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Research Article

Brief exposure to captivity in a songbird is associated with reduced diversity and altered composition of the gut microbiome

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Abstract

The gut microbiome is important for host fitness and is influenced by many factors including the host's environment. Captive environments could potentially influence the richness and composition of the microbiome and understanding these effects could be useful information for the care and study of millions of animals in captivity. While previous studies have found that the microbiome often changes due to captivity, they have not examined how quickly these changes can occur. We predicted that the richness of the gut microbiome of wild-caught birds would decrease with brief exposure to captivity and that their microbiome communities would become more homogeneous. To test these predictions, we captured wild house sparrows (*Passer domesticus*) and collected fecal samples to measure their gut microbiomes immediately after capture ("wild sample") and again 5–10 days after capture ("captive sample"). There were significant differences in beta diversity between the wild and captive samples, and captive microbiome communities were more homogenous but only when using nonphylogenetic measures. Alpha diversity of the birds' microbiomes also decreased in captivity. The functional profiles of the microbiome changed, possibly reflecting differences in stress or the birds' diets before and during captivity. Overall, we found significant changes in the richness and composition of the microbiome after only a short exposure to captivity. These findings highlight the necessity of considering microbiome changes in captive animals for research and conservation purposes.

Keywords: bird, captivity, diet, microbiome, *Passer domesticus*, stress

Introduction

The gut microbiome—the collection of microorganisms that reside in an animal's gastrointestinal tract—is important for immune development, nutrient absorption, and behavior of their host organism (Pan and Yu 2013, Vuong et al. 2017, Broom and Kogut 2018). This community of microorganisms is influenced by multiple factors including the genetics of the host animal and the environment. The extrinsic factors influencing the microbiome include diet, social relationships, and the physical environment (Bisanz et al. 2019, Sarkar et al. 2020a, Drobniak et al. 2022). Bringing a wild individual into captivity drastically alters these extrinsic factors and is likely to impact the gut microbiome (Dallas and Warne 2022). Animals are brought into captivity for many purposes and understanding the effects of captivity on the microbiome is important for animal welfare, conservation, and research.

Conservation programs often bring animals into captivity for rehabilitation or captive breeding for various lengths of time (Hanson et al. 2021). These programs are important tools used in conservation biology as the current biodiversity crisis continues (Strang and Rusli 2021, Barbanti et al. 2022, Pritchard et al. 2022). Although many conservation programs try to maintain natural condition for animals in captivity, they may not consider how to preserve the animals' natural microbiomes (Trevelline et al. 2019). Maintaining a healthy microbiome is important for the animal's welfare in captivity (Wienemann et al. 2011, Wasimuddin et al. 2017) and possibly the success of reintroduction to the wild. Captive animals reintroduced into the wild may experience increased risk of mortality and decreased reproductive success (Olsson 2007, Roe et al. 2010, Bennett et al. 2013, Ashbrook et al. 2016), which lowers reintroduction success and hinders conservation efforts. However, it is unknown what role the captive microbiome has on the success of a reintroduction. Therefore, understanding the changes of the microbiome in captivity, particularly as individuals enter captivity, may be important for improving the chances of reintroduction success.

Changes in the microbiome induced by captivity is also a concern for animals used in research. Studies often bring wild animals into captivity for observation and experimentation (e.g. Davidson et al. 2020, Moeller et al. 2020) and the animals may be in captivity for a few days to several years prior to experimentation (Daniels et al. 2019, Battaglia et al. 2022). Therefore, researchers should consider how the artificial captive environment will impact their study subjects (Calisi and Bently 2009). There are

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many studies documenting potential changes to the nervous and endocrine systems, as well as behavior in captive animals (Tanimoto et al. 2017, Li et al. 2019, DuRant et al. 2020, de Oliveira Terceiro et al. 2021, Walsh et al. 2022). However, the effect that the captive microbiome may have on research outcomes is not well-understood. For example, when the microbiome is altered with probiotics, it has been found to change hormone levels and working memory (Lv et al. 2015, Emge et al. 2016). Therefore, it may be important for researchers to understand the effect that captivity has on the microbiome when designing experiments that use captive individuals, even if an animal's time in captivity is brief.

While we have a growing understanding of how animal microbiomes change in captivity, it is important to consider the timescale over which these changes occur. A recent review summarized studies comparing captive and wild microbiomes in various taxa (Diaz and Reese 2021). Relatively few studies tracked the same individuals before and after introduction to captivity and those that did resampled individuals 2 weeks to 9 months after capture (Dhanasiri et al. 2011, Kohl and Dearing 2014, Kohl et al. 2014, 2017, Schmidt et al. 2019, Edenborough et al. 2020, Kelly et al. 2022, Song et al. 2022, Koziol et al. 2022). These studies tracked the microbiomes of various species of mammal, fish, bird, and reptile with varying results based on time in captivity. Lizards in captivity for 8 weeks did not have significant changes in their microbiome alpha diversity (Kohl et al. 2017), while birds of prey had significant alpha diversity decreases after 2 weeks (Song et al. 2022). Ecological niche also contributed to captivity's influence as rodents with specialist diets had greater microbiome composition changes in 6–9 months in captivity than generalist species (Kohl et al. 2014). Changes to the microbiome in the captive environment are often attributed to the change in diet, however, differences in environmental microbes also play a role (Teyssier et al. 2020, Bornbusch et al. 2022). These results indicate that captivity can impact the microbiome, but its effects vary by time and taxa. We are unaware of any studies that have explored changes in the captive microbiome over very short timescales (less than 2 weeks after introduction into captivity). Understanding the dynamics of the microbiome community soon after animals are introduced into captivity may be important for interpreting research outcomes.

