Undergraduate Journal of Experimental Microbiology and Immunology (UJEMI) Vol. 27:1-12

Captivity plays a role in shaping the gut microbiome of social mammals

Mariah Alyssa de Leon, Selena Li, Kathleen Tom, Matthew Yap

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY With its roles in nutrition, behavior and immunity, the gut microbiome is an important health factor among mammals living in captivity. Recent studies have shown that the composition of the mammalian gut microbiome is altered by environmental changes associated with built, enclosed environments; these changes could explain certain negative health outcomes experienced by captive mammals. Because of the novelty surrounding the impacts of captivity, our study takes interest in understanding the effects of captivity on mammals that typically live in social groups in the wild. We hypothesized that being held captive, away from the social structure of the wild, affects the gut microbiome composition of mammals, and that such differences would vary by sociality and social group size. Our diversity analyses captured significant differences between social and non-social mammals, and between captive and wild social mammals, with respect to gut microbial alpha diversity. Taxonomic analysis showed that captivity had a greater effect on the number of unique microbial phyla in social mammals compared to non-social mammals. Differential abundance analysis identified Proteobacteria, Actinobacteria, Euryarchaeota and Fusobacteria as the main phyla responsible for observed differences between the gut microbiomes of captive and wild social mammals, with potential health implications that could be explored.

INTRODUCTION

Living in captivity represents a drastic change for animals accustomed to life in the wild. While the built environment has the intention of improving animal welfare and survivability, many animals in captivity experience negative health outcomes, including stress, infections, and gastrointestinal dysfunction (1-3). With species becoming increasingly threatened by climate change and anthropogenic activities, maintaining animal health in captivity is of utmost urgency. Particularly critical to host health is the gut microbiome, which plays a key role in digestion of food, defense against pathogens, immune system function, and behaviour (4-5). Given its importance in many aspects of animal biology, the gut microbiome has been proposed to mediate host health in captive conditions (1, 6). Intriguingly, there is concurrent evidence showing a pattern of significantly altered gut microbiota in captive animals compared to their wild counterparts, raising the possibility that this may be a factor in the poor health outcomes observed in captive individuals (7-9). To make sense of this, much research has attempted to identify and better understand the drivers behind the altered gut microbial signatures of animals in captivity.

In their investigation of the broad effects of captivity on the mammalian gut microbiome, McKenzie *et al.* assembled a large-scale dataset of 41 mammal species across six orders by sequencing DNA extracted from fecal samples and collecting information on various host traits such as diet, body size, and sociality (7). When comparing the gut microbiomes of paired samples from wild versus captive conditions, they found alpha diversity results to be inconsistent; alpha diversity decreased with captivity in some mammalian families, increased in one, and remained unchanged in the rest (7). Beta diversity, however, exhibited a clearer trend in which significant differences between captive and wild conditions were observed for the majority of genera tested (7). This echoes similar findings from other studies that reported distinct gut microbial differences between captive and wild hosts (8-12). Although these variations in bacterial communities have been found to be most strongly predicted by host taxonomy and diet, environmental factors, which fewer studies have addressed, also have a

Published Online: September 2022

Citation: Mariah Alyssa de Leon, Selena Li, Kathleen Tom, Matthew Yap. 2022. Captivity plays a role in shaping the gut microbiome of social mammals. UJEMI 27:1-12

Editor: Andy An and Gara Dexter, University of British Columbia

Address correspondence to: https://jemi.microbiology.ubc.ca/

large role in shaping gut microbial composition (7, 13). Among a plethora of other novel environmental circumstances faced by captive individuals is reduced or lack of social interactions with other animals.

In humans and other primates, social interactions are well known to profoundly impact health and, in more recent evidence, affect the gut microbiome (14-16). Notably, a handful of studies have established a link between sociality and the gut microbiota of social animals living in the wild. For instance, social networks and social group membership predict gut microbial composition in wild baboons (14). This holds true even after controlling for confounding factors such as diet and spatial proximity (14, 17). Another study revealed that black howler monkeys have more similar gut microbial communities if they spend more time in social contact (18). In a separate study on wild lemurs, social groups with greater social connectedness had microbiomes more homogeneous in composition, and within social groups, more gregarious individuals possessed the greatest microbial diversity (17). In chimpanzees, social interactions are key sources of gut microbial diversity both within and between host generations (19). Collectively, these findings point to a promising link between sociality and the gut microbiome in the context of wild animals.

However, there is a marked absence of studies examining this link within the captive setting. The social interactions and relationships formed naturally in the wild are undoubtedly disrupted by captivity, but whether the disruption of these social dynamics directly contributes to the altered gut microbial signatures of captive animals has not been addressed to date. Most of the studies on sociality and the gut microbiome of wild animals also confined their investigations to one specific social species, limiting our ability thus far to generalize findings to social animals as a whole.

With these knowledge gaps in mind, we sought to explore the role of captivity in shaping the gut microbiota of social mammals. Previous studies have only focused on one species; however, using a large-scale dataset collated by McKenzie *et al.* allowed us to combine data from multiple mammalian species and analyze patterns of microbial differences between social and non-social mammals in captivity and in the wild (7). To carry out this objective, we aimed to determine whether there are significant gut microbial differences between social and non-social mammals, between social mammals in captivity and in the wild, and between non-social mammals in captivity and in the wild. To extend our analysis, we also performed a preliminary evaluation of whether gut microbial diversity varies according to social group size. We expected to see significant differences in gut microbial diversity between social and non-social mammals; considering that social interactions are sources of bacterial diversity in wild animal populations, we expected social mammals to have greater microbial diversity compared to non-social mammals. Additionally, we hypothesized that social mammals would exhibit larger differences in gut microbial composition between captive and wild groups compared to non-social mammals. Being held captive in a built environment, a stark contrast from the natural social structure that they would otherwise experience in the wild, would have greater effects on the gut microbiome composition of social mammals compared to non-social mammals. To summarize, our goals were to 1) investigate differences in gut microbial diversity between social vs. non-social mammals, captive vs. wild social mammals, and captive vs. wild non-social mammals, and 2) identify differentially abundant taxa between captive and wild hosts for social and non-social mammals.

