THE UNIVERSITY OF CHICAGO

GEOGRAPHIC VARIATION AND TEMPORAL DYNAMICS OF MIGRATORY BIRD MICROBIAL COMMUNITIES

A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES
AND THE PRITZKER SCHOOL OF MEDICINE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

COMMITTEE ON EVOLUTIONARY BIOLOGY

BY

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CHICAGO, ILLINOIS

MARCH 2022

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Acknowledgements

I chose to leave the acknowledgements as the final task to complete for the dissertation as this should be the easiest part to write, yet it is surprisingly difficult to put into words the profound gratitude I have for so many people. I am eternally thankful to my advisor, Dr. John Novembre, for his encouragement, insight, and willingness to advise someone who works with birds. To my committee members, Dr. Maureen Coleman, Dr. Greg Dwyer, Dr. Shannon Hackett, and Dr. Trevor Price, I am grateful for the diverse expertise they contributed to this interdisciplinary research. Dr. John Bates, an unofficial committee member who was always willing to answer questions. Thank you to Audrey Aronowsky and Carolyn Johnson, for logistical support and personal guidance. I am thankful for the input and guidance from many people at the University of Chicago and within the Committee on Evolutionary Biology community, Dr. Stefano Allesina, Dr. Michael Coates, Dr. Andrew Hipp, Dr. Dave Jablonski, Dr. Joseph Lampert, Dr. Zhe-Xi Lou, Dr. Cathy Pfister, Dr. Stephen Pruett-Jones, Dr. William Rando, and Dr Tim Wooton.

The Field Museum has been an integral part of my development as a scientist before and throughout this dissertation research. I am grateful to members of the Birds department including Dr. Chad Eliason, Josh Engel, Tom Gnoske, Mary Hennan, Kayleigh Kueffner, Peggy Macnamara, and Dr. Ben Marks. I am especially thankful to Dr. Dave Willard, without whom I would not have the foundation for two of my chapters. The Pritzker Lab is my home base at the museum. Thank you to Dr. Kevin Feldheim, Dr. Dylan Maddox, and Erica Zahnle for guidance, support, and never once commenting on my very normal levels of organization. I am also thankful to Dr. Felix Grewe, Dr. Larry Heany, Dr. Julian Kerbis, Dr. Matt Nelson, Dr. Bruce Patterson, and Dr. Rick Ree. Dr. Corrie Moreau, Dr. Manu de Oliveira Ramalho, and the rest of the Ant Lab; though no longer at

the museum your positive impact remains. A special thank you to Dr. Sushma Reddy, whose mentorship during the Master's program provided a solid foundation for success with the Ph.D.

The research presented here was funded by the Illinois Ornithological Society, Henry Hinds Fund, Pritzker Laboratory Summer Research Grants, Society for Systematic Biologists, ARCS Foundation of Illinois, National Geographic, Field Museum's Women in Science program, American Ornithological Society, International Ornithological Union, Chicago Chapter of Graduate Women in Science organization, Field Museum's Museum Collections Spending Fund, Brien O'Brien and Mary Hasten Scholarship, Field Museum Women's Board Field Dreams Awards, Grainger Bioinformatics Center, and the Microbiome Center of Argonne National Laboratory. The University of Chicago Center for Teaching and the Field Museum's Women in Science organization provided funding for two fellowships.

Regarding my first chapter, I first thank Dr. Nathan Cooper and Dr. Peter Marra for letting me join their ongoing Kirtland's Warbler research. Nathan was an exceptional collaborator, both in the field and throughout the course of drafting the publication. This work spanned multiple locations throughout Michigan and Cat Island, The Bahamas. I thank USGWS MI Ecological Services Field Office which supported tower construction in Michigan. I also thank USFWS, USFS, USDA Wildlife Services, and MI DNR for hosting towers and A. Makarewicz and the Davies and Sansote families for providing field housing in Michigan. I also thank Josie, M. Seymour, and F. Wolff for hosting towers and M. Tukeva and the Campaignes for providing field housing on Cat Island. Finally, I thank the many field technicians who helped collect samples, especially Adrienne Dale.

My second and thirds chapters would not have been possible without Dr. Dave Willard, the Chicago Bird Collision Monitors organization, and the Wednesday prep lab volunteers. There

are more people who contributed to these two chapters than I will ever know. I thank the incredible volunteers who walk downtown Chicago every morning during spring and fall migration collecting salvage birds which contribute to the museum's migratory bird specimen collection. I also thank the Wednesday prep lab volunteers, who prepped the birds and streamlined the process of collecting samples. I also thank Ethan Gyllenhaal, Brian Tsuru, and Kristen Wacker for their contributions to these projects. I am grateful to Courtney Brennan, Dr. Andy Jones, Dr. Brian Weeks, and Dr. Ben Winger for letting me join their field work so I could collect samples for the 'summer' portion of the thrushes' cycle. Finally, thank you to Kayleigh Kueffner for coming with me to Peru to collect the 'overwintering' period of the thrushes' cycle. Someday we will get those samples back to Chicago.

Completing a dissertation takes guidance, support, and often, emotional labor. The following people have my utmost gratitude: Mark Bitter, Benjamin Blanchard, Robert Burroughs, Jacob Cooper, Shane Dubay, Peter Flynn, Ryan Fuller, Daniel Hooper, Simon Lax, Jackie Lugmus, Joel Mercado-Díaz, Chloe Nash, Daniella Palmer, John Park, Arvind Pillai, Katherine Silliman, Tim Sosa, Rahul Subramanian, Supriya, Brooke Weigel, Alex White, and especially, Kristina Fialko, Valentina Gómez-Bahamón and Natalia Piland. Beyond the academic setting, I am surrounded by incredible friends; these people are my family. Tina and Karyl who provide unending support and encouragement; Lizzy who can relate and commiserate unlike anyone else I know, Stauchy for taking me hiking, MK for being in Chicago and in graduate school with me, Mike-thanks for the walks during over the past two years, the group from the bar who were by far the most excited as I started at UC, and Matt for giving me a backpack, a news article, and more bird jokes than necessary. A special thanks to so many more, most of who can now count our

friendship in decades: Ben, Brian, Bryon, Cameron, Christy, Katie, Laura, other Laura, Lauren, Maggie, Mandy, Megan, Mike, other Mike, Niki, Nina, and Sam.

Finally, I must acknowledge three people who have provided support and encouragement but who unfortunately are not here to celebrate as this dissertation work comes to an end. To my grandmother, Ginny Lamermayer; my stepfather, Paul Spink; and my very good friend, Lynika Strozier – thank you.

INTRODUCTION

Within each vertebrate there exists a complex microbial ecosystem, composed of millions of microorganisms that interact with each other, the host, and the outer environment. Microorganisms represent the vast majority of genetic diversity on the planet, driving critical ecosystem processes and influencing the evolutionary trajectories of many vertebrate species (Henry *et al.* 2021). Technological advancements in culture-independent molecular approaches have substantially increased our ability to identify and catalogue host-associated microorganisms (Caporaso *et al.* 2010, Caporaso *et al.* 2012). To illuminate key components in the natural history, overall biodiversity, and general health of wild animals, a more complete understanding of factors driving diversity and abundance of host-associated microbiota is necessary.

The research presented in this dissertation is motivated by the need for increased understanding of wild animal microbiota. Specifically, I aim to address how geographic and temporal variation influence change in the prevalence and diversity of host-associated microbiota in migratory birds. Animals that complete bi-annual migrations inherently experience substantial variety in diet and habitat throughout the annual cycle, as well as considerable physiological changes that often accompany extreme long-distance movements (Bauchinger *et al.* 2005, Bowlin *et al.* 2008). Change in diet, habitat, or physiological stress have previously been shown to impact the composition of host-associated microbiota of migratory animals, but these studies were generally limited in scope, focusing on a single time period within the annual cycle (Lewis *et al*. 2017, Cao *et al.* 2020, Capunitan *et al.* 2020). Migratory birds represent an ideal natural experiment in which specific variables, such as change in diet or habitat, can be investigated for their influence on microbiota.

Through both active collection of new samples and the use of museum accessioned specimens, I expand the scope of host-associated microbiota in migratory birds by conducting several extensive surveys in which I determine the how changing geographies and time periods affect variation in microbiota. The purpose of this introductory chapter is to provide an overview of the three primary subject areas of my dissertation: wildlife microbiology, disease ecology, and the use of natural history collections in research on host-associated microbiota.

Wildlife microbiology

Known collectively as the microbiome, the ecosystems of microbes that exist within vertebrates has quickly become recognized as a fundamental aspect of the host – stimulating immune system maturation, affecting behaviors, providing defense against pathogens, influencing mate choice and success, and directly impacting overall host health (Yeoman *et al.* 2012, Apajalahti and Vienola 2016, Broom and Kogut 2018, Davidson *et al.* 2020, Slevin *et al.* 2020, Taff *et al.* 2021). Of particular interest are the microbes that exist within the gastrointestinal tract of vertebrates, colloquially termed gut microbiota, as this community appears to have the most substantial impact on host biology (Kinross *et al.* 2011). The majority of gut microbiota has been conducted on humans and model organisms, with nearly 90% of published work on mammals (Grond *et al.* 2018). Although this research has greatly expanded our cumulative knowledge of the microbiota, it is becoming increasingly clear that avian microbiomes do not behave in the same manner as mammalian microbiomes (Hird 2017, Bodawatta, Hird *et al*. 2021). This is hypothesized to be in part due to the evolution of flight and the resulting physiological adaptations to flight that have occurred within the gastrointestinal tract (Song *et al.* 2020). These physiological adaptations are especially evident in birds that undergo bi-annual long-distance flights as part of

their annual migratory cycle. Increased understanding of the avian-microbe relationships, including the impact of migration, can be achieved through temporally and geographically diverse surveys of microbiota.