For this study, we examined whether the microbiome changes over very short timescales (less than 2 weeks) after animals are introduced into captivity. We used the house sparrow (*Passer domesticus*) as our model system. The house sparrow is a globally distributed species that is frequently used as a model in avian research (Hanson et al. 2020). A recent paper tracked changes in the cloacal microbiome of this species 8 weeks after captivity (Kelly et al. 2022) and found significant decreases in alpha diversity and changes to the composition of the microbiome community during this time. We compared the gut microbiomes of house sparrows immediately after capture ("wild sample") and 5–10 days after they were in captivity ("captive sample"). We predicted that the microbiomes of individual birds brought into captivity would change in community structure compared to their wild microbial community, (i.e. differences in beta diversity) and that there would be less variance in the microbiome communities of the group of captive animals due to the standardized captive environment. We also predicted that captive samples would have decreased species richness within each sample (i.e. lower alpha diversity). Lastly, we predicted that captive samples would have differences in the relative abundance of microbial taxa and functions present in their communities compared to wild samples.

Methods

Animals and housing

From March to July 2020, we captured 30 house sparrows from four sites [at least 0.5 km apart, 3.2 ± 1.9 km (mean ± standard deviation)] in College Station, Texas. Mistnets and baited potter traps were used to capture the birds. Birds were sexed based on plumage: males have black chests, gray crowns, and chestnut backs while females are duller brown with a strip behind the eye. Juveniles cannot be reliably sexed and were excluded based on paler plumage, bill color, and an enlarged, yellow gape (Summers-Smith 1988). Immediately after capture, birds we placed in a paper bag with a sterilized weightboat at the bottom to collect a fecal sample ("wild sample;" details of methodology in Knutie and Gotanda 2018). These fecal samples were used as a proxy for the microbial community present in the gut (Ingala et al. 2018, Berlow et al. 2020). Once the birds had defecated (within 5 min), the samples were transferred to an Eppendorf tube with a sterilized spatula. We also recorded the time on a 24-h clock when the sample was collected. Samples were placed on ice until they could be stored in a −80°C freezer (mean time to freezer 107 ± 63 min). At capture, weight and tarsus length was measured from each bird. We used Peig and Green's (2009) method to calculate the scaled mass index with the formula as follows: $\widehat{M_i} = M_i[\frac{L_0}{L_i}]^b$, where M_i is the weight, L_i is the tarsus length, L_0 is the average tarsus length of all birds measured, and b is the slope of standard major axis regression between mass and tarsus length. Birds were then housed in cages (0.6 m × 0.33 m × 0.3 m) at Texas A&M University (30° 36 N, 96° 21 W) in an indoor room ("housing room:" 5 m × 6.3 m). The birds were housed alone in their cage to minimize the transfer of microbes between individuals, although they were in visual and auditory contact with other birds. The birds received *ad libitum* water and commercial mix of bird seed (Royal Wing Wild Bird Food, Tractor Supply Co.). The housing room was kept on a 13 h:11 h light:dark cycle at a temperature of 24.0 ± 0.5°C (mean ± standard deviation). After 5–10 days in captivity (6.80 ± 1.72 days: mean ± standard deviation) another fecal sample ("captive sample") was taken from each bird in the same manner as above, including storage in a −80°C freezer (mean time to freezer 45 ± 24 min). There was variation in when the captive samples were collected because the birds were being used in other studies examining exploratory behavior and cognition; during these studies, the birds were tested individually to minimize microbe transfer and all the birds underwent the same experimental protocols (Florkowski and Yorzinski 2023). After fecal samples were collected, we weighed and measured the tarsus of the birds again. Once the procedures were concluded the birds were released to their capture sites. All methods were approved under the Texas A&M University's Institutional Animal Care and Use Committee (IACUC #2019–0219).

Microbiome methods

Using 0.25 g of each frozen fecal sample, we isolated DNA with QIAamp PowerFecal DNA Isolation Kits (Qiagen, Germany) following the manufacturer's protocol (except we increased sample incubation at 65°C from 10 min to at least 8 h in an effort to increase DNA yield). To verify sufficient DNA yield, we used a Qubit fluorometer (dsDNA HS Assay Kit, Invitrogen, Carlsbad, California) and then diluted each sample to a concentration of 5 ng/ul of DNA. The extracted DNA was then sent to the Michigan State University's genomics core and was processed and sequenced according to Kozich et al. (2013). In brief, libraries were constructed by amplifying the V4 region of the 16S rRNA gene using primers 515F and 806R with Illumina adapters and dual indices. Samples were

amplified using DreamTaq Master Mix (ThermoFisher, Waltham, Massachusetts). The PCR reaction was incubated at 95°C for 3 min, followed by 30 cycles of 45 s at 95°C, 60 s at 50°C, and 90 s at 72°C, then a final extension at 72°C for 10 min. PCR products were then pooled and were batch normalized using Invitrogen SequalPrep DNA Normalization plates (Invitrogen). Product recovered from the plates were concentrated using an Amicon Ultra centrifugal filter (Sigma-Aldrich, St. Louis, Missouri) and cleaned using AMPure XP magnetic SPRI beads (Beckman Coulter, Brea, California). The cleaned pools were sequenced on the Illumina MiSeq platform using v2 2 × 250 base pair kit (Illumina Inc, San Diego, California).

We performed initial quality control of raw sequences with *Trim Galore* (version 0.6.6), which was used to remove adaptors and trim reads with base quality below a Phred score of 20. We then processed trimmed sequences using the *Mothur* software (version 1.45.3; Schloss et al. 2009) using standard operating procedure (accessed May 2021). Briefly, the sequences were assembled into contigs and further quality trimmed. Identical sequences were merged, and singletons were removed. Remaining sequences were aligned against the SILVA database (Release 132). Chimeric sequences were removed using the UCHIME function. Any sequences classified as mitochondria, chloroplast, or eukaryote were also removed. Two negative control samples were sequenced along with the samples, sequencies that appeared in the negative controls were removed from the sample sequences based on their occurrence in the controls. Remaining sequences were clustered into Operational Taxonomic Units (OTUs) with 97% similarity. Samples with fewer than 1000 reads ($n = 5$) were dropped from subsequent analysis as the low sequence read count may skew downstream analysis (Momozawa et al. 2011). A rarefaction analysis was also conducted to confirm that samples with more than 1000 reads had sufficient depth. The paired sample from the same bird of the dropped samples was also removed so that each bird had two samples, therefore a total of 10 samples were removed from downstream analysis. We also calculated Good's estimator of coverage of the remaining samples and found the average was 99% (standard deviation +/− 2%) indicating adequate coverage (Good 1953). The raw sequence data are available from the National Center for Biotechnology Information under the project accession number PRJNA838556. Individual sample accession numbers are given in Table S1 (Supporting Information).