METHODS AND MATERIALS

Overview of dataset and metadata. All sequencing data and metadata were sourced from a dataset compiled by McKenzie *et al.* (7). In their study on the broad effects of captivity on the mammalian gut microbiome, they obtained fecal samples from 41 species of paired wild and captive mammals. After extraction of DNA from the samples, the 16S rRNA gene was amplified and sequenced for analysis. A wide range of host traits was also collected, including but not limited to diet type, body size, gut fermentation type, and sociality.

To support our preliminary investigation of how social group size can affect the gut microbiota of captive and wild mammals, we modified the metadata file to include an additional column for social group size category. The original metadata for the dataset included a column that contained a numerical value, ranging from 1 to 100, indicating social

group size for each sample. To increase the sample size for our analyses, we grouped numerical values together to create a new metadata category that indicated whether the social group size is small (3-10 individuals), medium (10-20 individuals), or large (>20 individuals). The range for each social group size category was chosen arbitrarily while ensuring that the three groups included similar numbers of samples.

Data processing using Quantitative Insights Into Microbial Ecology (QIIME) 2. The initial data processing steps were completed through the Quantitative Insights Into Microbial Ecology (QIIME) 2 pipeline in UNIX bash with the aims to import the dataset, perform demultiplexing and denoising, assign taxonomic information to sequence reads, and filter data based on our research questions (20). We imported the sequencing data through a manifest file that associated sample identifiers with absolute file paths of the single-end sequencing reads. Sequence reads were clustered based on sample identification in the form of barcode sequences based on the information provided in the metadata file, and the barcodes were removed once demultiplexing was completed. We visualized the demultiplexed data in a sequence base quality plot using QIIME 2 View (https://view.qiime2.org/) and selected a sequence read truncation of length of 150 nucleotides (nt) for quality control. A truncation length of 150 nt allowed us to retain at least 98% of the reads while also ensuring an acceptable minimum sequence base Phred quality score of 27, which corresponded to less than 1% chance of the occurrence of a base call error at any position in the 150 nt sequence (note that the study by McKenzie *et al*. trimmed sequence reads to 100 nt and selected a base quality score cut-off of 19) (7). We performed denoising on the dataset using the Divisive Amplicon Denoising Algorithm (DADA) 2 algorithm (21) with the selected truncation length to correct errors and remove low quality regions in the sequence read. By the end of the denoising step, we generated output files including a features table file and representative sequence file which contained information on retained amplicon sequence variants (ASVs), or unique sequence reads, that can be used in further data analyses.

Following denoising, we assigned taxonomic information to the ASVs aligning sequences using a pre-trained taxonomy classifier against reference sequences from the Greengenes 16S rRNA gene database (13_8 99% Operational Taxonomic Units from 515F/806R region of sequences) (22-24). Subsequently, we performed taxonomic analysis to produce taxa bar plots representing relative frequencies of identified taxa using the features table and the newly generated file containing taxonomic information for ASVs as input. In the final steps of the initial data processing stage, we filtered the features table to remove ASVs that aligned to chloroplasts and mitochondria sequences to prevent contamination that may lead to falsely interpreted increase in diversity. Lastly, we performed multiple filtering steps to sub-set the features table based on metadata categories including sample sociality (i.e., social vs. non-social) and captive or wild living status in preparation for downstream data analyses.

Alpha and beta diversity analyses. To address our research questions by comparing various diversity metrics, we generated alpha and beta diversity metrics for each subset of the feature table using the QIIME 2 pipeline (20). For the purpose of correcting for differences in sample library sizes, we selected sampling depths based on the alpha rarefaction curve and the number of features and samples retained as predicted by the visualizations and summaries of the features table subsets. We chose a sampling depth of 87442 for the complete features table and the social mammals features table for the comparison of social and non-social mammals and the comparison of captive social and wild social mammals, respectively; we chose a sampling depth of 98425 and 30307 for the comparison of different social group size categories in social captive mammals and in social wild mammals, respectively. We selected the sampling depths to retain at least 35% of features and 32% of samples, while also ensuring a minimum of six samples per group in each comparison. Each subset of the features table was used as input alongside its corresponding sampling depth and phylogenetic tree for alpha and beta diversity analyses, which outputted results for diversity metrics including Faith's phylogenetic diversity distance (25), Pielou's evenness index (26), Jaccard index (27), Bray-Curtis dissimilarity (28), unweighted UniFrac distance (29), and weighted UniFrac distance (30). We selectively re-created box plots for the alpha diversity metrics in R for better visualization of the results.

Taxonomic analysis for identification of unique and shared taxa. To compare the gut microbiota composition based on the presence of microbial phyla, we identified unique and shared taxa for captive and wild groups in social and non-social mammals. We visualized taxonomic bar plots generated as part of the QIIME 2 pipeline using QIIME 2 View and downloaded the tabulated data with taxonomic level set to level 2. We separated the data for social mammals and non-social mammals into different spreadsheets and sorted samples based on their captive or wild living status. We identified shared and unique phyla for each group by comparing the total abundance of each listed phyla between the captive and wild groups: phyla with total abundance greater than zero in one group and zero in the other group were classified as unique to the non-zero group; phyla with total abundance greater than zero in both groups were classified as shared taxa.

