (min: 10,560, max: 104,960).

2.3 | Statistical analyses

We used generalized linear models (GLMs) with Gaussian errors to determine the effect of host species and site on bacterial diversity and morphological measurements and the relationship between bacterial diversity and finch morphology. Data were log-transformed as appropriate to meet assumptions of normality. Probability values were calculated using log-likelihood ratio tests using the Anova function in the car package (Fox & Weisberg, 2002). Pairwise post-hoc t -tests were used to determine differences among variables. Analyses were conducted in R (2017, version 3.4.3) and all figures were made in PRISM (2017, version 7).

We determined the effect of host species and site on bacterial community structure and membership with Bray–Curtis and Jaccard dissimilarity matrices, respectively, using PERMANOVA+ (2008, version 1.0.1; with 999 permutations) in PRIMER (2008, version 6.1.11). Ordination plots of community membership and structure are presented as distance-based redundancy analyses (dbRDAs), which were calculated in PRIMER. Unweighted scores represent bacterial community membership, which is based on the presence or absence of bacterial taxa, whereas weighted scores represent bacterial community structure, which also takes into account the relative abundance of bacterial taxa. Relative abundances (arcsine square root transformed; Shchepkova, Nagaraja, & Kumar, 2010; Kumar, Mason, Brooker, & O'Brien, 2012) of bacterial phyla and genera were

	Species	Site	Interaction
Body mass	$\chi^2 = 290.49, df = 1,$ $p < 0.001$	$\chi^2 = 9.71, df = 2,$ $p < 0.01$	$\chi^2 = 10.25,$ $df = 2, \mathbf{p < 0.01}$
Bill length	$\chi^2 = 411.08, df = 1,$ $p < 0.001$	$\chi^2 = 1.09, df = 2,$ $p = 0.58$	$\chi^2 = 3.01, df = 2,$ $p = 0.22$
Bill depth	$\chi^2 = 436.15, df = 1,$ $p < 0.001$	$\chi^2 = 4.91, df = 2,$ $p = 0.09$	$\chi^2 = 4.54, df = 2,$ $p = 0.10$
Shannon index	$\chi^2 = 1.70, df = 1,$ $p = 0.19$	$\chi^2 = 1.78, df = 2,$ $p = 0.41$	$\chi^2 = 6.64, df = 2,$ $p = 0.03$
Simpson index	$\chi^2 = 1.46, df = 1,$ $p = 0.23$	$\chi^2 = 1.70, df = 2,$ $p = 0.43$	$\chi^2 = 8.38, df = 2,$ $p = 0.01$

Bold text highlights statistical significance of $p < 0.05$.

analysed in JMP (2016, version 13) using one-way analyses of variance (ANOVAs) with host species and site as independent variables. For all analyses, p -values were corrected using the false discovery rate correction for multiple comparisons.

3 | RESULTS

3.1 | Morphological measurements

Body mass differed between bird species, among sites, and with an interaction between site and species (Figure 1; Tables 1 and S1). Small ground finches were, on average, 34% lighter than medium ground finches (small ground finch: $n = 49$, mean \pm SE 13.78 ± 0.14 g, 11.9–16 g; medium ground finch: $n = 47$, 20.75 ± 0.43 g, range: 17.2–28.5 g). For medium ground finches, birds at the site without human presence were, on average, 13% and 7% lighter than birds at the intermediate site and site with human presence, respectively (human presence: $n = 13$, 20.85 ± 0.87 g; intermediate: $n = 16$, 22.23 ± 0.89 g; no human presence: $n = 18$, 19.36 ± 0.28 g). For small ground finches, birds at the intermediate and site without human presence, respectively, were 6% and 7% lighter than birds at the site with human presence (human presence: $n = 18$, 14.39 ± 0.18 g; intermediate: $n = 15$, 13.33 ± 0.22 g; no human presence: $n = 16$, 13.53 ± 0.23 g).

Bills of medium ground finches were larger than bills of small ground finches (Table S1). The bill length of medium ground finches was 29% larger and bill depth was 34% larger than small ground finches (medium ground finch: $n = 47$, length: 11.79 ± 0.15 mm, range: 9.95–14.5 mm, depth: 10.74 ± 0.17 mm, range: 8.90–13.70 mm; small ground finch: $n = 48$, length 8.42 ± 0.08 mm, 7.20–9.50 mm, depth: 7.05 ± 0.06 mm, range: 6.25–7.70 mm). Bill size did not differ significantly among sites for either species (Tables 1 and S1).