Statistical analysis

We imported files into RStudio (version 4.1.2) using the *phyloseq* R package (version 1.36.0; McMurdie and Holmes 2013). We then applied variance stabilization transformation to the OTU counts to account for the differences in library size across samples using the *Deseq2* R package (version 1.32.0; Love et al. 2014) and used these data for all analyses. To determine the differences in the microbial communities between the wild and captive samples, we calculated beta diversity measures using Bray–Curtis dissimilarity (Bray and Curtis 1957), Jaccard distances (Jaccard 1912), and unweighted and weighted UniFrac distances (Lozupone et al. 2011), which measure community differences based on abundances, presence/absence, presence/absence weighted by phylogenetic distance, and abundances weighted by phylogenetic distance, respectively. We plotted these measures on a PCoA plot to visualize the differences. We then used the adonis2 function to perform a PERMANOVA in the *vegan* R package (version 2.5–7; Oksanen et al. 2020) set at 999 permutations. This function compares the centroids of microbial communities of different groups with Bray–Curtis dissimilarity, Jaccard distances, unweighted and weighted UniFrac distances as the independent variables. The dependent variables were sample type (wild or captive), the sex of the bird, and scaled mass index. Adonis2 cannot accommodate mixed effect models so capture site (one of the four sites), date of capture, and time of day we collected the fecal sample were included as fixed effects. We could not include bird identity in the model due to overfitting; however, this repeat sampling without controlling for individual identity has been previously used with this test (e.g. Escallón et al. 2019). We also ran separate PERMANOVAs to determine if the number of days in captivity had a significant impact on the Bray–Curtis dissimilarity, Jaccard distances, unweighted and weighted UniFrac distances of the captive samples. The dependent variables were the number of days between the two samples, sex, change in scaled mass index during captivity, capture site, date of capture, and time of day we collected the fecal sample. Only samples collected after captivity were included in this analysis. To compare the dispersion of the wild and captive samples, we used the betadisper function in *vegan* (Anderson et al. 2006).

To quantify alpha diversity, we calculated Shannon's diversity index (Shannon 1948) and Chao1 (Chao and Chiu 2016) using *phyloseq* and Faith's phylogenetic diversity using the *picante* package (Kembel et al. 2010), which evaluates the community's richness and evenness, richness corrected for missing rare taxa, and the sum of the branch lengths of a phylogenetic tree connecting all species, respectively. We then used these diversity measures as independent variables in mixed effect linear models to determine variables that predict diversity. The dependent variables were sample type (wild or captive), sex, and scaled mass index. Random effects used in the model were capture site, date of capture, time of day we collected the fecal sample, and bird identity. We were also interested in determining whether there was an effect of the number of days in captivity on alpha diversity. The independent variable were the alpha diversity values of the samples collected in captivity. The dependent variables were the number of days the birds were in captivity at sampling, sex, and change scaled mass index during captivity. Random effects used in the model were capture site, date of capture, and time of day we collected the fecal sample.

We used a Wilcoxon test in the stats R package (version 4.1.2) to determine which bacterial genera are significantly more abundant in captive or wild sample types. Comparisons were corrected with the Benjamini–Hochberg correction (Benjamini and Hochberg 1995).

To generate predictions about the functions of the microbiota communities we used the *Tax4Fun2* R package (version 1.1.5; Wemheuer et al. 2020), which calculates a functional profile by associating OTUs with KEGG orthologue functional genes and their functional pathways. *Tax4Fun2* it is considered more accurate at determining microbial functions than other programs (Wemheuer et al. 2020), however, it is a prediction rather than direct measurement of the metagenome and must be interpreted with caution, especially in nonmodel species like house sparrows (Gil and Hird 2022). We used the LEfSe function on the Galaxy server (http://huttenhower.org/galaxy) to determine which functional pathways are differentially abundant between wild and captive microbiome samples. The results from the analysis were adjusted for multiple comparisons with the Benjamini–Hochberg correction (Benjamini and Hochberg 1995).

Results

We compared the microbiomes from wild and captive house sparrows ($n = 30$) by sequencing the 16S rRNA gene. From the fecal samples collected, the sequencing output resulted in 1 869 626 raw reads. After quality control (which resulted in us omitting the samples of five birds due to low read counts), we processed the fecal samples of 25 birds with an average number of 27 995 ± 11 087 (mean ± standard deviation) sequencing reads clustered into a total of 10 470 OTUs. Between collection of first and second fecal samples, all birds lost weight, on average of 3.2 +/− 1.8 g representing a 12.8 +/− 6.9% loss in body mass, which is an acceptable reduction based on animal welfare recommendations (Talbot et al. 2020).

There were differences in beta diversity between wild and captive samples measured using Bray–Curtis dissimilarity ($P = .001$), Jaccard distances ($P = .002$), unweighted-UniFrac distances ($P = .004$), and weighted-UniFrac distances ($P = .001$) (Table 1). Principal components one and two explained 5.5% and 3.9%, 3.9% and 3.1%, 7% and 3.3%, and 8.3% and 6.5% of the variation, for Bray–Curtis dissimilarity, Jaccard distance, unweighted-UniFrac distances, and weighted-UniFrac distances, respectively (Fig. 1).

We found that number of days in captivity had no significant impact on microbiome community composition using Bray–Curtis dissimilarity ($P = .26$), Jaccard distances ($P = .26$), unweighted-UniFrac distances ($P = .22$), and weighted-UniFrac distances ($P = .64$) (Table S2, Supporting Information).

There were significant differences in dispersion in captive and wild samples. When comparing Bray–Curtis dissimilarities and Jaccard distances, wild samples had greater dispersion, but with weighted-UniFrac distances and unweighted-UniFrac distances wild and captive samples had equal dispersion (Fig. 2 and Table 2).