Migratory birds experiencing substantial change in diet and habitat across the annual cycle may have corresponding shifts in host-associated microbial diversity. In birds, phenotypic flexibility associated with migration invokes numerous changes to the birds' digestive systems, including atrophication of the intestinal tract (Piersma and Gill 1998, McWilliams and Karasov 2001). These changes may reduce birds' the overall abundance of colonized gut microbiota and promote increase presence of transient bacterial ingested from the local environmental pool, as suggested in a study of migratory passerines on stopover after crossing the Gulf of Mexico (Lewis *et al.* 2016). Exposure to different environmental microbial pools at stopover sites could artificially inflate avian gut microbial diversity as birds may acquire up to 25% of their gut microbiota from environmental sources (Chen *et al*. 2020). Shifts in food sources and flocking behaviors throughout the annual cycle may similarly impact gut microbiota (Grond *et al.* 2018, Davidson *et al.* 2020). Comparisons of microbiota within the same host species across multiple periods of the annual cycle will reveal how variable the microbial abundance and diversity is over time and provide insight into specific factors driving this variation.

Exploring the impact of migratory bird movement on gut microbial dynamics provides an opportunity to enhance knowledge of the host-microbe relationship within the context of changing environments and a known, recurrent physiological stress. Previous research has indicated the environment and diet are more influential than host genetics in shaping avian gut microbiota, with host taxonomy playing a weakly significant role compared to abiotic factors (Hird *et al.* 2015, Grond *et al.* 2018, Capunitan *et al.* 2020, Song *et al.* 2020). Characterization of migratory bird

microbiota across the annual cycle will best support identification of drivers of variation in diversity and abundance.

Disease ecology

While the majority of host-associated microorganisms are mutualistic or commensalistic, some are parasitic and have detrimental effects on the host. Increased insight into host-associated microbiota will be achieved through studies of mutualistic, commesalistic, and pathogenic microbes within the same host systems. A primary focus of disease ecology is to understand the distribution and abundance of pathogens across scales. This includes identifying ecological causes of disease patterns, for instance – how host population density influences pathogen transmission rates. Insight into the ecology of bird populations is necessary for understanding of the epidemiology of bird-associated diseases.

Migratory birds can become long-distance vectors for numerous pathogenic microbes and are believed to be at least partially responsible for the broad geographic distributions of diseases that impact human health, including West Nile Virus, avian influenza and *Borrelia burgdorferi*, which can cause Lyme disease. Of particular interest are a group of pathogenic microbes in Order Haemosporidia, which include the causative agent of the disease malaria. Haemosporidians infect nearly all terrestrial vertebrate, with especially detrimental effects on humans (Asghar *et al.* 2015, Galen *et al.* 2018). Avian haemosporidians are globally distributed and are primarily classed within three genera: *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* (Valkiūnas 2004). Prevalence of avian haemosporidians is highly variable in wild populations across the world (Louiseau *et al.* 2012, Lutz *et al.* 2015, Fecchio *et al.* 2021). *Haemoproteus, Plasmodium,* and *Leucocytozoon* share several traits, including near worldwide distribution, transmission via

arthropod vector, and frequent host switching (Valkiunas 2004). While they share those similarities, they differ in biogeographic distribution patterns and are vectored by different arthropods (Valkiunas 2004, Clark *et al.* 2014, Fecchio *et al.* 2021). One notable difference is that although *Haemoproteus* and *Plasmodium* can be found to infect nearly all terrestrial vertebrates, *Leucocytozoon* is so far only known from birds (Outlaw and Ricklefs 2011). Identifying patterns in pathogen prevalence within their host communities can yield insights into the forces that shape and determine complex host-pathogen associations (Dunn *et al.* 2010, Louiseau *et al.* 2010, Salkeld *et al.* 2013). This is especially true of vector-transmitted pathogens where host and vector density can impact transmission rates across individuals.

Research of avian haemosporidians has repeatedly demonstrated that host genetics and ecologies as well as environmental characteristics impact the prevalence and diversity in birds throughout the world. However, most of these studies focus on single locations or time periods. Investigations into the temporal dynamics of avian haemosporidians are lacking, with few studies spanning more than a single season or year. The ecological dynamics of host-pathogen systems often change over time, typified in many cases by systemic temporal variation in pathogen prevalence (Altizer *et al.* 2006). Long term datasets are crucial to understanding the temporal dynamics of natural populations, allowing for a systemic approach in identifying variability of prevalence and diversity over time. Logistical challenges associated with collecting the necessary samples for temporal studies often inhibit generating datasets necessary to assess temporal dynamics with statistical confidence. Understanding the dynamics of avian haemosporidians in migratory birds may elucidate mechanisms of pathogens which impact multiple avian communities across geographically disparate areas and throughout the annual cycle.

Natural History Collections

Housed with natural history museums are massive biorepositories spanning time and geography. Historically, vertebrate specimens consisted primarily of prepared skins or skeletons, used in studies driven by morphological variations. With the advent of sequencing technologies, preservation and accession of genetic specimens became common. In 2013, the concept of the extended specimen was introduced at the American Ornithological Union's annual conference, which describes the numerous uses of a single specimen, enabled in large part by advancements in technology. Within the framework of the extended specimen, novel avenues of scientific inquiry opened (Webster 2017). Of relevance to this dissertation, was the increased use and preservation of museum specimens for research of host-associated microorganisms. This promoted research on host-associated microorganisms, including investigations into co-evolutionary dynamics of ectoparasites and their hosts, descriptions of new species of intestinal helminths, surveys of the prevalence and diversity of blood pathogens in communities of wild animals, and characterization of gut microbiota of vertebrates. Museum accessioned specimens provide a powerful resource for establishing the spatial and temporal distribution of global biodiversity, including that of microbial communities.

Conclusion

In this dissertation, I present results from several extensive surveys of microbial communities and blood pathogens of migratory birds, focusing on how diversity and prevalence varies across time and geography. First, I examined the extent to which changing habitats impacted the within-individual composition of the gut microbiota of a migratory habitat specialist. Second, I characterized the microbiome of four species of widespread migratory birds on spring and fall

migration as well as the start of the breeding season. Using observations replicated over three years, I found that microbial community diversity is significantly different across different periods of the annual cycle, and community composition is more similar within than across years. Third, I generated a dataset of avian malaria and malaria-like pathogens from migratory bird specimens collected during spring and fall migration over a 24-year time period. I used hierarchical statistical modeling to infer periodicity in pathogen prevalence and density-dependent disease modeling to explain plausible transmission mechanisms. The overarching results from this research demonstrate that significant and often recurrent shifts occur in host-associated microbiota arise throughout the annual cycle and over multi-year periods.

1 Repeated sampling of individuals reveals impact of tropical and temperate habitats on microbiota of a migratory bird[1]

1.1 ABSTRACT

Migratory animals experiencing substantial change in diet and habitat across the annual cycle may have corresponding shifts in host-associated microbial diversity. Using automated telemetry and radio tags to recapture birds, we examined gut microbiota structure in the same population and often same individual of Kirtland's Warblers (*Setophaga kirtlandii*) initially sampled on their wintering grounds in The Bahamas and subsequently resampled within their breeding territories in Michigan, USA. Initial sampling occurred in March and April and resampling occurred in May, June, and early July. The composition of the most abundant phyla and classes of the warblers' microbiota is similar to that of other migratory birds. However, we detected notable variation in abundance and diversity of numerous bacterial taxa, including a decrease in microbial richness and significant differences in microbial communities when comparing the microbiota of birds first captured in The Bahamas to that of birds recaptured in Michigan. This is observed at the individual and population level. Furthermore, we found that 22 bacterial genera exhibit heightened abundance within specific sampling periods and are likely associated with diet and environmental change. Finally, we described a small, species-specific shared microbial profile that spans multiple time periods and environments within the migratory cycle. Our research highlights that the avian gut microbiota is dynamic over time, most significantly impacted by changing environments associated with migration. These results support the need for full annual cycle monitoring of

[1] A version of this chapter has been published as: Skeen, H.R., N.W. Cooper, S.J. Hackett, J.M. Bates and P.P. Marra. 2021. Repeated sampling of individuals reveals impact of tropical and temperate habitats on microbiota of a migratory bird. Molecular Ecology 30(22): 5900-5916.

migratory bird microbiota to improve understanding of seasonal host movement ecologies and response to recurrent physiological stressors.

1.2 INTRODUCTION

A healthy gut microbiome is thought to be both resilient and flexible (Voolstra and Ziegler 2020, Bodawatta, Freiberga *et al.* 2021) and may be heavily affected by a variety of extrinsic and intrinsic factors, including host genetics, habitat, and diet (Hird *et al.* 2015, Rothschild *et al.* 2018). The composition of a healthy microbiome may change as animals undergo recurrent physiological stressors, such as migration or changing climates across seasons (Sommer *et al.* 2016, Carey and Assadi-Porter 2017, Risely *et al.* 2018). Increased understanding of both resilience and flexibility of gut microbial communities relating to recurrent physiological stressors can further elucidate host adaptation to repetitive stress. Here, we ask what changes and what remains consistent within the gut microbiota of a migratory bird species across multiple time points and locations within the annual cycle.