3.2 | Bacterial alpha diversity

Shannon and Simpson indices did not differ significantly between species or among sites (Figure 2; Tables 1 and S1). However, there was an effect of the interaction between species and site on both indices (Tables 1 and S1; Figure 2a, b). For medium ground finches, individuals at the site with human presence had lower bacterial diversity

TABLE 1 Effect of species, site, and their interaction on morphology and gut bacterial diversity indices using general linear models

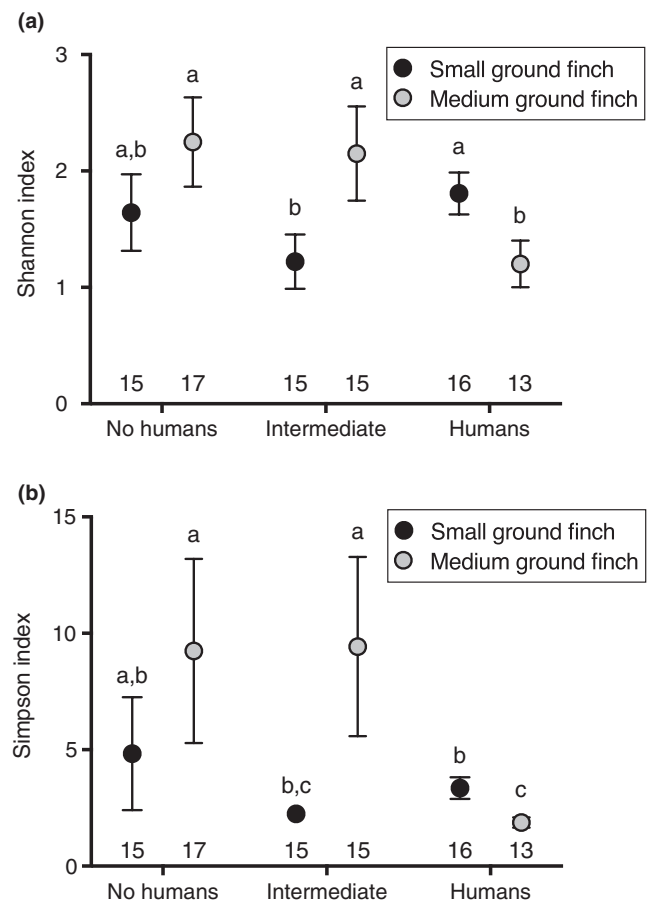


FIGURE 2 The Shannon index (a) and Simpson index (b) for the gut bacterial community of small and medium ground finches across sites. Letters indicate whether there is a significant difference (different letters; $p < 0.05$) or no significant difference (same letters; $p > 0.05$) between and within species across sites. Numbers represent the sample size

than the other two sites but this difference was only statistically significant for the Simpson, but not the Shannon, index (Shannon, human presence vs. no human presence: $p = 0.06$, human presence vs. intermediate: $p = 0.08$; Simpson: $p = 0.04$ for both analyses). For small ground finches, the Shannon index was higher in individuals at the site with human presence compared to the intermediate site

($p = 0.03$), and this difference was not significant for the Simpson index ($p = 0.09$). Small ground finches had higher bacterial diversity than medium ground finches at the site with human presence (Shannon index: $p = 0.03$, Simpson index: $p = 0.003$). Mass did not correlate with diversity indices in small ground finches (Shannon: $\chi^2 = 0.11$, $df = 1$, $p = 0.74$, Simpson: $\chi^2 = 0.60$, $df = 1$, $p = 0.44$) or medium ground finches (Shannon: $\chi^2 = 0.33$, $df = 1$, $p = 0.56$, Simpson: $\chi^2 = 1.02$, $df = 1$, $p = 0.31$).

3.3 | Bacterial community structure

Bacterial community structure differed between species ($F_{1,90} = 2.79$, $p = 0.02$; Figure 3a) and among locations, but this difference was not statistically significant ($F_{2,90} = 1.56$, $p = 0.08$). The interaction between species and location did not significantly affect community structure ($F_{2,90} = 0.94$, $p = 0.44$). Species, location, and the interaction between species and location affected bacterial community membership (species: $F_{1,90} = 1.25$, $p = 0.03$, location: $F_{2,90} = 1.58$, $p = 0.01$, interaction: $F_{2,90} = 1.20$, $p = 0.01$; Figure 3b).