Differential abundance analysis. Generated QIIME 2 artifacts (features table, phylogenetic tree, taxonomic classifications) and the metadata file were imported into R (Version 2021.09.2) to create two phyloseq objects, one for social mammals and the other for non-social mammals, using the phyloseq package (Version 1.38.0) (7, 20, 31-32). We filtered the two phyloseq objects by the chosen sequencing depth for quality control, 103804 and 93885 for social and non-social mammals, respectively. We removed low abundant features that represent less than 0.05% of total sequencing reads. Next, we created two DESeq2 objects for social and non-social mammals using the DESeq2 package (Version 1.34.0) to express differences in abundance in mammals living in captivity relative to the wild (33). Differential abundance plots were visualized using the ggplot2 package (Version 3.3.5) (34). We used GraphPad Prism (Version 9.3.1) to combine differential abundance plots of social and non-social mammals into one plot. Statistically significant different taxonomic groups, evaluated using the Wald test (35), were determined using an adjusted p-value of 0.05.

RESULTS

Gut microbial diversity differed between social and non-social mammals, and between captive and wild social mammals. We generated alpha and beta diversity metrics and obtained adjusted p-values for diversity comparisons between social vs. non-social mammals, captive vs. wild social mammals, and captive vs. wild non-social mammals. Although we did not find any metadata-based clustering with respect to all four beta diversity metrics, we found some statistically significant differences in alpha diversity (Table 1). There were differences observed in Pielou's evenness, but not in Faith's phylogenetic diversity for comparisons between social vs. non-social mammals, and captive vs. wild social mammals. Our results suggested that observed gut microbiota diversity differences among the sampled mammal groups were likely driven by evenness rather than phylogenetic distance.

Additionally, we generated alpha and beta diversity metrics for comparing mammals that live with social groups of different sizes. Similar to the other comparisons, our results did not show significant differences between groups in terms of beta diversity. However, social group size appeared to have an effect on the alpha diversity in the gut microbiota of wild but not captive mammals, as suggested by the statistically significant difference in Faith's phylogenetic diversity observed between large and small social groups and between medium and small social groups (Table 1).

TABLE. 1 Results of Kruskal-Wallis test to compare the alpha diversity of the gut microbial composition of social and non-social mammals living in captivity or in the wild. Abbreviations: L-M = large-medium; LS = large-small; MS = medium-small. * indicate statistical significance (adjusted p-value < 0.05).

	Social vs. non-social	Social captive vs. social wild	Non-social captive vs. non-social wild	Social group size					
				Captive			Wild		
				L-M	L-S	M-S	L-M	L-S	M-S
Faith's PD	1.2×10^{-1}	8.3×10^{-1}	8.3×10^{-1}	5.9×10^{-1}	5.9×10^{-1}	5.9×10^{-1}	9.0×10^{-1}	1.5×10^{-2}*	5.0×10^{-3}*
Pielou's evenness	8.2×10^{-5}*	4.2×10^{-2}*	6.1×10^{-1}	2.7×10^{-1}	2.7×10^{-1}	2.7×10^{-1}	4.5×10^{-1}	3.1×10^{-1}	4.0×10^{-1}

Decreased gut microbial diversity in non-social mammals and social captive mammals was driven primarily by evenness. Significant differences in alpha diversity of the gut microbiota were observed between social and non-social mammals and between captive and wild social mammals. As seen in Figure 1A, non-social mammals showed significantly decreased diversity in terms of evenness compared to social mammals. When zeroing in on social mammals, the gut microbiota of those living in captivity had lower diversity in terms of evenness compared to their wild counterparts (Figure 1B). This difference was found to be statistically significant by Kruskal-Wallis pairwise comparison (36). In combination, our findings revealed a pattern of decreased gut bacterial diversity in non-social mammals compared to social mammals, and in social mammals in captivity compared to social mammals in the wild. Therefore, this may suggest that lack of or reduced social interaction, either in the form of living without a social group or living in captivity, is associated with decreased mammalian gut microbial diversity.

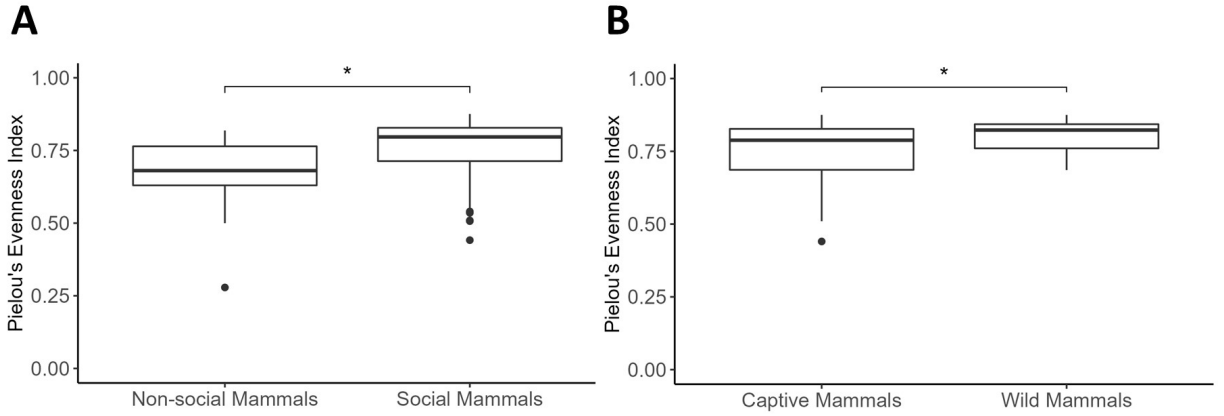

FIG. 1 Gut microbiota of social mammals and social wild mammals showed higher evenness than non-social mammals and social captive mammals, respectively. Gut microbiota diversity was assessed in terms of Pielou's evenness index in (A) non-social (n = 25) vs. social (n = 95) mammals and (B) social captive (n = 54) vs. social wild (n = 24) mammals. Adjusted p-values based on Kruskal-Wallis pairwise comparisons are 8.2×10^{-5} for (A) and 4.2×10^{-2} for (B), as indicated in Table 1.