Sample type, either captive or wild, significantly predicted alpha diversity for the Shannon diversity index ($P = .02$), Chao1 ($P = .02$), and Faith's phylogenetic diversity ($P = .01$). The average diversity of samples taken after captivity had lower diversity compared to the wild samples in both Shannon (wild mean = 5.41, captive mean = 4.84), Chao1 (wild mean = 314.5, captive mean = 151.5) and Faith's phylogenetic diversity (wild mean = 16.93, captive mean = 10.01) (Fig. 3 and Table 3). Overall, the microbiomes of most individuals (Shannon: 76%, Chao1: 76%, and Faith's phylogenetic diversity: 76%) decreased in alpha diversity after introduction into captivity.

We found that the number of days the birds were in captivity did not predict the change in alpha diversity for the Shannon diversity index ($P = .21$), Chao1 ($P = .06$), or Faith's phylogenetic diversity ($P = .06$) (Table S3, Supporting Information).

A total of nine genera were significantly differentially abundant between captive and wild microbiome samples. There were seven genera that were significantly more abundant in the wild microbiome samples, *Sphingomonas*, *Rhizobiaceae*, *Microvirga*, *Methylobacterium*, *Enterococcus*, and *Bacillaceae*, and two that were significantly more abundant in the captive microbiome samples, *Rothia* and *Catellicoccus* (Fig. 4).

The microbiome predicted functional analysis resulted in a total of 368 predicted pathways. *Tax4Fun2* only utilizes sequences that match their reference database to predict functions; on average 89.7 +/− 18.3% (mean ± standard deviation) of sequences per sample were utilized. A total of 33 functional pathways were significantly more abundant in wild samples and 18 functional pathways were significantly more abundant in captive sam-

Table 1. Results of PERMANOVA investigating the influence of sample type, sex, scaled mass index, capture site, and date of capture on beta diversity. Significance indicated by an asterisk.

Bray–Curtis	Degrees of freedom	SS	R2	F-value	P-value
Sample type (wild or captive)	1	0.62	0.02	1.46	.001*
Sex	1	0.41	0.01	0.99	.51
Scaled mass index	1	0.45	0.02	1.06	.15
Capture site	3	1.36	0.06	1.07	.04*
Date of capture	1	0.47	0.02	1.11	.07
Time of sampling	1	0.44	0.02	1.04	.27
Residual	41	17.35	0.81		
Total	49	21.34	1.00		
Jaccard	**Degrees of freedom**	**SS**	**R2**	**F-value**	**P-value**
Sample type (wild or captive)	1	0.57	0.02	1.24	.002*
Sex	1	0.45	0.01	0.99	.51
Scaled mass index	1	0.47	0.02	1.03	.18
Capture site	3	1.43	0.04	1.04	.64
Date of capture	1	0.48	0.06	1.06	.64
Time of sampling		0.46	0.02	1.02	.22
Residual	41	18.80	0.82		
Total	49	22.82	1.00		
Unweighted UniFrac	**Degrees of freedom**	**SS**	**R2**	**F-value**	**P-value**
Sample type (wild or captive)	1	0.43	0.02	1.36	.004*
Sex	1	0.32	0.02	1.02	.32
Scaled mass index	1	0.35	0.02	1.10	.09
Capture site	3	1.00	0.06	1.05	.16
Date of capture	1	0.31	0.01	0.98	.51
Time of sampling	1	0.35	1.02	1.10	.10
Residual	41	13.07	0.81		
Total	49	16.05	1.00		
Weighted UniFrac	**Degrees of freedom**	**SS**	**R2**	**F-value**	**P-val**
Sample type (wild or captive)	1	0.13	0.03	1.71	.001*
Sex	1	0.08	0.02	0.13	.17
Scaled mass index	1	0.09	0.02	1.18	.11
Capture site	3	0.27	0.06	1.18	.04*
Date of capture	1	0.07	0.01	0.97	.54
Time of sampling	1	0.09	0.02	1.25	.07
Residual	41	3.17	0.79		
Total	49	4.00	1.00		

Table 2. Results of betadisper test to determine the average distance from the centroid of wild and captive gut microbiome samples. Significance indicated by an asterisk.

	Average distance to centroid of wild samples	Average distance to centroid of captive samples	P-value
Bray–Curtis	0.62	0.51	.008*
Jaccard	0.65	0.58	.003*
Unweighted-UniFrac	0.59	0.59	.75
Weighted-UniFrac	0.33	0.40	.01*