Species experiencing seasonal variation in habitat, diet, or physiological stressors often exhibit correlated alterations in their microbiota (Maurice *et al.* 2015, Sommer *et al.* 2016, Ren *et al.* 2017, Smits *et al.* 2017, Drovetski *et al.* 2018). Migratory animals may undergo seasonal fluctuations in metabolic needs that, in combination with changing habitats and diets, result in variable microbiota composition across their annual cycles, but the extent to which this occurs remains unclear (Jenni and Jenni-Eiermann 1998, Grond *et al.* 2018). Exploring the impact of movement of migratory birds on gut microbial dynamics provides an opportunity to increase understanding of the host-microbe relationships within the context of changing environments and a known, recurrent physiological stress.

Migratory birds are exposed to a variety of habitats and associated novel environmental microbial suites across the annual cycle, which may impact overall gut composition. Recently, several studies have illustrated the effect that environment and movement can have on structuring the host microbiota (Hird *et al.* 2014, Wu *et al.* 2018, Grond, Santo Domingo *et al.* 2019). For example, the microbial community differed between the wintering and breeding grounds in migratory geese (Wu *et al.* 2018), between spring and fall migrants in two passerines (Lewis *et al.* 2016), and between migratory and non-migratory barn swallows (*Hirundo rustica;* Turjeman *et al.* 2020). Additionally, Corl *et al.* (2020) showed that increased movement, with exposure to more varied environments, results in increased diversity of the gut microbiota in breeding barn owls (*Tyto alba*), and Lewis *et al.* (2017) found that host microbial communities of birds at a migration stopover site begin to converge with the local environmental microbial suite within 24 hours. Contradicting these results, a study of migratory red-necked stints (*Calidris ruficollis*) concluded that only 0.1% of gut microbiota are sourced directly from the environment, and that individual stints in different environments exhibited weak variation in microbial composition (Risely *et al.* 2017). Further comparison of recent migrants to individuals that had remained on the non-breeding grounds for a full year identified the bacterial genus *Corynebacterium* as significantly more abundant in migratory individuals than non-migratory individuals (Risely *et al.* 2017). Similarly, *Corynebacterium* and the genus *Mycoplasma* were identified as more abundant between a comparison of migratory and non-migratory sympatrically occurring subspecies of barn swallows (Turjeman *et al.* 2020).

In addition to the potential impact of novel habitats associated with long-distance movement, physiological adaptations of migratory birds, such as intestinal atrophication, could further affect gut microbiota, possibly by reducing the number of/volume of bacteria that the birds

harbor (Grond *et al.* 2018) or by allowing functionally relevant bacteria to proliferate during active migration (Risely *et al.* 2018). In addition to environmental factors, host characteristics, such as diet (Song *et al.* 2020), species (Capunitan *et al.* 2020), and age (Kreisinger *et al.* 2017) may play additional roles in structuring the microbiota.

Given the variability of gut microbiota, intrinsic variables such as host genetics, and strong environmental effects, it may be difficult to directly correlate variation in gut microbiota to ongoing biological processes, specific host traits, or environmental factors without temporal sampling across different time points of the annual cycle (Hird *et al.* 2014, Capunitan *et al.* 2020, Song *et al.* 2020). Here, we sampled individuals on their subtropical wintering grounds and recaptured them multiple times on their temperate breeding grounds to better understand local and temporal variability in gut microbiota by reducing sources of variability known to be associated with sampling different individuals and different populations (Flores *et al.* 2014, Hird *et al.* 2014, Baxter *et al.* 2015).

Until now, no migratory songbird has been sampled at multiple time points and locations across their annual cycles. Migratory birds have complicated annual cycles that involve twice-annual movements often spanning thousands of kilometers between stationary breeding and wintering periods. Once captured, researchers typically have no way to relocate or recapture the same individuals outside of the original capture site, especially for species with expansive wintering and breeding ranges and with populations that may number in the millions. This inhibits sampling from the same population, let alone the same individual, at multiple points in the annual cycle. As a result, one must attempt to measure and control for confounding factors, such as between population differences, and account for high inter-individual variability (Flores *et al.* 2014, Hird *et al.* 2014, Baxter *et al.* 2015). This inability to study the same individuals across the

annual cycle has impeded identification and understanding of variation within birds associated with seasonal movement.

The Kirtland's Warbler (*Setophaga kirtlandii)* provides an unusual opportunity for studying changes across the annual cycle in a migratory species. Their small population size as well as restricted breeding and wintering ranges (Cooper, Ewert, *et al.* 2019) make it feasible to relocate individuals across seasons (Cooper *et al.* 2018, Cooper and Marra 2020). Following substantial population declines, only 167 singing males were recorded in 1974 and again in 1987, based on breeding surveys (Kepler *et al.* 1996). Through extensive conservation management efforts, the population has increased to approximately 2,300 singing males of which 97% breed across a relatively small area in Michigan's Lower Peninsula. This species winters primarily in the scrub forests of The Bahamas (Cooper, Ewert, *et al.* 2019), more than 2,000 km south of the breeding grounds. For this study, we radio-tagged individuals on the wintering grounds and then relocated and recaptured the same birds on the breeding grounds in Michigan through the use of automated telemetry towers. We used 16S rRNA next generation sequencing technologies to catalogue the bacterial communities of individuals. Our goals were to: (1) characterize the bacterial diversity of Kirtland's Warblers at three distinct periods of the annual cycle at the population and individual level; (2) evaluate host sex, age, period of annual cycle, and location effect on abundance and diversity of gut microbiota; and (3) determine if a shared bacterial profile for Kirtland's Warblers exists and if so, establish a species-specific pattern.

1.3 MATERIALS AND METHODS

1.3.1 Initial sample collection in The Bahamas

We captured Kirtland's Warblers on Cat Island, The Bahamas, in March and April of 2017 and 2018 using vocalization playback and mist nets. We classified individuals into two age categories (SY = second calendar year or ASY = after second year), sexed individuals following Pyle (1997), and attached a USGS metal band and three plastic, colored bands. We then fitted a coded radio-tag (0.35g, Model = NTQBW-2, Lotek Wireless, Inc.) using a modified leg-loop harness (Rappole and Tipton 1991). Tags emitted coded pulses at regular intervals (29.3s), which allowed for individual identification through handheld or automated telemetry receivers (Taylor *et al.* 2017). After attaching the radio tags, we collected fecal samples by placing birds in a wax paper bag for up to ten minutes. We transferred fecal materials from the bag to Whatman FTA Cards (Whatman, Florham Park, NJ) using Whatman sterile swabs (Whatman, Florham Park, NJ). Following sample transfer, we stored FTA Cards in airtight containers at room temperature until transportation to and processing within the molecular laboratory. Whatman FTA Cards are stable long term at room temperature, therefore ideal for field work where traditional methods of flash freezing may be unavailable (da Cunha Santos 2018). The microbial composition recovered from fecal samples stored on FTA cards are comparable to that of flash freezing and storage at -20°C methods (Song *et al.* 2016).

1.3.2 Relocation and recapture in Michigan

We used 11 automated telemetry towers in Michigan erected as part of ongoing Kirtland's Warbler management and research (Cooper *et al.* 2018, Cooper and Marra 2020) to detect tagged individuals as they arrived at the majority of breeding sites. Birds arrived between May 9 and June 3. We downloaded tower data daily and used handheld telemetry to search the few areas not well covered by towers at least every three days. We used these data to determine arrival dates in

Michigan. Following initial detection, we used handheld telemetry to locate each individual's territory and target them for recapture. Birds were captured an average of 7.7 (SD ± 8.1). days after their first detection in Michigan. We also attempted to recapture individuals towards the end of the breeding season in early July. In May of 2018, we also captured and sampled non-tagged Kirtland's Warblers in Michigan to compare microbial variation in individuals known to be from Cat Island with birds that may have wintered on other islands. Regardless of timing, we used identical capture and sampling protocols as those used in The Bahamas (see above).

1.3.3 Molecular Methods

We isolated DNA from fecal samples stored on Whatman FTA Cards using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's extraction protocol. We included six blank negative controls to account for possible contamination during extraction and polymerase chain reaction (PCR). Following standardized procedures of the Earth Microbiome Project, we used PCR to amplify the V4 region of the 16S microbial small subunit ribosomal RNA (rRNA) gene using the EMP universal primers 515F/806R (Caporaso *et al.* 2012). We then used the Illumina MiSeq sequencing platform to obtain paired-end 150 base pair reads. We performed DNA extractions at the Field Museum of Natural History. All subsequent molecular work was conducted at the IGM Genomics Center of the University of California, San Diego.