Captivity affected the number of unique phyla in the gut microbiota of social mammals more than that of non-social mammals. We performed taxonomic analysis at the phylum level to compare unique and shared taxa between captive and wild mammals. Our results indicated that, regardless of sociality, most taxa present in the gut microbiota were shared between captive and wild mammals (Figure 2). However, while the captive group in social mammals showed a smaller number of unique taxa compared to its wild counterpart (Figure 2A), the captive group in non-social mammals showed a similar or slightly greater number of unique taxa than the wild group (Figure 2B). This observation may suggest that the amount

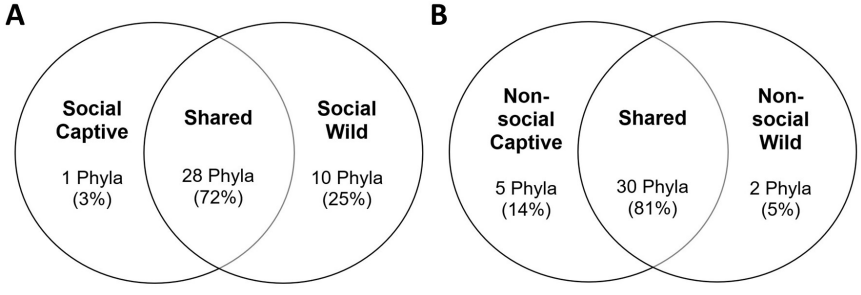

FIG. 2 Social mammals showed greater difference in the number of unique phyla present in the gut of captive vs. wild mammals. Comparison between (A) social captive (n = 75) and social wild (n = 146) mammals and (B) non-social captive (n = 36) and non-social wild (n = 39) mammals was based on the presence or absence of taxa at the phylum level.

of social interaction that can contribute to changes in the gut microbiota composition was not remarkably different between captive and wild state for non-social mammals; captivity did not have a major effect on non-social mammals since social interaction may have already

been minimal for these mammals that often live in simpler social networks even in the wild. On the other hand, the amount of social interaction for social mammals that often live in more complex social networks in the wild may have experienced a notable decline when they live in captivity; limitations associated with smaller and restricted living space may have forced the social mammals to interact less with others, including animals of the same species within their social groups and other animals that often live in the same community or ecosystem as them in the wild. As a result of experiencing a more drastic change in the simplicity of their social networks, there was a more noticeable difference between captive and wild social mammals in the number of gut microbial phyla identified compared to non-social mammals.

Differentially abundant microbes differed between social and non-social mammals. To explore the characterization of the microbial gut composition of social and non-social mammals living in captivity or in the wild, we performed differential abundance analysis. We did this to determine differentially abundant microbes in captivity or in the wild (control) under different sociality conditions. Social mammals exhibited different abundant phyla than non-social mammals as indicated by the greater abundance of Actinobacteria, Euryarchaeota, and Proteobacteria in captivity relative to the wild (Figure 3). On the other hand, the phylum Fusobacteria was significantly less abundant in captivity than wild non-social mammals (Figure 3). Our results suggest opposite trends, where identified phyla in social mammals are more abundant in captivity, whereas the identified phylum in non-social mammals are less abundant in captivity.

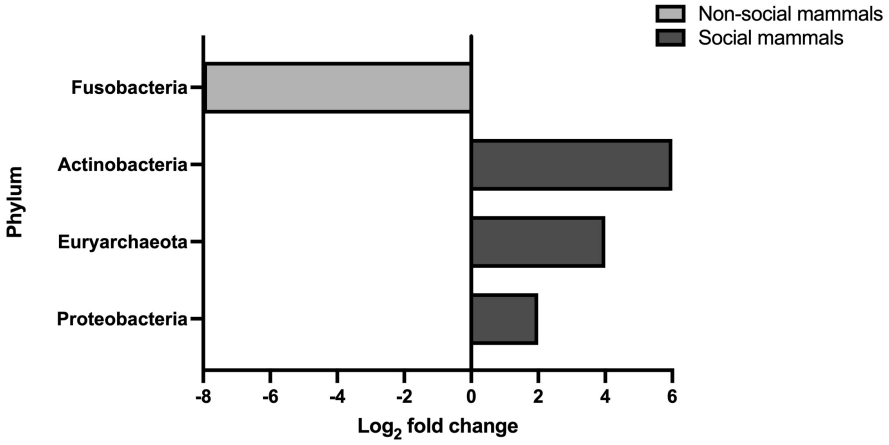

FIG. 3 The phyla Fusobacteria, Actinobacteria, Euryarchaeota, and Proteobacteria differed significantly in abundance between captive vs. wild mammals. Significantly differentially abundant microbes were detected using DESeq2 extension contained in a phyloseq package. Abundances are expressed as log-transformed differences in abundance in the group captive relative to the group wild for both social and non-social mammals. Bars represent the log_2FoldChange values of significantly (adjusted $p < 0.05$ based on Wald test) different phyla in captive vs. wild groups.