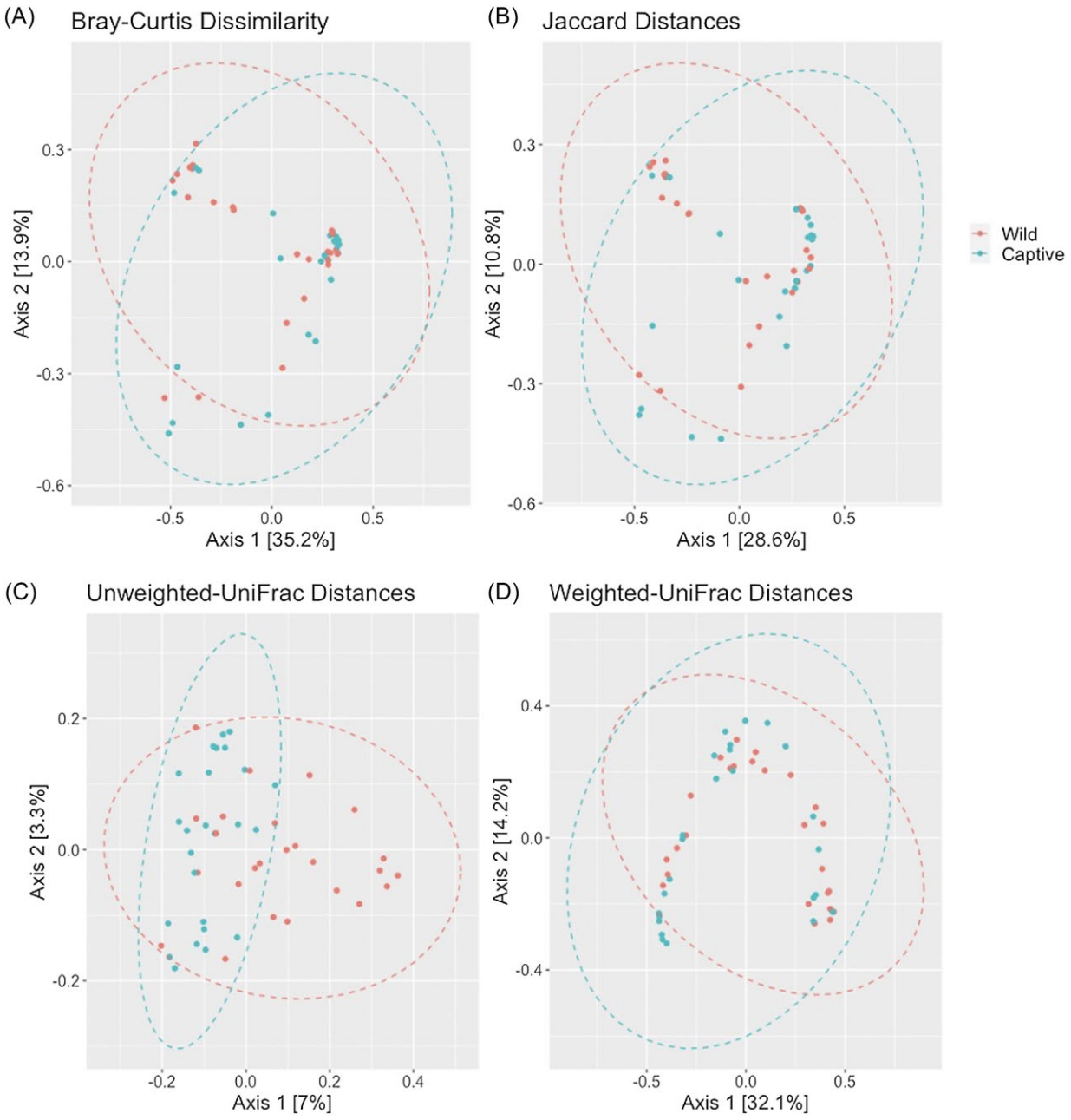

Figure 1. Principal component analysis of (A) Bray–Curtis dissimilarity, (B) Jaccard, (C) unweighted-UniFrac, and (D) weighted-UniFrac distances between captive (blue) and wild (red) microbiome samples. Circles indicate 95% confidence intervals.

ples. The majority of functional pathways that were enriched in these groups were related to metabolism (83% of pathways enriched in captive samples versus 87% of pathways in wild samples), with the rest being cellular processes (5.5% of pathways enriched in captive samples versus 12% of pathways in wild samples), organismal systems (5.5% of pathways enriched in captive samples versus 0% of pathways in wild samples), and environmental information processing (5.5% of pathways enriched in captive samples versus 0% of pathways in wild samples) (Fig. 5).

Discussion

We found that there were differences in beta diversity between the gut microbiome of wild birds sampled at capture and after a short exposure (5–10 days) to captivity. This is among the first studies to show that captivity induced changes in the microbiome can occur over this short of a timescale. Additionally, alpha diversity of the microbiome decreased after the birds lived in captivity compared when they were sampled in the wild and resulted in changes in the relative abundance of some of the genera and predicted functions found in the birds' microbiomes.

These changes we found in the birds' alpha and beta diversity agree with other studies comparing captive and wild bird populations (San Juan et al. 2021, Madden et al. 2022). They are also consistent with another study that sampled house sparrows at capture and again after 8 weeks in captivity (Kelly et al. 2022). However, we find that changes in the microbiome happen much sooner than previous studies have explored. After only 5–

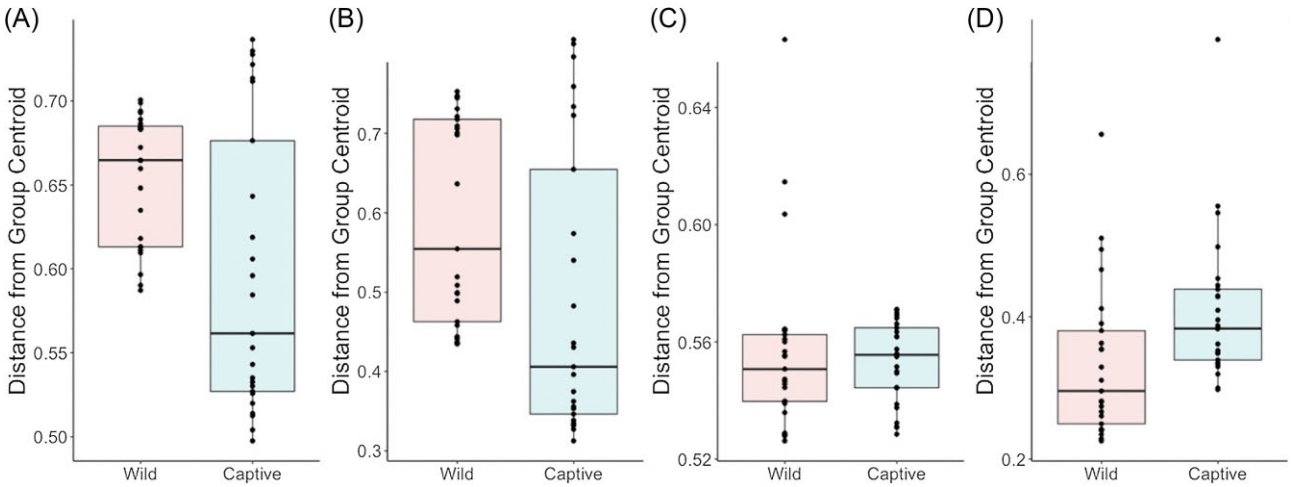

Figure 2. Boxplots show distance to group centroid of each sample using (A) Bray–Curtis dissimilarity, (B) Jaccard, (C) unweighted-UniFrac, and (D) weighted-UniFrac distances.