1.3.4 Sequence Processing

We processed raw sequence data with the Quantitative Insights Into Microbial Ecology (QIIME2 version 2019.1) pipeline (Caporaso *et al.* 2010, Bolyen *et al.* 2019). In QIIME2, following standard

demultiplexing and quality filtering, we generated amplicon sequence variants (ASVs) using the Divisive Amplicon Denoising Algorithm (DADA2) (Callahan *et al.* 2016). The DADA2 toolkit statistically infers sample sequences and implements quality control elements including exclusion of singletons, chimera removal, and sequence error elimination. Using a quality score threshold of 33, we trimmed all sequences outside base pair positions 13 and 145. We based the threshold of quality score on visual assessment of the quality score plots and recommendations in Mohsen *et al.* (2019). We classified ASV taxonomies using the Silva reference database (Quast *et al.* 2012, version 132). After classification, we removed all ASVs identified as Archaea, chloroplasts and mitochondria. We aligned all sequences using MAFFT (Katoh and Standley 2013) and then inferred a phylogeny of all bacterial sequences with FastTree (Price *et al.* 2010). We identified bacterial contaminants with the R package *decontam* (Davis *et al.* 2018). We used six DNA extraction blanks processed in parallel with all other samples as negative controls with the default parameters for frequency-based contaminant determination. Quality control measures resulted in the removal of 10 libraries for poor DNA or PCR yield and 52 contaminant ASVs from the overall dataset. Of the 176 total fecal samples, 166 were analyzed in the final dataset.

1.3.5 Normalization of microbial data

Normalization of microbial data accounts for biases introduced by differing library size, technical variability and sampling bias. However, normalization can lead to data loss and may be detrimental to interpretation of results (McMurdie and Holmes 2014). To ensure that rarefaction does not bias results, we conducted all alpha and beta-diversity analyses on normalized and non-normalized data. For normalization, we rarefied all libraries to 7,000 reads. Results from the normalized data did not qualitatively differ from the non-normalized data. Therefore, we present and discuss the

results of rarefied data for diversity analyses. Identification of shared microbes across individuals and sampling periods was conducted on non-normalized data as rarefaction may be ill-suited for detection of ASVs with low abundance in individuals.

1.3.6 Statistical Analysis

We analyzed community alpha diversity using the natural log of observed ASV richness and the Shannon diversity index. For modeling diversity, we used a linear mixed model as implemented in the R package *lme4* (Bates *et al.* 2007) and evaluated the importance of different variables, taking into account the repeated sampling of some birds. We included host age (SY or ASY), sex (male or female), year (2017 or 2018) and sampling period (The Bahamas, first recapture in Michigan, and second recapture in Michigan) as fixed effects and individual host as a random effect. Using *lmerTest* (Kuznetsova *et al.* 2015), we generated an ANOVA table from the linear model analysis, and subsequently conducted *a posteriori* pairwise tests to compare the three sampling periods. Additionally, we conducted a pairwise t-test to assess differences between tagged and randomly caught birds within the first recapture period of 2018. We tested for the influence of outliers, which appeared to cause a deviation from normality in ASV richness (Shapiro-Wilks test), by repeating the analyses with outliers omitted and obtained very similar results. We also tested for the effect of individual-level random effects with a likelihood ratio test comparing the model with and without individual ID as the random effect term, and we found individuals did not consistently differ from each other. Finally, we used a generalized additive model (GAM) to test the impact of time on alpha diversity of recently arrived birds in Michigan following the end of spring migration with the R package *mgcv* (Wood 2012). We used GAMs on Shannon diversity index and the natural log of observed ASV richness of individuals that had been

present in Michigan for nine days or less. We determined the day of arrival in Michigan from tower data. We applied the GAM to fit a smoothed curve for days after arrival in Michigan, the predictor variable, to the estimate of variance explained in the alpha diversity metric, the response variable. We determined optimal smoothing parameters for our GAM by examining the minimized generalized cross-validation score of the GAM for all possible smoothing parameters (k = 3-10). The GAM estimated a smoothing function of k = 3, though all possible k values produced results that are effectively identical. Results presented here, including in Figure 1.3, are from k = 3 for both observed ASV richness and Shannon diversity index.

To examine community differences in the microbiome (beta diversity), we applied permutational multivariate analysis of variance (PERMANOVA) of Bray-Curtis dissimilarity and unweighted UniFrac distances, calculated among individual samples (Anderson 2014). For variables that showed significant differences in the PERMANOVA analyses, we conducted an *a posteriori* test to assess differences in dispersion or centroids using PERMDISP. We visualized beta diversity of significant variables using non-metric multidimensional scaling (nMDS) ordination of the Bray-Curtis measurements. Diversity calculations were implemented using the R packages *vegan* and *phyloseq* (Oksanen *et al.* 2007, McMurdie and Holmes 2013).

Finally, to ask which taxa differ in abundance across sampling periods, we implemented analysis of composition of microbes with bias correction (ANCOM-BC; Mandal *et al.* 2015, Lin and Peddada 2020). ANCOM-BC uses the underlying structure of the microbiota data to identify differentially abundant taxa between categories while controlling for false discovery rates. This method applies a library-specific offset term estimated from the observed abundance which is incorporated into a linear regression model, providing the bias correction. We used the R package

ANCOMBC to test for differences in abundance of bacterial genera with a significance of $p < 0.05$ with Bonferroni corrections (Lin and Peddada 2020).

1.3.7 Shared microbial profile

We identified a common, species-specific and temporally persistent microbial profile for Kirtland's Warblers following Risely (2020). We defined the species-specific shared microbial profile as ASVs present in over 50% of all individuals in each of the three sampling periods (Astudillo-García *et al.* 2017, Grond *et al.* 2017). We studied the shared microbial profile at multiple taxonomic levels using *Phylocore* (Ren and Wu 2016). We also identified a temporally persistent microbial profile in birds sampled in triplicate, defined as ASVs found at all three sampling periods within the same bird (Shade *et al.* 2012). We calculated the proportion of temporally persistent ASVs to those that are transient and not found at all three sampling periods to identify the average proportion of ASVs that are retained over time.

1.4 RESULTS

We collected a total of 166 fecal samples from 116 Kirtland's Warblers at locations throughout Cat Island, The Bahamas (n = 92), where we collected 92 samples, and Michigan's Lower Peninsula (first recapture n = 43, second recapture n =18). Thirty-four birds were sampled twice, once during initial capture in The Bahamas and a second time during first recapture in Michigan. Of those birds, nine individuals were sampled a third time during the second recapture period in

Table 1.1 Individuals sampled per time period, including age (SY = second calendar year, ASY = after second calendar year) and sex (M = male, F = female) breakdown. Numbers reflect libraries included in analyses and do not include those removed for poor sequencing or PCR yield.

		Samples	Age			Sex		
Sampling Period	**Date**	**Collected**	**SY**	**ASY**	**Unk.**	**M**	**F**	**Unk.**
Initial Capture (CIB)	March 29 – April 16, 2017	41	18	22	1	38	3	0
	March 23 – April 24, 2018	51	37	14	0	36	15	0
First Recapture (MI1)	May 20 – June 6, 2017	19	10	9	0	19	0	0
	May 13 – June 26, 2018	24	18	6	0	23	1	0
Non-tagged Birds (MI1)	May 13 - 20, 2018	13	6	6	1	12	0	1
Second Recapture (MI2)	July 2 - 10, 2017	8	4	4	0	8	0	0
	July 1 - 11, 2018	10	7	3	0	8	2	0
	Total	**166**	**100**	**64**	**2**	**144**	**21**	**1**

Michigan (Table 4.1). Additionally, 13 non-tagged Kirtland's Warblers were sampled in May 2018 in Michigan. Our final dataset is composed of 166 sequenced libraries (Table 1.1) which totaled 5,007,844 reads, with an average 30,168 reads per library (range: 7,022 – 100,856). We detected 7,426 unique ASVs across all sampled with a mean of 107.3 ± 96.7 (standard deviation [SD]) per library.

1.4.1 Bacterial community composition and diversity

Across all samples, bacteria from 37 phyla were detected. Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria composed 91.13% of the total reads; 5.8% of the reads belonged to the 33 remaining phyla and 3.07% of reads did not align to any known bacterial phyla (Figure 1.1A). *Clostridia* (Phylum Firmicutes), *Gammaproteobacteria* (Phylum Proteobacteria), and *Bacteroidia* (Phylum Bacteroidetes) were the most abundant classes, representing 70.16% of all