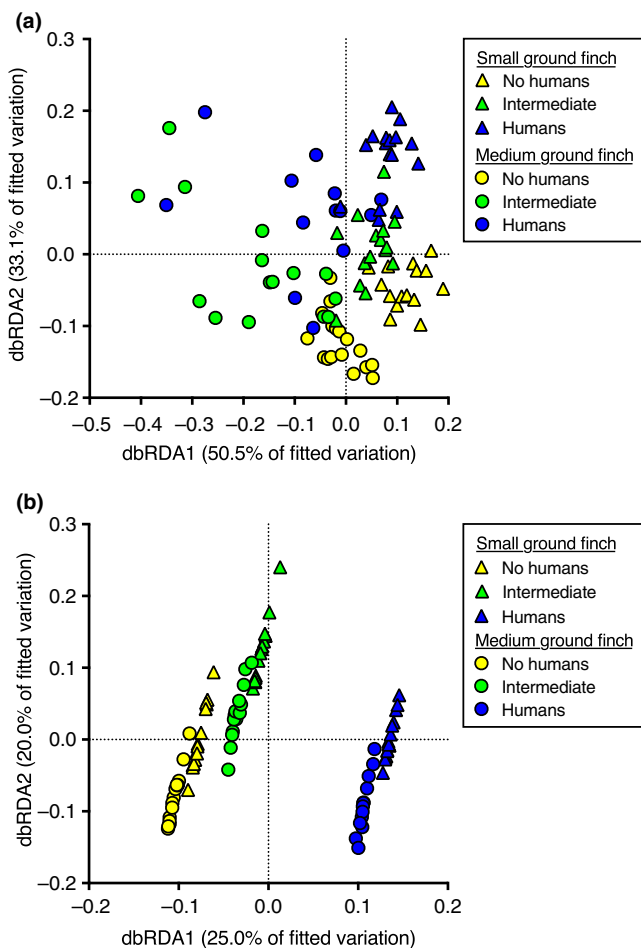


FIGURE 3 Bacterial community structure and membership based on the (a) Bray-Curtis dissimilarity matrix and (b) Jaccard dissimilarity matrix, respectively, from the guts of female small and medium ground finches across sites [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | Relative abundance of taxa

Relative abundances of bacterial phyla did not differ significantly between species and for medium ground finches across sites (Figure S1). For small ground finches, relative abundances of phyla Acidobacteria and Planctomycetes were lower in birds at the intermediate site compared to the sites with and without human presence (Acidobacteria: $F = 12.72$, $p = 0.03$, human presence: 0.10%, intermediate: 0.03%, no human presence: 0.10%; Planctomycetes: $F = 9.54$, $p = 0.05$, human presence: 0.50%, intermediate: 0.20%, no human presence: 0.60%). Additionally, relative abundances of Chlamydiae and Cyanobacteria were higher in small ground finches at the site with human presence compared to the intermediate site and the site without human presence (Chlamydiae: $F = 12.10$, $p = 0.03$, human presence: 0.005%, intermediate: 0.001%, no human presence: 0.002%; Cyanobacteria: $F = 11.27$, $p = 0.04$, human presence: 38.67%, intermediate: 24.78%, no human presence: 16.71%). Lastly, the relative abundance of Chloroflexi was higher at the site with human presence (0.05%) compared to the intermediate site (0.02%; $F = 10.53$, $p = 0.04$).

For genera, the relative abundance of *Lactobacillus* sp. was nearly twice as high in small ground finches (69%) compared to medium ground finches (37%) ($F = 15.73$, $p = 0.03$). For small ground finches, the relative abundance of *Saccharothrix* sp. was lower in birds from the intermediate site (0%) compared to the site with human presence (0.02%) and site without human presence (0.01%; $F = 20.26$, $p = 0.01$). Additionally, the relative abundance of *Steroidobacter* sp. was higher at the site with human presence (0.08%) compared to the intermediate site (0%) and site without human presence (0.01%; $F = 19.44$, $p = 0.01$). For medium ground finches, the relative abundance of genera did not differ significantly across sites.