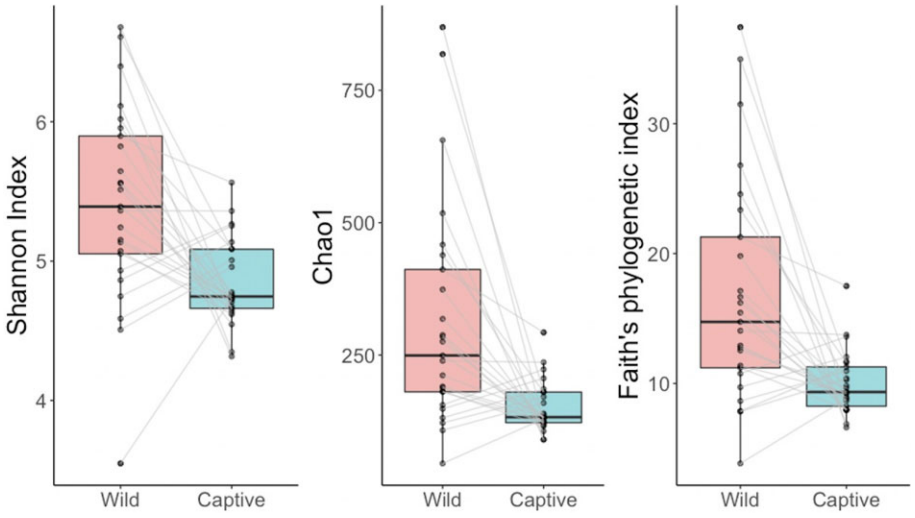

Figure 3. Comparing alpha diversity measured with Shannon index (A), Chao1 (B), and Faith's phylogenetic index (C) between wild and captive samples. Data points from the same individuals are connected with a line.

10 days in captivity, the gut microbiomes of the house sparrows in our study were altered. Birds generally have fast gut transit times, which may have contributed to rapid changes in the microbial community after the change in environment (McWhorter et al. 2009). We did not see any significant impact of the number of days in captivity (5–10 days) on microbiome diversity, therefore more frequent sampling of the individuals will be required to capture the dynamics of the shift of community composition.

We predict that these changes to the microbiome occurred due to the captive environment, however, it may also be due to temporal stochasticity. Studies of microbiome stability over time in the wild are rare and have found varying results (Stevenson et al. 2014, Baxter et al. 2015, Kreisinger et al. 2015, Lavrinienko et al. 2020). However, studies in captive animals have found that, without perturbation, the animal microbiomes can be stable over long periods of time (Schloss et al. 2012). In this study, we used microbial communities from fecal samples to characterize the microbiome, which may be more less stable than the microbiome of the gut, however, the microbiome sampled directly from the gut have been found to change at similar short time scales (Warzecha et al. 2017). Diurnal cycles can also impact the microbiome (Thaiss et al. 2015), and we did not standardize the time each sample was collected. However, the samples taken before and after captivity spanned the same range of times and we did not find time of day as a significant predictor of microbiome composition. Potential factors that could have contributed to the microbiome change in captivity include changes in uptake of microbes from the environment, diet, social environment, and stress levels. To understand the relative importance of each of these factors, additional studies that independently manipulate each variable would be needed.

Diet influences the microbiome by influencing which resources are available in the gut. After introduction into captivity, many animals are fed a simplified diet, which may impact the diversity and structure of their microbial communities (Leeming et al. 2019). A recent study in rhesus macaques (*Macaca mulatta*) found that there were limited differences in the microbiomes of wild and captive individuals when they ate similar diets (Kuthyar et al. 2022), however, this result may depend on the species as feed-