DISCUSSION

In the present study, we aimed to explore the effects of captivity on the gut microbiome composition of mammals that typically live in social groups using a large-scale dataset previously published by McKenzie *et al.* (7). Following a bioinformatics-centered approach, we used alpha diversity, taxonomic, and differential abundance analyses to dissect gut microbial differences between social and non-social mammals in captivity and in the wild.

Captivity affects the gut microbiome of social mammals more than non-social mammals. We sought to determine whether there exist significant gut microbial differences between social and non-social mammals, between social mammals in captivity and in the wild, and between non-social mammals in captivity and in the wild. Congruent with our hypothesis, results from our comparison between social and non-social mammals revealed alpha diversity to be significantly higher in social mammals. This is unsurprising, given the known effects of social relationships and interactions on the gut microbiota of numerous social species in the wild (14, 17-19). Animal social groups are reservoirs of gut bacterial diversity; microbes are transmitted between members through physical contact between individuals, shared environments, shared diets, and kinship (14-15). Our observed differences in alpha diversity between social and non-social mammals were found to be driven specifically by evenness (Figure 1A) and not phylogenetic distances (Table 1, Supplemental Figure 1A). The basis of

this evenness-driven difference in gut microbiome diversity between social and non-social mammals is unknown; however, we know that social mammals have more frequent social interactions and, as a result, more microbial exchange between mammalian hosts (14, 17-19). As a consequence, within an individual host, microbes must adapt in order to coexist with other microbes. Over time, this may result in members of the host microbial community having more similar relative abundances, giving rise to the higher evenness observed in the gut microbiota of social mammals. Conversely, for non-social mammals, the lack of social interaction means there is very little microbial exchange between hosts; without the need to accommodate for other microbes, members of the microbial community may instead be in competition with each other, resulting in, for instance, more dominant members achieving higher relative abundances. If relative abundances vary greatly, this may lead to lower community evenness, as we saw for non-social mammals.

As hypothesized, our study also found that captivity had a greater effect on the gut microbiome of social mammals than that of non-social mammals. Gut microbiome evenness was significantly altered in captivity among social mammals (Figure 1B), whereas evenness did not significantly differ with captivity in non-social mammals (Supplemental Figure 2B). Differences in phylogenetic distances were also not significant between captive and wild groups for both social mammals (Supplemental Figure 1B) and non-social mammals (Supplemental Figure 2A). Additionally, there was a larger difference between captive and wild groups in the number of unique microbial phyla found in the gut for social mammals compared to non-social mammals (Figure 2). All of these results converge to a common finding: captivity affects the gut microbiome of social mammals. Previous studies have concluded that social connectedness and the amount of social interaction are factors that can affect gut microbiome diversity in lemurs and mice, with more socially active individuals acquiring more diverse microbiomes (17, 37). The same may apply for social mammals in general. As such, a possible explanation for our findings may be that social mammals most likely encounter more social interactions in the wild, with more opportunities for physical contact and transmission of gut microbes; in captivity, such interactions are limited, or sometimes even non-existent. Non-social mammals, on the other hand, encounter few, if any, social interactions in the wild; as they are well-adjusted to solitary living, being in captivity is not associated with reduced social interactions and thus does not reduce the chances of microbe transmission for non-social mammals as much as for social mammals.

Our preliminary analysis on social group size showed a significant difference in gut microbiome diversity for wild mammals based on Faith's phylogenetic distance between large and small social groups and between medium and small social groups (**Table 1**). Interestingly, we did not observe any significant results for captive mammals. It should be noted that it was unclear whether the data in the social group size metadata category reflected the group size of the animal in captivity or its presumed group size if it were in the wild. Despite our resulting inability to pursue further investigations into group size, social group size remains an important topic, as it is a major determinant of individual fitness in wild animal populations (38).

Four microbial phyla are responsible for differences observed between the gut microbiomes of social and non-social mammals. We performed differential abundance analysis to determine the effect of sociality status on the mammalian gut microbiota composition, depending on their living status. That is, we determined differentially abundant microbes of social and non-social mammals living in captivity relative to the wild. Our results show distinct differentially abundant microbes between social and non-social mammals.

In order of decreasing prevalence, Actinobacteria, Euryarchaeota, and Proteobacteria were the most dominant phyla among social mammals living in captivity relative to the wild (Figure 3). Our findings for the bacterial phyla Actinobacteria and Proteobacteria differed from the study by McKenzie *et al.* They found that Actinobacteria and Proteobacteria, particularly Alpha- and Betaproteobacteria, were more abundant in wild counterparts (7). However, it is important to note that their findings only pertained to mammals living in either the captive or wild state, without the sociality aspect (7). To explain this observation, having more social interactions with other mammals increases the abundance of Actinobacteria and Proteobacteria in the gut microbiomes of captive mammals. Furthermore, the archaeal

phylum Euryarchaeota was abundant in social captive mammals. Findings related to the abundance of Euryarchaeota in mammals living in captivity or in the wild are inconsistent between different studies. One study showed no significant differences in relative abundance of Euryarchaeota between captive and wild white-lipped deer (11). Meanwhile, another study found higher abundance of Euryarchaeota in captive rhinos than wild rhinos, which aligns with our results (10). Similarly, these studies did not take sociality into account. Although these studies focused on different mammalian taxa than our study, we were able to discern conflicting findings. As a result, analysis of Euryarchaeota abundance between captive and wild mammals, along with sociality status, should be performed to determine any replicable results.