4 | DISCUSSION

Our study found that the gut microbiota and body mass of breeding female small and medium ground finches differed across sites but these differences depended on the site and finch species. As predicted, both finch species had higher body mass at the site with humans compared to the site without humans. Overall, bacterial community structure and the relative abundance of certain genera, such as *Lactobacillus* sp., but not bacterial phyla and diversity, differed between finch species. Instead, finch species differed in their gut bacterial diversity across sites but with different patterns. Medium ground finches had lower bacterial diversity at sites with humans compared to sites without humans; small ground finches had higher gut bacterial diversity at sites with humans compared to intermediate sites but did not differ from sites without humans. Interestingly, small and medium ground finch gut bacterial diversity was least similar at the site with human presence and more similar at the site without human presence. These results contradicted what we predicted given the results of de León et al. (2018), which showed that niche overlap of finches was lowest in areas without

human presence and highest in areas with human presence. Overall, these results suggest that Darwin's finches are affected similarly (body mass) and differently (gut microbiota) by localities that vary in the presence of humans, which might have implications for the evolutionary ecology of the different finch species.

If differences in diet are driving differences in the gut microbiota of finches, we would predict the microbiota to be most similar at the site with human presence as compared to the site without human presence. However, generally, we found that the gut bacterial community structure differed among sites between species. We also found that the gut bacterial diversity of small and medium ground finches was more similar at the site without human presence as compared to the site with human presence. These results might be explained by competition between medium and small ground finches at the site with human presence. Human food availability might be limited to the period when the humans are at the site (i.e., generally midday). Thus, if medium ground finches, which are larger, can outcompete the small ground finches for the human food, the small ground finches might have to rely more on their natural diet. Additionally, during the breeding season (January–May) both small and medium ground finches will feed on the fruits and invertebrates at the site without human presence (de León et al., 2014). The key differences in diet between finch species is more pronounced during drier months (e.g., June–December) or years when fruits and invertebrates are not available. Therefore, the lack of differences in the microbiota between small and medium ground finches at the site without human presence could be due to the larger overlap in diet during months (e.g., January–May) or years with higher rainfall.

For medium ground finches, gut bacterial diversity was lower at the site with human presence compared to the intermediate and site without human presence. These results corroborate recent studies that found that the gut bacterial diversity of birds was lower in sites with the presence of humans as compared to sites without human presence (Furst et al., 2018; Teyssier et al., 2018). In contrast, Phillips et al. (2018) found that white-crowned sparrows (*Zonotrichia leucophrys*) from sites with human presence had higher gut bacterial diversity than sites without human presence. Two different hypotheses could explain these conflicting studies. First, the presence of humans might not have the same effect on diet across host species. Thus, different sites with humans could have differential effects on gut microbiota, depending on the diet composition and site in which we captured the finches. Second, gut microbiota of the hosts can be affected by multiple interacting factors, such as diet, environment, physiology and behaviour, which can vary based on geographical location of the sites with humans (Alberti et al., 2017; Brans et al., 2017; Kern & Langerhans, 2018; Merckx, Kaiser, & Van Dyck, 2018). Because of the highly variable environment across islands and years in the Galapagos, the Darwin's finch system could provide an opportunity system to explore these causal interactions.

In contrast to Michel et al. (2018), we found that the gut microbiota of two closely related species of Darwin's finches differed, depending on the site. Specifically, they found that only the gut microbiota of blood-feeding vampire finch (*Geospiza septentrionalis*)

differed from the rest of the Darwin's finches, including small and medium ground finches. However, the study found that the gut microbiota of small and medium ground finches differed between dry and wet seasons, which was hypothesized to be related to shifts in diet composition. This study focused on finches in natural areas with no human presence, which might explain why the researchers did not find a difference in the gut microbiota of small and medium ground finches. Our results suggest that sampling Darwin's finch populations from areas with the presence of humans could present a different picture of the similarities and dissimilarities of the gut microbiota across finch species.

Host genetics, rather than environmental factors, can explain differences in gut microbiota (Knowles, Eccles, & Baltrunaite, 2019). However, Darwin's finches, especially ground finches, are quite closely related (Chaves et al., 2016; Lamichhane et al., 2015; McKay & Zink, 2015), suggesting that the genomic underpinnings of the observed differences in the microbiota are unlikely. These genetic similarities, coupled with the study by Michel et al. (2018) showing that the microbiota of small and medium ground finches do not differ across locations without humans, suggest that our results are driven primarily by the presence of humans and their accompanying foods items, rather than genetic relatedness or natural characteristics of the environment. However, our study only included one site per habitat type and therefore future replication is needed to establish broad patterns across human-inhabited islands (e.g., on the islands of San Cristobal, Floreana or Isabela).