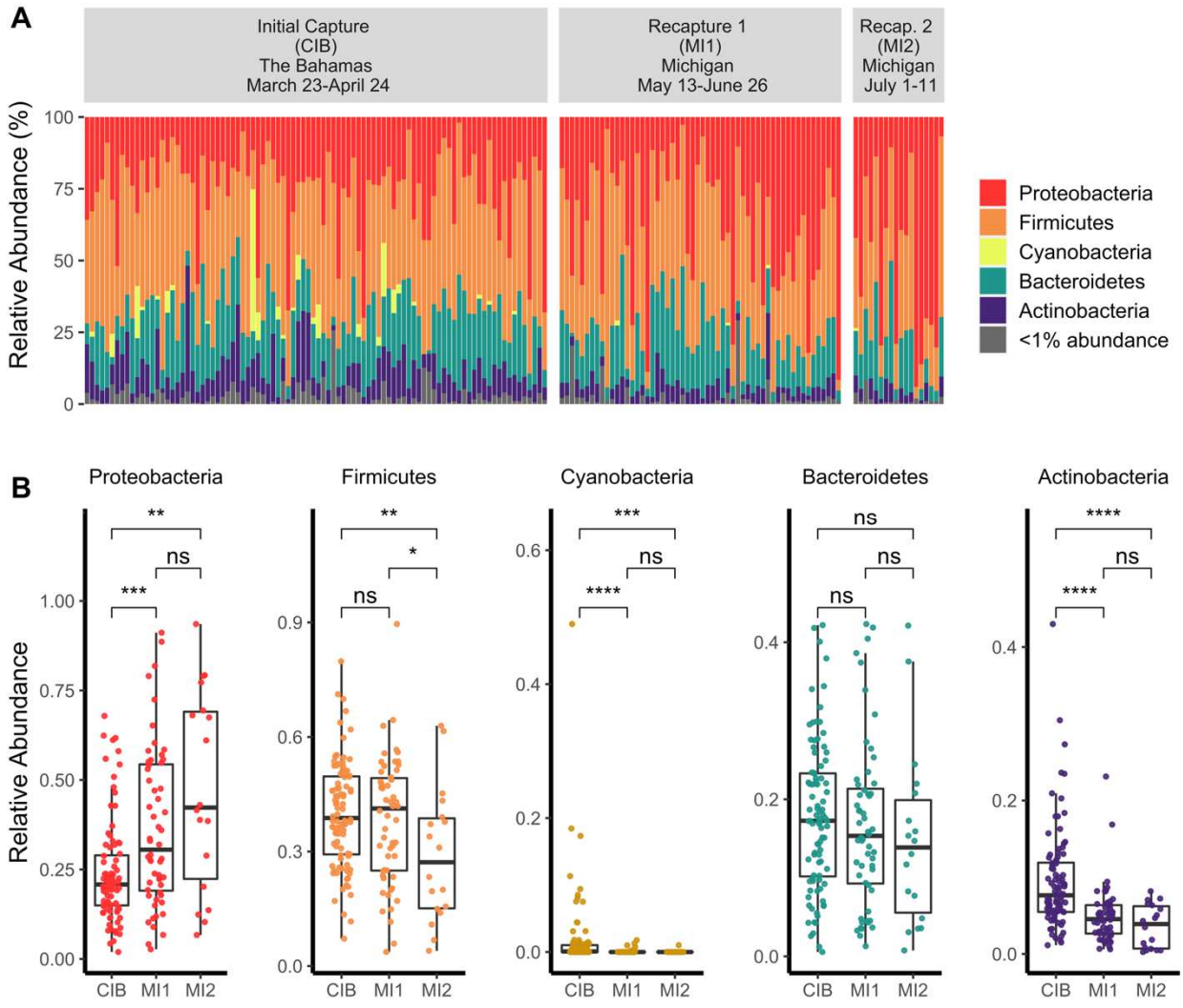


Figure 1.1 Relative abundance of bacterial phyla. (**1.1A**) Stacked barplots showing the relative abundance of each phylum with each column representing one individual sample, ordered by day of capture and separated by sampling period. Phyla with total abundance less than 1% and unclassified phyla are represented by gray. (**1.1B**) Relative abundance boxplots of the five most common phyla per individual by sampling period representing the change in relative abundance from Cat Island, The Bahamas (CIB) to the first Michigan recapture period (MI1) and the second Michigan recapture period (MI2). Individual points represent the relative abundance of each phyla per individual per sampling period. Significance levels are pairwise comparisons between sampling periods are shown (ns: $p>0.05$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$).

Table 1.2 Results of the linear mixed model analyses of alpha diversity values for observed ASV richness (1.2A) and Shannon Diversity Index (1.2B). Model factors include sex (male or female), age (second year or after second year), year (2017 or 2018), and sampling period (initial capture in The Bahamas, first recapture in Michigan, or second recapture in Michigan). Asterisks denote statistically significant results of model, $p < 0.05$.

Table 1.2A Alpha diversity comparisons of Observed ASV Richness

Model factors	Sum Sq	Mean Sq	F value	Pr(>F)
Sex	0.001	0.001	0.027	0.871
Age	<0.001	<0.001	0.001	0.970
Year	0.080	0.080	2.003	0.160
Sampling Period	1.178	0.589	14.764	<0.001*

Table 1.2B Alpha diversity comparisons of Shannon Diversity Index

Model factors	Sum Sq	Mean Sq	F value	Pr(>F)
Sex	<0.001	<0.001	0.000	0.987
Age	0.140	0.140	0.382	0.538
Year	0.557	0.557	1.524	0.220
Sampling Period	21.348	10.674	29.221	<0.001*

reads. The mean abundance of most phyla and classes differed between initial sampling in The Bahamas and subsequent samplings in Michigan (Figure 1.1B, Table 4.2). The birds shifted from a Firmicutes dominated microbiota in The Bahamas (mean abundance per individual 39.82% [SD, ±13.97%]) and Michigan following arrival (38.12% [SD, ±16.41%]) to Proteobacteria as the most abundant phylum in the second Michigan recapture period (47.07% [SD, ±27.90%]). Bacteroidetes and Actinobacteria were also proportionally more

abundant in The Bahamas than in the second Michigan recapture period. Notably, Cyanobacteria represented 1.91% (SD, ±5.93%) of the total microbiota in The Bahamas, but decreased to 0.05% (SD, ±0.23%) by the second recapture period in Michigan.

Alpha diversity was not significantly affected by year, host age or host sex (Table 1.2). However, the three sampling periods significantly differed (Type III ANOVA with Satterthwaite's

method; observed richness: $F_{2,116.34} = 14.76$, $P < 0.0001$; Shannon diversity: $F_{2,126.91} = 29.22$, $P < 0.0001$). All Bonferroni corrected pairwise comparisons on the fitted values from the linear model were significantly different from each other (observed: Bahamas vs. each recapture period both $P < 0.0001$, first vs. second recapture period, $P = 0.002$; Shannon diversity: all comparisons: $P < 0.0001$). Birds in The Bahamas showed higher bacterial diversity compared to either recapture period in Michigan, demonstrated through a comparison of all samples (Figure 1.2A) as well as with paired sampling of the same individuals (Figure 2B). In the birds sampled in triplicate, alpha diversity varied between first and second Michigan recaptures (Figure 1.2C). A comparison of tagged and randomly captured birds in the first Michigan sampling period of 2018 revealed no significant differences in alpha diversity (pairwise t-test; observed: p = 0.13, Shannon diversity: p = 0.22). Generalized additive models showed that 38.4% of the deviance in observed diversity (*P*-value = 0.004) and 10.8% of the deviance in Shannon diversity measures (*P*-value =0.354) could be explained by amount of time spent in Michigan following arrival after spring migration. In the 27 individuals that were found within nine days of arrival to the breeding grounds, we observed a decrease in alpha diversity through the first four to five days followed by an increase in alpha diversity through the ninth day (Figure 1.3), with each bird represented once in the analysis.

Our results indicate that beta diversity was not significantly affected by age or sex of the birds within the full dataset or individual sampling periods (Table 1.3), with the exception of age in the second Michigan resampling period (unweighted UniFrac: PERMANOVA p = 0.0128, PERMDISP p = 0.2213). Community composition of the microbiota significantly differed by year in the full dataset and at each sampling period (Table 1.3, Figure 1.4A, Figure 1.5). Additionally, our PERMANOVA results suggest that microbiota composition differed significantly between sampling periods (Bray-Curtis: p = 0.0002, R^2=0.025; unweighted UniFrac:

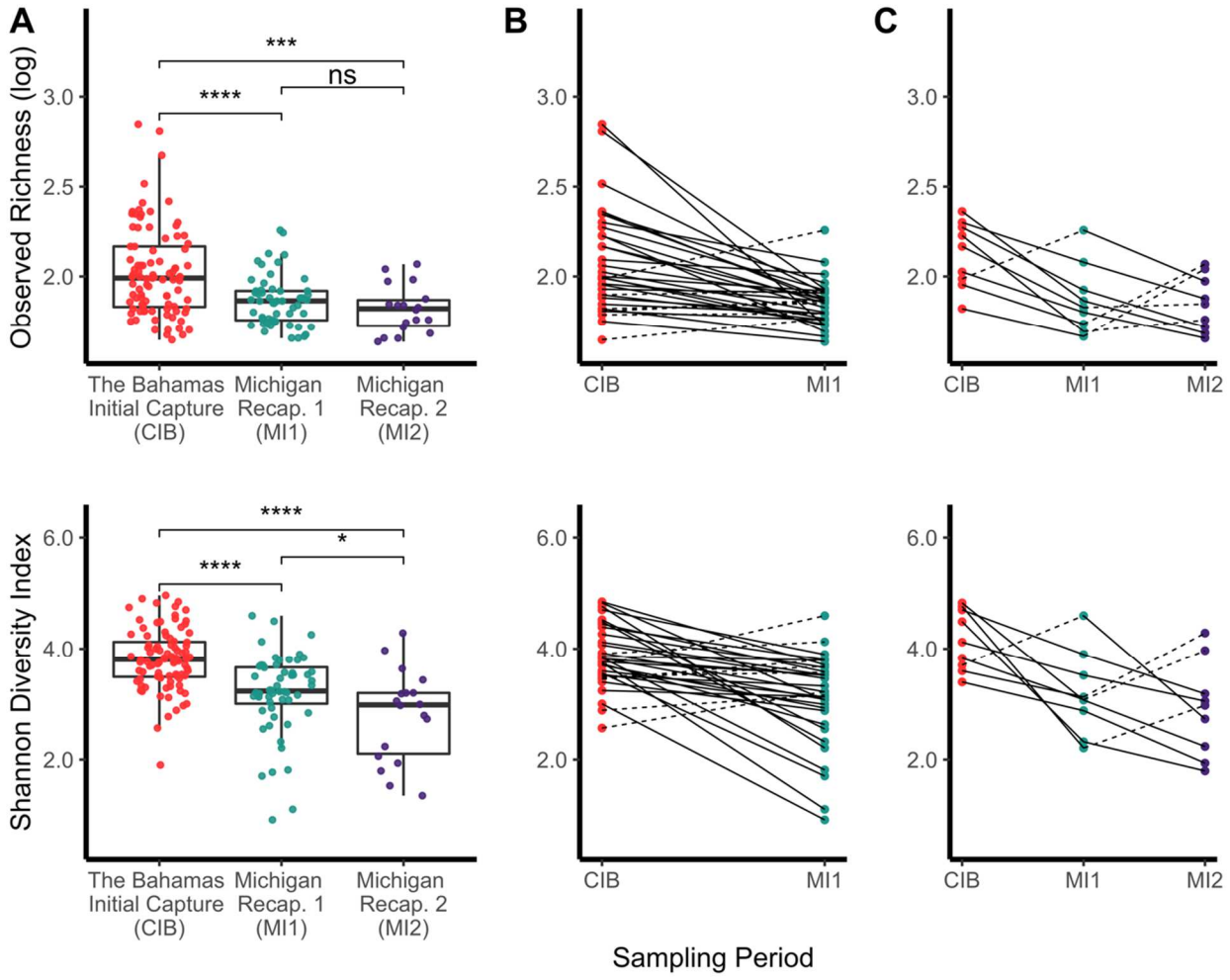


Figure 1.2 Alpha diversity measurements of amplicon sequence variants (ASVs) including observed ASV richness (log transformed, top row) and Shannon diversity index (bottom row) Boxplots of alpha diversity at each sampling period (Column A). Individual points represent the alpha diversity measure of the individual at that period. Significance levels are pairwise comparisons between sampling periods are shown (ns: $p>0.05$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$). Alpha diversity change over time in the individuals sampled two (Column B) or three times (Column C). Each line connects the measurements of the same individual between the respective sampling periods. Continuous lines represent a negative change in alpha diversity and dotted lines represent a positive change.

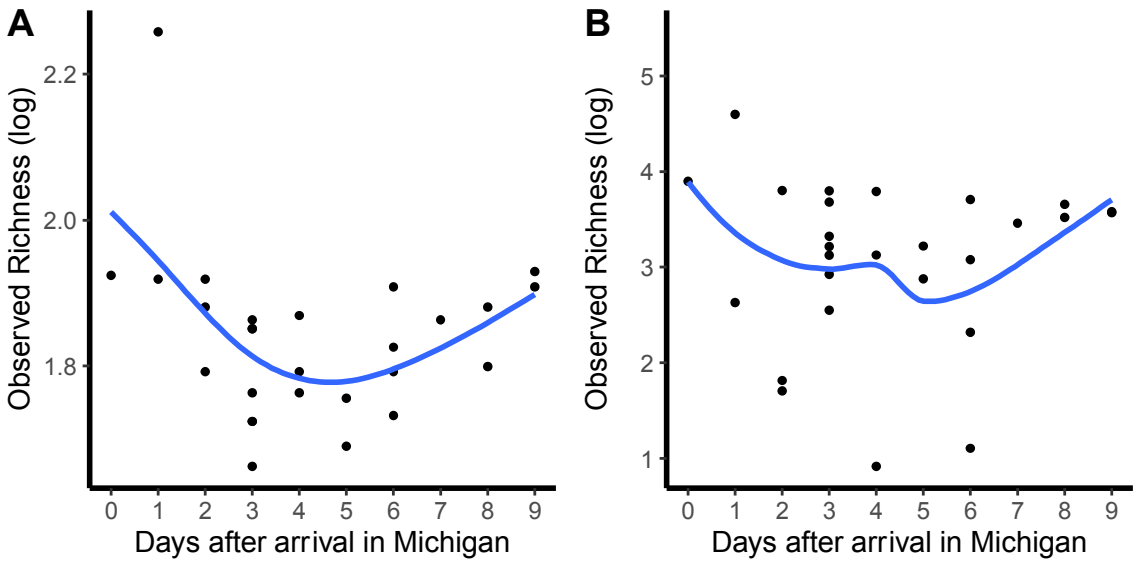


Figure 1.3 Generalized additive model smoothed time series comparing diversity measures (observed richness, top panel; Shannon diversity Index, bottom panel) against day(s) after arriving in Michigan. Each dot represents the diversity measure of an individual bird. The blue line represents the moving average change in diversity over time with the gray area corresponding to a 95% confidence interval.

p = 0.0001, R^2=0.024), though the significant unweighted UniFrac result can be explained through variation in spread of the sample composition, rather than with significantly different centroids such as with the Bray-Curtis dissimilarity matrix (PERMDISP; Bray-Curtis p = 0.7104, unweighted UniFrac p = 3.71e-6). This indicates that although the abundances of microbiota are significantly different during sampling periods, the taxonomic variation of bacterial lineages present are not. The effect of sampling period on the gut microbiota explained 2.5% and 2.4% of the variation in microbiota composition for Bray-Curtis and unweighted UniFrac respectively. Taken together, all variables tested (Sampling period, Year, Sex, Age) explained less than 5% of the total variation in the microbiota (Bray-Curtis: 4.91%, unweighted UniFrac: 4.6%). No consistent changes were observed in the beta diversity of the birds sampled in triplicate (Figure 1.4B).

Table 1.3 Results of permutational multivariate analysis of variance (PERMANOVA) tests indicating if ASV beta diversity measures are significantly different for the tested variable based on Bray-Curtis dissimilarity (1.3A) and unweighted UniFrac distance metrics (1.3B). Results reported for full dataset and within sampling periods for variables year, sex, and age. Asterisks denote statistically significant results of PERMANOVA with Bonferroni correction, $p < 0.05$. PERMDISP analysis results reported when PERMANOVA results significant. All tests conducted with 999 permutations.

Table 1.3A PERMANOVA Results with Bray-Curtis dissimilarity

	PERMANOVA			**PERMDISP**	
Variable	*Pseudo-F*	R^2	*Pr(>F)*	*f-value*	*P-value*
Sampling Period	2.058	0.025	<0.001*	0.343	0.710
Year (full dataset)	1.900	0.011	0.002*	1.659	0.200
Year (CIB Only)	1.485	0.016	0.019*	0.936	0.336
Year (MI1 Only)	2.474	0.044	<0.001*	0.304	0.583
Year (MI2 Only)	2.223	0.172	0.003*	0.000	0.984
Sex (full dataset)	1.203	0.007	0.137		
Sex (CIB Only)	1.035	0.011	0.345		
Sex (MI1 Only)	1.169	0.216	0.482		
Sex (MI2 Only)	1.425	0.082	0.082		
Age (full dataset)	0.929	0.006	0.595		
Age (CIB Only)	0.927	0.010	0.681		
Age (MI1 Only)	0.926	0.017	0.586		
Age (MI2 Only)	1.020	0.060	0.343		

Table 1.3B PERMANOVA Results with unweighted UniFrac distances metrics

	PERMANOVA			**PERMDISP**	
Variable	*Pseudo-F*	R^2	*Pr(>F)*	*F*	*P-value*
Sampling Period	2.001	0.024	<0.001*	13.514	<0.001
Year (full dataset)	1.314	0.008	0.121		
Year (CIB Only)	2.027	0.022	0.003*	0.180	0.673
Year (MI1 Only)	1.295	0.025	0.003*	0.7258	0.398
Year (MI2 Only)	1.541	0.088	0.007*	0.001	0.974
Sex (full dataset)	1.159	0.007	0.074		
Sex (CIB Only)	0.939	0.010	0.737		
Sex (MI1 Only)	0.862	0.016	0.695		
Sex (MI2 Only)	0.862	0.016	0.695		
Age (full dataset)	1.131	0.007	0.099		
Age (CIB Only)	0.887	0.010	0.925		
Age (MI1 Only)	1.279	0.023	0.013*	1.532	0.221
Age (MI2 Only)	0.942	0.056	0.615		