Fusobacteria was found to be an uncommon phylum for non-social mammals living in captivity relative to the wild (Figure 3). Our results were inconsistent with the findings of the study by McKenzie *et al.* (7). They found that Fusobacteria were not differentially abundant in mammals living in the captive or wild state, without considering the sociality of the mammals. In this regard, it is possible that the lack of social interaction of mammals living in captivity drives the decrease in abundance of Fusobacteria in their gut.

Although more experiments on mammalian gut ecophysiology must be conducted to precisely determine the health effects of captivity-associated microbiome changes in social and non-social mammals, the differentially abundant microbial phyla we identified in our study have a variety of implications on mammalian health. These four phyla show an interesting combination of potential positive and negative consequences on host health. Recent developments have noted the potential use of Actinobacteria and Euryarchaeota in probiotic treatments. In contrast, Proteobacteria and Fusobacteria have been associated with unfavourable health conditions.

Members of the phylum Actinobacteria such as *Bifidobacterium*, which are found in the human gut microbiome, have shown probiotic effects due to their ability to aid in digestion, immunity and vitamin synthesis (39-41). There is also evidence that *Bifidobacteria* supplementation as probiotic treatment could improve symptoms in humans who suffer from irritable bowel disease, a common gastrointestinal order (42). In addition, these bacteria also show anti-inflammatory properties in the gut by negatively regulating pro-inflammatory cytokine mRNA levels (43). Similarly, members of the phylum Euryarchaeota such as methanogens have demonstrated promising effects of reducing methanol toxicity as well as preventing accumulation of cardiovascular disease agents (i.e. triethylamine and triethylamine N-oxide) in mouse models (44).

Diet influences the presence of Proteobacteria in the gut composition of mammals, which aids in protein, carbohydrate, cofactor and vitamin metabolism (45). Given the finding that Proteobacteria is more abundant in social captive mammals, this must mean that these mammals have an altered microbial gut composition than social wild mammals to allow them to have a high protein and carbohydrate diet, and be proficient in converting what they consume into energy. Although Proteobacteria has functional contributions in the mammalian gut, it is important to note that high abundances of Proteobacteria is associated with dysbiosis in mammals with metabolic or inflammatory disorders (45). It is possible that social captive mammals, due to the built environment influencing their gut composition, are more prone to acquiring disease. Moreover, Fusobacteria has been commonly detected in animal and human colorectal tumor sites, and has been found to activate host inflammatory responses that promote tumor formation (46). As such, non-social mammals that live in captivity are more likely to be protected from tumor formation derived from Fusobacteria.

Ultimately, captivity-associated changes in the abundance of the aforementioned microbial phyla seem to steer towards improving the health of their host mammals, as indicated by the presence or absence of the indicated phyla. However, correlation analysis between the indicated phyla and the health of mammals should be conducted to observe the direct effects of captivity in influencing the microbial gut composition, which may aid in our efforts towards improving mammalian well-being in the built environment. Future explorations of mammalian inflammatory profiles and cytokine expression levels in relation to microbe abundance could provide additional insights into the health effects of captivity-associated microbial changes.

Limitations The dataset compiled by McKenzie *et al.* investigated wild and captive samples from mammalian taxa across six orders, and thus, one limitation of our study is that the findings and trends we observed only pertain to those mammalian orders. This limitation cannot be addressed because we were only provided one dataset and therefore, were unable to explore other mammalian orders. Furthermore, we were limited to the information given in the dataset, and a number of the metadata categories provided, such as social group size as previously mentioned, required further clarification to prevent false interpretations. It is worth mentioning that the dataset did not include any information about the life history of the mammals. For instance, the length of time in captivity, whether the mammals have been relocated, or the use of antibiotics in captive mammals were not included. Therefore, these confounding variables were not accounted for, which may have affected the mammalian gut microbiome. Additionally, the small sample size used in our analyses may not be representative of the entire population. Similarly, we used uneven sample sizes when comparing alpha diversity metrics for social and non-social mammals, and for social captive and social wild mammals. Because of the small and uneven sample sizes used, there is a greater chance of accepting a false premise as true, known as Type II error, which may reduce the power of our study.

Conclusions In our study, we aimed to investigate whether captivity affects the gut microbiota of mammals that normally live in social groups. Alpha diversity analysis results indicated that the gut microbiota of social mammals and social wild mammals showed higher evenness than non-social mammals and social captive mammals, respectively. Based on our taxonomic analysis, captivity had a greater impact on the number of unique microbial phyla in social mammals than in non-social mammals. Social captive mammals had less unique phyla present in their gut composition than non-social captive mammals. Lastly, our differential abundance analysis revealed that the phylum Fusobacteria were less abundant in non-social mammals living in captivity than in the wild. Actinobacteria, Euryarchaeota, and Proteobacteria were more abundant in social mammals living in captivity than in the wild. Altogether, our findings support our hypotheses that social and non-social mammals have observed differences in gut microbial diversity, and that social mammals have larger gut microbial composition differences between captive and wild groups than non-social mammals.