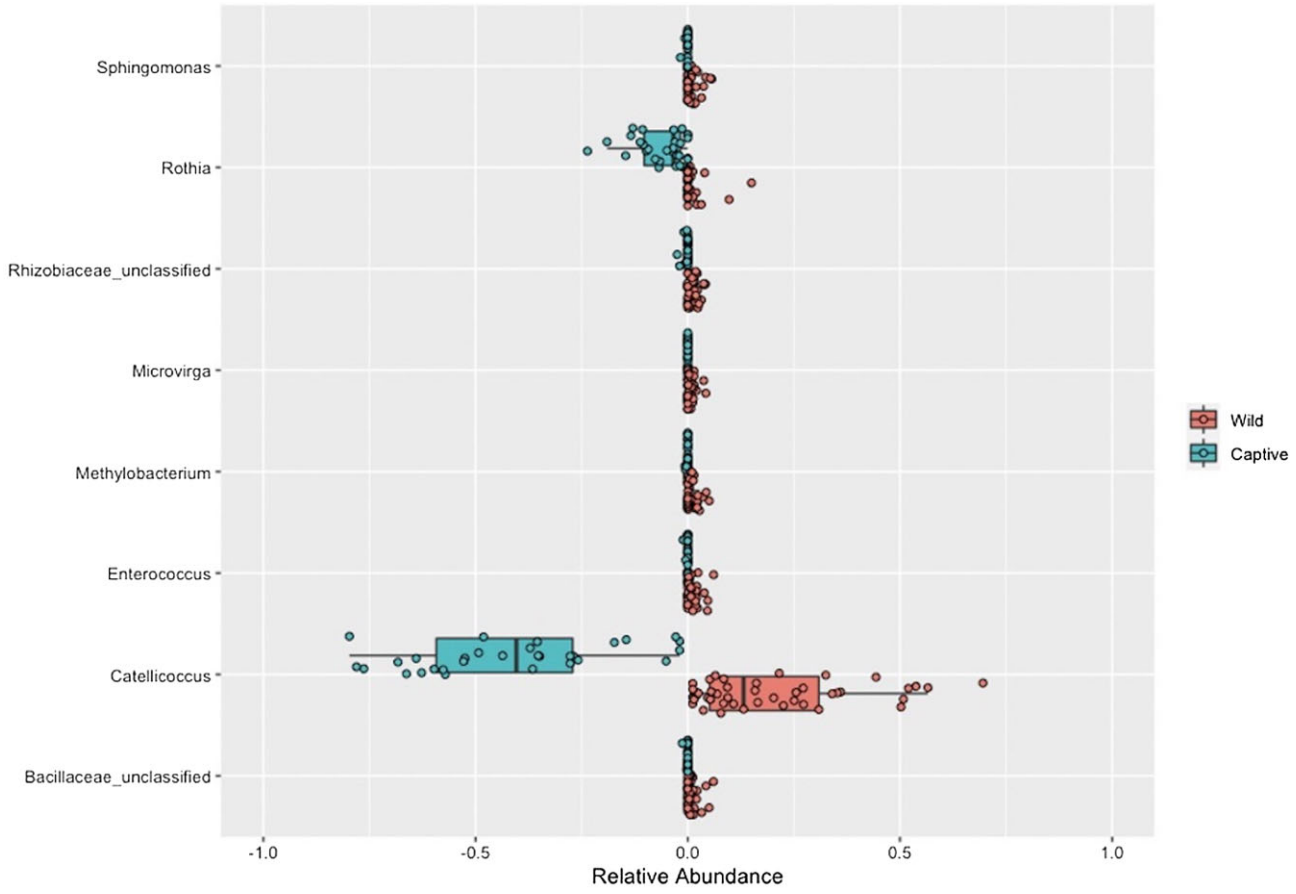

Figure 4. Genera that are differentially abundant in wild versus captive samples. The relative abundances of each genus in a sample are represented by a point. Relative abundances in captive samples are represented by negative numbers.

ing strategy can impact the effects of captivity on the microbiome (Bornbusch et al. 2019, Greene et al. 2019). In the wild, house sparrows are opportunistic and eat a varied diet consisting of seeds, other plant material, insects, and human refuse (Summers-Smith 1988). In captivity, we fed the house sparrows commercial seed mix, possibly contributing to the observed change in microbial community structure and decreases in alpha diversity (Leeming et al. 2019). Due to the rapid change in response to captivity, we may predict that birds would rapidly return to a wild-type microbiome after release. However, studies in birds and rodents have shown that dietary changes can result in the loss of taxa that prevents full recovery of the microbiome (Sonnenburg et al. 2016, Bodawatta et al. 2021). These animals did not reacquire microbial taxa from their food, suggesting the nutrient composition of the diet and not microbes ingested with the food were major contributors to the microbiome change. However, sequencing the microorganisms on an animal's food to see how microbes from food influence an animal's microbiome would be an interesting future study.

In the captive house sparrows, we observed changes in the relative abundance of several taxon as well as predicted functions that may be a result of the changes in diet. Birds were fed a mixture of seeds that likely had more carbohydrates and less protein and fats than a typical wild bird diet, which can include up to 10% invertebrates as well as meat from human food waste (Wilman et al. 2014, MacGregor-Fors et al. 2020). We observed an increase in predicted functions for carbohydrate metabolism in captive samples compared to wild samples. We also found there were more functions for protein and lipid metabolism in wild samples. This may reflect plasticity in the microbiome in response to the birds' diet in captivity. Wild microbiome samples also had greater abundances of *Methylobacterium*, which is associated with the microbiomes of birds fed an exclusively insect diet (Davidson et al. 2020). This change may reflect the lack of insects available to the captive birds. Based on the methods used in this study, we are only able to make predictions about the functionality of the microorganisms identified in the birds' guts. Therefore, further experimentation to determine the impact of these changes on the host birds is necessary.

Microorganisms are often shared across social groups in animals (Sarkar et al. 2020b) and individuals with closer connections have more similar microbiomes (Tung et al. 2015). House sparrows are social birds, congregating in foraging flocks of up to 45 individuals (Griesser et al. 2011). The house sparrows in this study were singly housed, which prevented social interaction and the sharing of microorganisms between individuals. This decrease in microbial input from social contact may have contributed to decreases in alpha diversity and changed community structure. At capture, we did not see strong clustering of individuals from the same capture sites, however, many individuals were collected on different days from these same sites and it unknown if they interacted before capture. Therefore, additional research is needed to see how captivity disrupts the microbiomes of social groups.