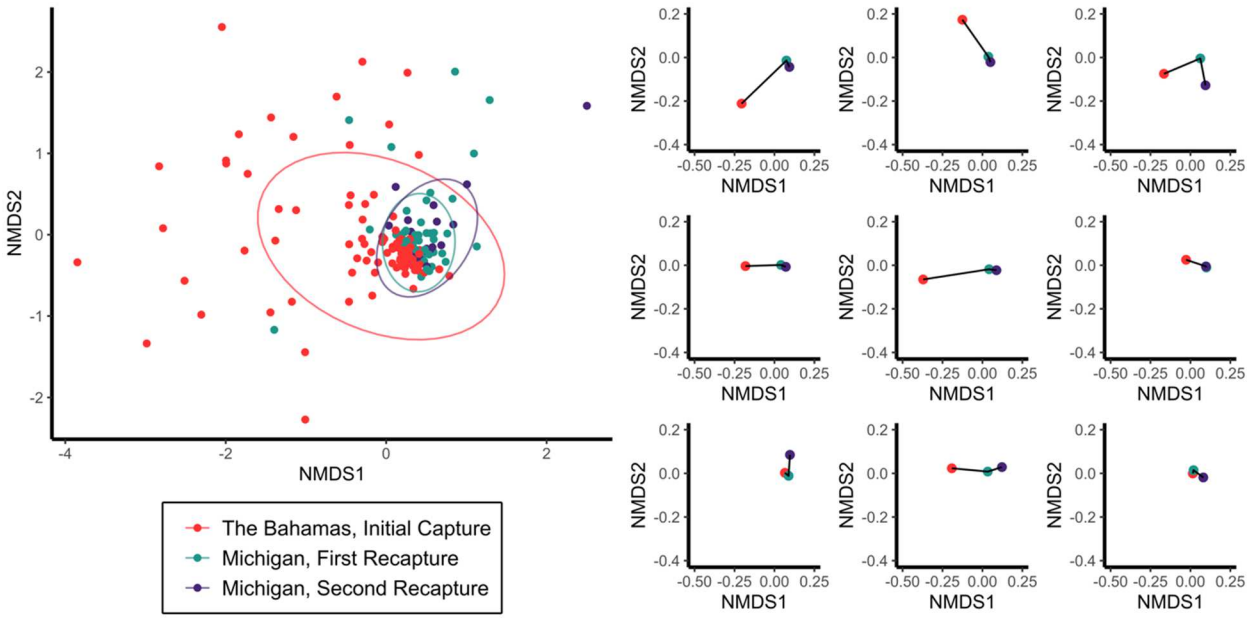


Figure 1.4 Non-metric multidimensional scaling (nMDS) ordination of Kirtland's Warbler gut microbiome community by sampling period, compared using Bray-Curtis dissimilarity (stress = 0.124, left panel). Ellipses show 95% confidence intervals around the centroid of each sampling period. Ordination of individual birds sampled in triplicate placed within the nMDS space of all samples, highlighting intra-individual change over time (right panel).

Across the three sampling periods, 22 bacterial genera were identified by ANCOM-BC as differentially abundant, with the majority being significantly more abundant in The Bahamas (Figure 1.6, Table 4.3). Ten genera in Phylum Actinobacteria were elevated in The Bahamas with one genus, *Streptomyces*, at higher abundance in the second recapture period in Michigan. Five genera of Phylum Firmictures were significantly more abundant in individuals from The Bahamas. *Bryocella* (Phylum Acidobacteria) was found at higher frequency in the first Michigan sampling period. Phylum Proteobacteria had genera differentially and significantly abundant at all three sampling periods, with *Aureimonas, Lysobacter* and an uncultured genus of *Beijernickiaceae* at elevated abundances in The Bahamas, *Candidatus* Hamiltonella in the first Michigan sampling period, and *Serratia* at the second Michigan sampling period.

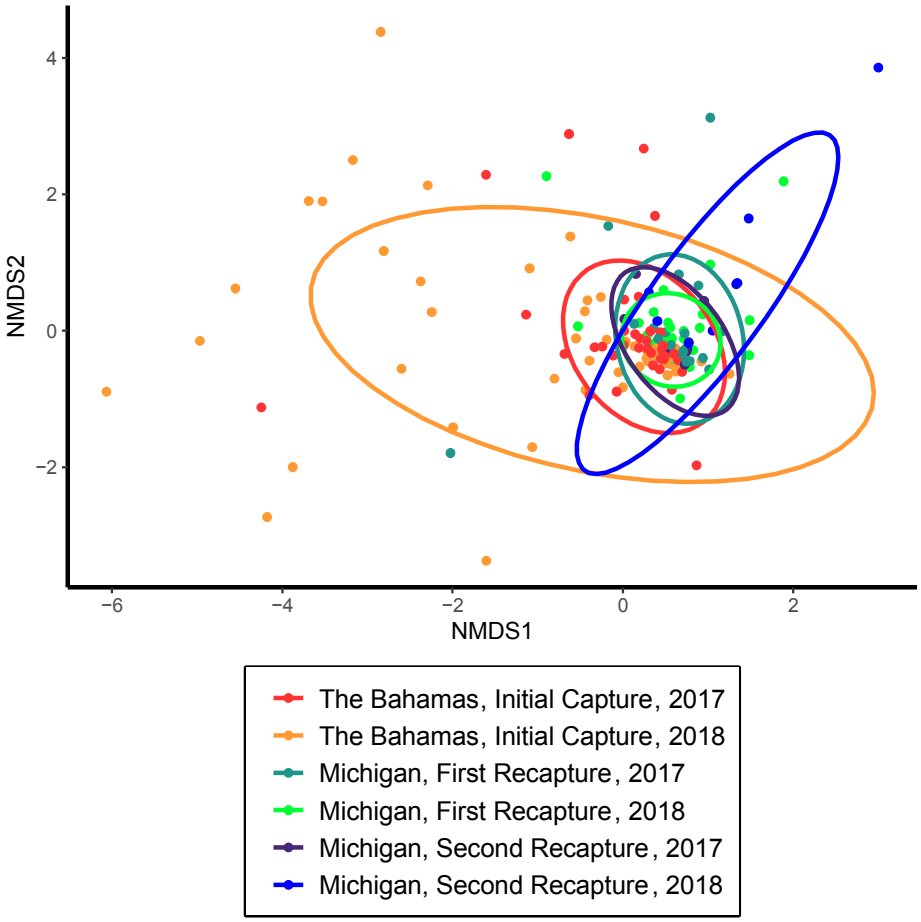


Figure 1.5 Non-metric multidimensional scaling (nMDS) ordination of Kirtland's Warbler gut microbiome community by sampling period and year, compared using Bray-Curtis dissimilarity. Ellipses show 95% confidence intervals around the centroid of each sampling year.

1.4.2 Shared microbial profile

We identified 28 ASVs as representing the species-specific shared microbiota of Kirtland's Warblers (Table 4.4). Two ASVs were from genera *Bifidobacterium* and *Collinsella* of Phylum Actinobacteria. The genus with the most shared ASVs was *Bacteroides* (Phylum Bacteroidetes) with eight. Fourteen ASVs are members of Firmicutes and are from genera *Blautia* (1 ASV), *Eubacterium eligens* (1 ASV), *Eubacterium hallii* (2 ASVs), *Fusicatenibacter* (1 ASV), *Roseburia* (2 ASVs), *Faecalibacterium* (3 ASVs), *Subdoligranulum* (2 ASVs), and two ASVs unclassified at the generic level. Finally, four ASVS from Phylum Proteobacteria are shared with

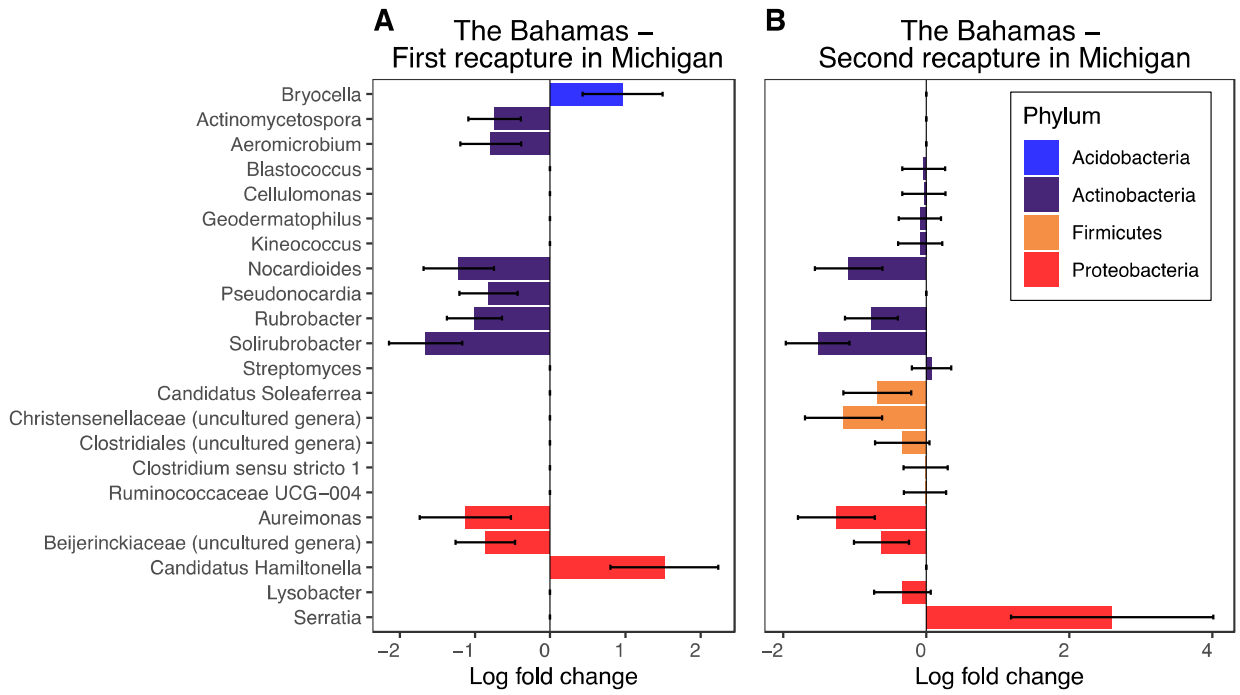


Figure 1.6 Analysis of composition of microbiomes with bias-correction (ANCOM-BC) identified bacterial genera that were differentially abundance at sampling periods. Bars correspond to the effect size (log fold change) of relative abundance of each genera, with negative values associated with an increase in abundance in The Bahamas (both panels) and positive values associated with an increase in abundance in the first recapture period (A) or second recapture period (B) in Michigan. Black bars represent the 95% confidence intervals. Adjusted p-values and confidence bounds can be found in Table S5.

one ASV from each genera *Ralstonia, Sutterella, Escherichia-Shigella* and *Alkanindiges*. We also identified the temporally persistent ASVs in the birds sampled at all three sampling points. Individuals retained 18-26 ASVs, present at each sampling period, which represented an average of 25.06% (range: 8.58%-50.00%) of ASVs detected per individual per time point.