Future Directions Future research can conduct further analyses, such as correlational analysis, on the current dataset to continue deconstructing the relationship between the sociality and captive or wild living status of mammals. Due to the nature of our project, we were highly constrained to the information provided in the dataset, and our findings were limited to the small and uneven sample sizes we used. Hence, a longitudinal study can be conducted to observe the changes in the gut microbiota of mammals that switched from captive to wild state or alternatively, wild to captive state over a certain period of time. Detailed information on the environmental conditions under which the samples were collected should also be carefully documented to enable better understanding of the effects of specific captive conditions on the gut microbial composition of mammals. In addition, to address our limitations with sample size, this study could conduct similar analyses with a larger sample size and evenly distributed samples to verify the effect of captivity. To further delve into sociality and its effects, future research can explore and investigate other related factors such as social group size, which we did not fully address in the present study, on the mammalian gut microbial composition. Any prospective work utilizing the social group size data from the dataset assembled by McKenzie *et al.* may wish to contact the authors to clarify details relating to the social group size variable. There is also a gap in knowledge about the impact of living space size on the gut microbiome of social mammals, which can be addressed and pursued by other studies.

ACKNOWLEDGEMENTS

We would like to thank McKenzie *et al.* for providing the dataset used in this study. We would like to express our gratitude to Dr. Evelyn Sun, Divya Kriti, and Zakhar Krekhno for their mentorship, guidance, and support throughout this study. We also thank the Department of

Microbiology and Immunology at the University of British Columbia for providing the tools and resources to carry out this study.

CONTRIBUTIONS

All listed authors were involved in the data analysis and manuscript-writing phases of the study; all authors participated equally in the maintenance of the lab notebook and discussion about interpretations of experimental results. Mariah completed the differential abundance analysis in R and re-created the related figure; she was responsible for drafting study limitations, conclusion, and future directions in addition to all the subsections related to differential abundance analysis in the manuscript. Selena completed the initial data processing and diversity metrics generation in QIIME 2, and re-generated the related figures in R; she co-wrote the results and discussion sections and drafted the subsections related to QIIME 2 and taxonomic analysis in the methods section. Kathleen aided in diversity metrics generation, explored additional analyses that can be done in R, drafted the introduction and overview of the dataset and co-wrote the results and discussion sections. Matthew explored additional analyses that can be done in R, drafted the abstract and co-wrote subsections related to alpha diversity, evenness and the health implications of our findings.

REFERENCES

1. **Diaz J, Reese AT.** 2021. Possibilities and limits for using the gut microbiome to improve captive animal health. *Animal Microbiome* **3**:89.
2. **Mason GJ**. 2010. Species differences in responses to captivity: stress, welfare and the comparative method. *Trends in Ecology & Evolution* **25**:713–721.
3. **Morgan KN, Tromborg CT**. 2007. Sources of stress in captivity. *Applied Animal Behaviour Science* **102**:262–302.
4. **McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Nealson K, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho M, Sanders JG, Tautz D, Wernegreen JJ**. 2013. Animals in a bacterial world, a new imperative for the life sciences. *PNAS* **110**:3229–3236.
5. **Suzuki TA**. 2017. Links between Natural Variation in the Microbiome and Host Fitness in Wild Mammals. *Integrative and Comparative Biology* **57**:756–769.
6. **Bahrndorff S, Alemu T, Alemneh T, Lund Nielsen J.** 2016. The Microbiome of Animals: Implications for Conservation Biology. *Int J Genomics* **2016**:5304028.
7. **McKenzie VJ, Song SJ, Delsuc F, Prest TL, Oliverio AM, Korpita TM, Alexiev A, Amato KR, Metcalf JL, Kowalewski M, Avenant NL, Link A, Di Fiore A, Seguin-Orlando A, Feh C, Orlando L, Mendelson JR, Sanders J, Knight R**. 2017. The Effects of Captivity on the Mammalian Gut Microbiome. *Integrative and Comparative Biology* **57**:690–704.
8. **Clayton JB, Vangay P, Huang H, Ward T, Hillmann BM, Al-Ghalith GA, Travis DA, Long HT, Tuan BV, Minh VV, Cabana F, Nadler T, Toddes B, Murphy T, Glander KE, Johnson TJ, Knights D.** 2016. Captivity humanizes the primate microbiome. *PNAS* **113**:10376–10381.
9. **Borbón-García A, Reyes A, Vives-Flórez M, Caballero S**. 2017. Captivity Shapes the Gut Microbiota of Andean Bears: Insights into Health Surveillance. *Front Microbiol* **8**:1316.
10. **Gibson KM, Nguyen BN, Neumann LM, Miller M, Buss P, Daniels S, Ahn MJ, Crandall KA, Pukazhenthi B.** 2019. Gut microbiome differences between wild and captive black rhinoceros – implications for rhino health. *Sci Rep* **9**:7570.
11. **Li B, Gao H, Song P, Liang C, Jiang F, Xu B, Liu D, Zhang T**. 2022. Captivity Shifts Gut Microbiota Communities in White-Lipped Deer (*Cervus albirostris*). *Animals* **12**:431.
12. **Trevelline BK, Moeller AH**. 2022. Robustness of Mammalian Gut Microbiota to Humanization in Captivity. *Front Ecol Evol* **9**:785089.
13. **Narat V, Amato KR, Ranger N, Salmona M, Mercier-Delarue S, Rupp S, Ambata P, Njouom R, Simon F, Giles-Vernick T, LeGoff J**. 2020. A multi-disciplinary comparison of great ape gut microbiota in a central African forest and European zoo. *Sci Rep* **10**:19107.
14. **Tung J, Barreiro LB, Burns MB, Grenier J-C, Lynch J, Grieneisen LE, Altmann J, Alberts SC, Blekhman R, Archie EA**. 2015. Social networks predict gut microbiome composition in wild baboons. *eLife* **4**:e05224.
15. **Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI**. 2012. Human gut microbiome viewed across age and geography. *Nature* **486**:222–227.
16. **Degnan PH, Pusey AE, Lonsdorf EV, Goodall J, Wroblewski EE, Wilson ML, Rudicell RS, Hahn BH, Ochman H**. 2012. Factors associated with the diversification of the gut microbial communities within chimpanzees from Gombe National Park. *Proceedings of the National Academy of Sciences* **109**:13034–13039.
17. **Perofsky AC, Lewis RJ, Abondano LA, Di Fiore A, Meyers LA**. 2017. Hierarchical social networks shape gut microbial composition in wild Verreaux's sifaka. *Proceedings of the Royal Society B: Biological Sciences* **284**:20172274.