The relative abundances of only a few bacterial taxa varied between species. For example, the relative abundance of *Lactobacillus* spp. was twice as high in small ground finches compared to medium ground finches across sites, which, interestingly, was not observed in Michel et al. (2018). *Lactobacillus* spp. bacteria can function to convert sugar to lactic acid and thus the differences in the relative abundance of *Lactobacillus* spp. could be related to differences in finch species overall diet. *Lactobacillus* spp. have also been linked to host resistance to pathogens in chickens by directly affecting pathogens or improving immune system of the host (Baba, Nagaishi, Fukata, & Arakawa, 1991; Li, Zhao, & Wang, 2009; Watkins & Miller, 1983). Because finches are exposed to a variety of pathogens and parasites in the Galapagos (Gottdenker et al., 2008; Wikelski, Fofopoulos, Vargas, & Snell, 2004), future studies could explore whether *Lactobacillus* spp. could be influencing variation in host resistance mechanisms, such as the immune system, against these parasites in each finch species.

The body mass of both small and medium ground finches was higher at the site with human presence compared to the site without human presence. Age was unlikely to influence mass differences across sites because morphology of finches is nearly fixed after the first year after fledging (Boag, 1984). Instead, these results are probably due to changes in diet of the finches across sites (de León et al., 2018) because “junk food” can cause an increase in mass in mammalian species (Bayol et al., 2007; French et al., 2001; Hill & Peters, 1998). McNew et al. (2017) also found that medium ground finches had higher body mass in areas with human presence compared to areas without humans, which was thought to be related to

differences in diet as well. Although we found an effect of site on both body mass and gut microbiota, we did not find a relationship between mass and microbiota. Many studies have found a relationship between body mass and gut microbiota (Backhed et al., 2004; Ley et al., 2005; Turnbaugh et al., 2006), but other studies have not found a relationship between gut microbiota and body mass. Even though diet can affect both body mass and gut microbiota, environmental factors might independently affect gut microbiota and body mass.

We did not find a significant difference in bill size within finch species across sites. However, we found a nonsignificant trend for the effect of site on bill size of small and medium ground finches; birds living at the site with human presence had, on average, smaller bills than birds living in the other two sites. These results suggest that we are perhaps observing the beginning of selection towards different sized bills across sites (Hendry et al., 2006; de León et al., 2011), probably resulting from food availability at the different sites (de León et al., 2018). McNew et al. (2017) found that medium, but not small, ground finches had bigger bills (length, depth, width) in the town of Puerto Ayora compared to finches at the site without human presence, which was the opposite direction of the trend that was found in our study. One explanation is that different selective pressures act on small and medium ground finches in Puerto Ayora compared to the El Garrapatero beach due to the permanent and transient presence of humans, respectively. Future studies could quantify the environmental factors that differentially affect finch bill size in the town and beach and determine whether smaller bill size is evolving at the beach within the next 5–10 years.

Our study suggests that the presence of humans in the environment can affect the gut microbiota of Darwin's finches but these effects depend on the bird species. These results further suggest that the closely related small and medium ground finches are distinct ecophenotypes, which can be affected differently by their environment. Our results also show that individual finches that are relatively near each other geographically can have different gut microbiota communities and therefore we caution against drawing broad conclusions based on single populations. Darwin's finches face tremendous challenges in the Galapagos from invasive parasites (Fessl et al., 2010; Knutie, 2018; Knutie et al., 2016; Koop, Kim, Knutie, Adler, & Clayton, 2016; Wikelski et al., 2004) and predators (Gotanda, 2019) to dynamic annual changes in natural and novel food availability (Grant & Grant, 1995; de León et al., 2018). The implications of the variation in gut microbiota and morphology in the evolutionary ecology of Darwin's finches has exciting potential for future functional work (Grond et al., 2018; Kohl, 2018).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

S.A.K. and K.M.G. conceived the study and collected data, S.A.K. conducted the bioinformatics and data analyses, S.A.K. and K.M.G. wrote the manuscript, and J.C. provided logistical support. All authors revised and approved the manuscript.

DATA ACCESSIBILITY

Supporting information has been made available online. Data are available at FigShare (Knutie, Chaves, & Gotanda, 2019) and sequences have been uploaded to GenBank (BioProject accession number: PRJNA528277).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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