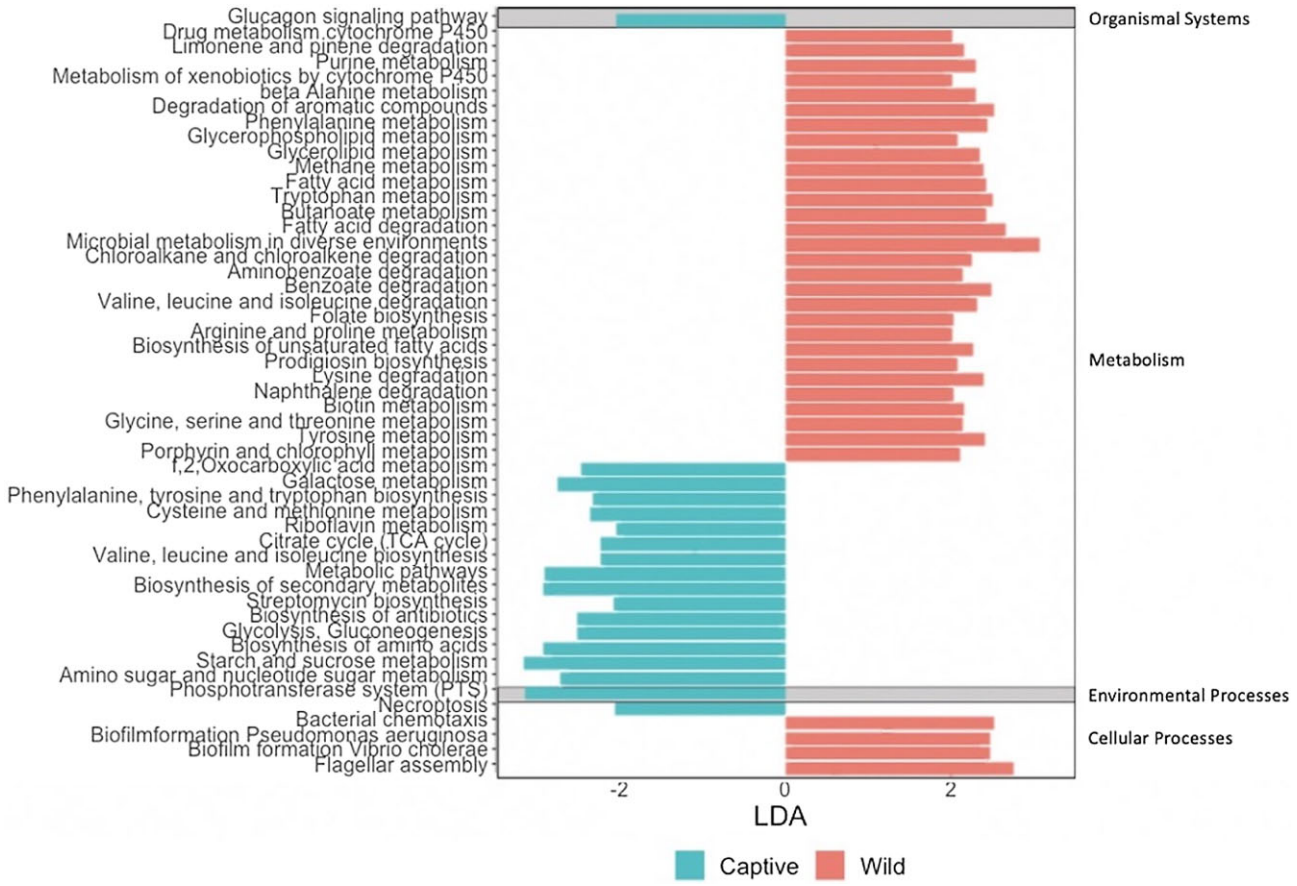

Figure 5. Linear discriminant analysis effect size results identifying functional pathways differentially abundant in the gut microbiotas of wild and captive samples.

Finally, captivity is a stressful environment for most animals and stress can alter the microbiome (Bailey 2018, Fischer and Romero 2019). The house sparrows in this experiment lost weight despite *ad libitum* access to food, suggesting they experienced stress in the captive environment. Corticosterone, the stress hormone in birds, administered to wild birds altered the abundance of several taxa in the microbiome, including decreasing the abundance of *Microvirga* (Noguera et al. 2018), this taxon also decreased in the microbiomes of the individuals after they were introduced to captivity in this study. Stress-induced corticosterone levels in captive house sparrows also correlated with changes in the community composition of their microbiome (Madden et al. 2022). Therefore, the stress of captivity may have contributed to changes in the microbiome.

Captive microbiome communities tended to be more homogenous than the microbiomes of wild birds. There was reduced diversity and *Catelliccocus*, a common avian gut symbiont, had increased dominance in the captive samples (Benskin et al. 2010, Grond et al. 2014, Kreisinger et al. 2015, Laviad-Shitrit et al. 2019). Interestingly, when community composition was weighted by phylogenetic distance, the microbiome of captive had similar or higher dispersions compared to wild samples. This may be due to wild microbiome samples having taxa that are more phylogenetically similar than captive samples. However, regardless of measurement type, there is still considerable variability in the captive samples suggesting that something other than extrinsic factors is maintaining variation in the microbiome. For example, host genotype could contribute to complex gene by environmental interactions that result in individual responses of the microbiome to captivity. Genes such as the highly variable major histocompatibility complex genes that code for proteins on cell surfaces of the adaptive immune system correlate with variation in the microbiome of wild organisms (Davies et al. 2022). We did not collect genotype data on the birds in this study, therefore, further research is needed to understand genetic or other factors that interact with the captive environment to influence the microbiome.

This study found significant changes in the diversity and composition of the gut microbiomes of house sparrows after living 5–10 days in captivity. This suggests a rapid restructuring of the avian microbiome after a change in environment, although more frequent sampling will be needed to capture the dynamics of this restructuring. This finding hints at the possibility that birds released from captivity back to the wild can quickly regain a natural microbiome, but this idea must be tested, and sampling individuals after reintroduction into the wild is logistically challenging. These results also suggest that changes in the microbiome should be considered in studies where individuals are brought into captivity from the wild, even if only for a few days. Many researchers allow animals to acclimate to captivity prior to an experiment, however, researchers may need to manage the tradeoff between allowing animals to adapt to captivity and maintaining their natural microbiome. Further experimentation on this topic will also be needed to disentangle the effect the different aspects of the

Table 3. Results of a linear mixed effect model investigating the influence of sample type, sex, and scaled mass index on Shannon's index, Chao1, and Faith's phylogenetic diversity.

Shannon's index	**Degrees of freedom**	**Estimate**	**SE**	***T*-value**	***P*-value**
Sample type (wild or captive)	1	−0.47	0.20	−2.34	.02*
Sex	1	0.27	0.15	1.70	.09
Scaled mass index	1	0.03	0.04	0.69	.48
Random effects	*Variance*	*SD*			
Capture date	0.0	0.0			
Bird ID	0.0	0.0			
Capture location	0.0	0.0			
Time of sampling	0.0	0.0			
Chao1	**Degrees of freedom**	**Estimate**	**SE**	***T*-value**	***P*-value**
Sample type (wild or captive)	1	−110.17	45.77	−2.40	.02*
Sex	1	23.24	32.61	0.71	.81
Scaled mass index	1	2.12	8.83	0.24	.48
Random effects	*Variance*	*SD*			
Capture date	27 147	164.8			
Bird ID	0.0	0.0			
Capture location	0.0	0.0			
Time of sampling	0.0	0.0			
Faith's phylogenetic index	**Degrees of freedom**	**Estimate**	**SE**	***T*-value**	***P*-value**
Sample type (wild or captive)	1	−5.46	2.21	−2.46	.01*
Sex	1	1.40	1.51	0.92	.36
Scaled mass index	1	0.19	0.41	0.46	.64
Random effects	*Variance*	*SD*			
Capture date	35.57	5.96			
Bird ID	0.0	0.0			
Capture location	0.0	0.0			
Time of sampling	2.45	1.56			

captive environment has on the microbiome as well as how this change will impact the host organism.

Authors' contributions

Melanie R. Florkowski (Conceptualization, Formal analysis, Methodology, Writing – original draft), Sarah A. Hamer (Conceptualization, Resources, Writing – review & editing), and Jessica L. Yorzinski (Conceptualization, Methodology, Resources, Writing – review & editing)

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Supplementary data

Supplementary data are available at *FEMSEC Journal* online.

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