18. **Amato KR, Van Belle S, Di Fiore A, Estrada A, Stumpf R, White B, Nelson KE, Knight R, Leigh SR**. 2017. Patterns in Gut Microbiota Similarity Associated with Degree of Sociality among Sex Classes of a Neotropical Primate. *Microb Ecol* **74**:250–258.
19. **Moeller AH, Foerster S, Wilson ML, Pusey AE, Hahn BH, Ochman H**. 2016. Social behavior shapes the chimpanzee pan-microbiome. *Sci Adv* **2**:e1500997.
20. **Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG**. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* **37**:852–857.
21. **Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP**. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**:581–583.
22. **Robeson MS, O'Rourke DR, Kaehler BD, Ziemski M, Dillon MR, Foster JT, Bokulich NA**. 2021. RESCRIPt: Reproducible sequence taxonomy reference database management. *PLoS Comput Biol* **17**:e1009581.
23. **Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory Caporaso J**. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **6**:90.
24. **McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P**. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* **6**:610–618.
25. **Faith DP**. 1992. Conservation evaluation and phylogenetic diversity. *Biol Cons* **61**:1–10.
26. **Pielou EC**. 1966. The measurement of diversity in different types of biological collections. *J Theor Biol* **13**:131–144.
27. **Jaccard P**. 1901. Distribution de la flore alpine dans le Bassin des Drouces et dans quelques regions voisines. *Bulletin de la Société Vaudoise des Sciences Naturelles* **37**:241–272.
28. **Bray JR, Curtis JT**. 1957. An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecological Monographs* **27**:325–349.
29. **Lozupone C, Knight R**. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol* **71**:8228–8235.
30. **Lozupone CA, Hamady M, Kelley ST, Knight R**. 2007. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Appl Environ Microbiol* **73**:1576–1585.
31. **R Core Team**. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
32. **McMurdie PJ, Holmes S**. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **8**:e61217.
33. **Love M, Huber W, Anders S**. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**:550.
34. **Wickham H**. 2009. ggplot2. Springer, New York, NY. http://link.springer.com/10.1007/978-0-387-98141-3. Retrieved 15 April 2022.
35. **Wald, Abraham**. 1943. Tests of Statistical Hypotheses Concerning Several Parameters when the Number of Observations Is Large. *Trans Am Math Soc* **54**:426–482.
36. **Kruskal WH, Wallis WA**. 1952. Use of Ranks in One-Criterion Variance Analysis. *J Am Stat Assoc* **47**:583–621.
37. **Raulo A, Allen BE, Troitsky T, Husby A, Firth JA, Coulson T, Knowles SCL**. 2021. Social networks strongly predict the gut microbiota of wild mice. *ISME J* **15**:2601–2613.
38. **Price EE, Stoinski TS**. 2007. Group size: Determinants in the wild and implications for the captive housing of wild mammals in zoos. *Applied Animal Behaviour Science* **103**:255–264.
39. **Servin AL**. 2004. Antagonistic activities of *lactobacilli* and *bifidobacteria* against microbial pathogens. *FEMS Microbiol Rev* **28**:405–440.
40. **Gronlund MM, Gueimonde M, Laitinen K, Kociubinski G, Gronroos T, Salminen S, Isolauri E**. 2007. Maternal breastmilk and intestinal bifidobacteria guide the compositional development of the *Bifidobacterium* microbiota in infants at risk of allergic disease. *Clinical and Experimental Allergy* **37**:1764–1772.
41. **Turroni F, Ribbera A, Foroni E, van Sinderen D, Ventura M**. 2008. Human gut microbiota and *bifidobacteria*: from composition to functionality. *Antonie van Leeuwenhoek* **94**:35–50.

42. **Lewis ED, Antony JM, Crowley DC, Piano A, Bhardwaj R, Tompkins TA, Evans M**. 2020. Efficacy of *Lactobacillus paracasei* HA-196 and *Bifidobacterium* longum R0175 in Alleviating Symptoms of Irritable Bowel Syndrome (IBS): A Randomized, Placebo-Controlled Study. *Nutrients* **12**:1159.
43. **Okada Y, Tsuzuki Y, Hokari R, Komoto S, Kurihara C, Kawaguchi A, Nagao S, Miura S**. 2009. Anti-inflammatory effects of the genus *Bifidobacterium* on macrophages by modification of phospho-IκB and SOCS gene expression. *Int J Exp Pathol* **90**:131–140.
44. **Lurie-Weinberger MN, Gophna U**. 2015. Archaea in and on the Human Body: Health Implications and Future Directions. *PLoS Pathog* **11**:e1004833.
45. **Moon CD, Young W, Maclean PH, Cookson AL, Bermingham EN**. 2018. Metagenomic insights into the roles of Proteobacteria in the gastrointestinal microbiomes of healthy dogs and cats. *MicrobiologyOpen* **7**:e00677.
46. **Kelly D, Yang L, Pei Z**. 2018. Gut Microbiota, Fusobacteria, and Colorectal Cancer. *Diseases* **6**:109.