1.5 DISCUSSION

Significant variation in both the diversity and community composition of Kirtland's Warblers microbiota was observed in individuals and the population as birds migrate from their wintering grounds in The Bahamas to breeding territories in Michigan. Repeated sampling at multiple points

across the annual cycle was only possible because we were able to capture, sample, and radio-tag individuals on the wintering grounds and then use automated telemetry to relocate the same individuals thousands of kilometers away on the breeding grounds (Cooper and Marra 2020). Through the resampling of individuals we removed potential biases associated with sampling multiple populations. Therefore, the effects observed can be attributed to true changes within individuals and our study population. We found that the overall diversity of the microbiota differed significantly between sampling periods and warblers on their wintering grounds had a significantly different and more diverse community of gut microbiota than those on their breeding grounds. We also documented a common, shared microbial profile of Kirtland's Warbler that persisted throughout multiple portions of the annual cycle.

1.5.1 Community composition

The overarching composition of Kirtland's Warbler microbiota is consistent with that of most wild bird surveys to date, with members of Phyla Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria comprising the majority of all bacteria detected (Dewar *et al.* 2014, Lewis *et al.* 2016, Grond *et al.* 2018). However, the relative abundances of all phyla changed, sometimes dramatically, as the birds migrated from The Bahamas to Michigan and over time in Michigan. Shifts in major bacterial taxa have been observed previously in migratory birds throughout different points of the annual cycle (Kreisinger *et al.* 2015, Lewis *et al.* 2016), though this is the first study to resample the same individuals at different points in the annual cycle of a migratory passerine. Population and individual level variation across the annual cycle may reflect difference in presence or abundance of environmental bacteria (Wu *et al.* 2018) and/or responses to altered diets (Góngora *et al.* 2021) that in turn favor some bacteria over others or vary with host

characteristics and requirements (Kers *et al.* 2018). Below, we consider plausible examples of each.

1.5.2 Environmental effect

The avian gut microbiota frequently reflects the local environment (Hird *et al.* 2014*,* Hird *et al.* 2018, Gillingham *et al.* 2019*,* Grond, Santo Domingo *et al.* 2019, Cao *et al.* 2020, but see Risely *et al.* 2017), even in cases when migratory birds maintain narrow dietary niches throughout the annual cycle (Wu *et al.* 2018). We observed evidence of environmental sourcing of microorganisms within the gut microbiota of Kirtland's Warblers. Cyanobacteria*,* found in marine and brackish waters (Sivonen 1996), was common in birds in The Bahamas but nearly absent from most individuals in Michigan. Cyanobacteria has previously been found in the gut microbiota of island birds (García-Amado *et al.* 2018) and is known to be acquired through food (Birrenkott *et al.* 2004). Kirtland's Warblers likely acquire environmentally derived Cyanobacteria in The Bahamas via food consumption, as most birds were captured within 2km of the ocean and much of the groundwater on the island is brackish. The most common class of Cyanobacteria detected in Kirtland's Warblers, *Oxyphotobacteria*, is an oxygenic phototroph (Shih *et al.* 2017). *Oxyphotobacteria* has previously been described in an avian host but is unlikely to provide a host associated function, suggesting the presence of this class is transient and the result of environmental sourcing (Zhu *et al.* 2020). In addition to *Oxyphotobacteria*, we detected several common environmental, soil-associated bacterial genera, including *Acitomycetospora*, *Aureimonas*, *Solirubrobacter* and *Nocardioides*, as more abundant in birds in The Bahamas (Topp *et al.* 2000, Janssen 2006). The presence and abundance of various groups of bacteria associated

with The Bahamas when compared to Michigan indicate a strong environmental effect on the gut composition of Kirtland's Warblers.

Variation in microbial community composition of birds is associated with a variety of intrinsic and extrinsic factors, including diet, host genetics, and the environment. Across all samples, we found that sampling period, including movement from The Bahamas to Michigan, accounted for 2.5% (Bray-Curtis) and 2.4% (unweighted UniFrac) of the variation observed. This proportion of dissimilarity between locations is smaller than reported in previous studies (Risely *et al.* 2017, Grond, Santo Domingo *et al.* 2019) yet is the most significant explanatory factor for observed differences in community composition. Our results contrast with a study which compared the microbiota of co-occurring migratory and resident red-necked stints which identified only slight compositional variation between distinct environments (Risely *et al.* 2017). This indicates that the response of the avian gut community is not consistently or uniformly impacted by the local environmental suite of microbes.

During migration birds are exposed to varying environments at stopover sites where they could acquire novel microbes (Lewis *et al.* 2017), possibly resulting in temporarily inflated diversity. However, it is unknown if microbial diversity increases or decreases during active migration or how the microbiota changes following arrival at breeding grounds. Possible adaptations to long distance flight, such as relatively shorter intestinal length and atrophication of intestines during active migration, might result in decreased microbial diversity (McWilliams and Karasov 2005, Caviedes-Vidal *et al.* 2007). Using the ability to determine what day individuals arrive in Michigan following migration, we observed variation in microbial diversity over the first nine days following the end of migration, including a slight decrease over the first three days before slowly increasing through day nine. During the first few days at their breeding grounds birds may

shed transient microbes acquired at stopover sites. This suggests that during spring migration microbial diversity increases due to exposure at stopover sites rather than decreases as an adaptation to long-distance flight. However, sample size per day is small and additional research with larger sample sizes are needed to further assess these results.

Gut microbiota are dynamic, displaying influence of novel microbial pools within 24-48 hours of exposure (Lewis *et al.* 2017, Grond *et al.* 2019, Capunitan *et al.* 2020). Two of our findings further support rapid acclimation to local microbiota. First, we observed no significant variation in the gut microbial diversity in birds sampled during the first recapture period in Michigan when comparing the microbiota of birds known to be from Cat Island and the 12 non-tagged birds that may have wintered on other islands. This implies rapid turnover of microbiota sourced from the local Michigan habitat. Second, we observed significant variation in beta diversity between 2017 and 2018 in the full dataset, as well as within each sampling period (Table 1.2, but for example: PERMANOVA of first Michigan recapture period: Bray Curtis $p < 0.001$, $R^2 = 0.044$; unweighted UniFrac $p = 0.003$, $R^2 = 0.025$). Similar results have been observed in greater flamingos (*Phoenicopterus roseus)* during the breeding season, with significant microbial variability within the same site at different years (Gillingham *et al.* 2019). Environmental microbes often exhibit high turnover over time (Faust *et al.* 2015). As such, our observations further support significant influence of local environment on the gut microbiota. This highlights the continued need for long term monitoring of microbiota as community-wide differences between years are demonstrable within the same geographic regions.

Though some individuals underwent substantial fluctuations in the gut microbiota structure and diversity over time, the community dissimilarity of the Kirtland's Warblers weakly varied between the first and second recapture periods in Michigan. Large individual fluctuations with

community-wide relative stability have been observed in other host species (Ren *et al.* 2016, Risely *et al.* 2017, Hicks *et al.* 2018). These fluctuations observed within a single location may result from changes in host diet, individuals becoming infected with a pathogen or changing physiological demands across seasons.

1.5.3 Host diet

Dietary shifts throughout the annual cycle often correspond to changes in gut microbiota (Ren *et al.* 2016, Smits *et al.* 2017, Drovetski *et al.* 2019). Kirtland's Warblers shift from a fruit-rich diet in The Bahamas to a diet composed primarily of insects in Michigan (Deloria-Sheffield *et al.* 2001, Wunderle *et al.* 2010, Wunderle *et al.* 2014). Firmicutes and Actinobacteria, which are often associated with frugivorous diets and known to aid in digestion through cellulose and carbohydrate degradation, were more abundant in The Bahamas where the warblers are consuming more fruit (Anand *et al.* 2012, Segawa *et al.* 2019). Class *Melainobacteria* (Phylum Cyanobacteria) is prevalent in the guts of herbivorous mammals where it aids in the digestion of plant materials (Di Rienzi *et al.* 2013). *Melainobacteria* was found in small abundances in some warblers where it may provide a similar role in digesting fruits.

Proteobacteria, often abundant in insectivorous birds and bats (Ben-Yosef *et al.* 2017, Edenborough *et al.* 2020) more than doubled in relative abundance from The Bahamas to the second Michigan recapture period. Specific lineages within this phylum support association with an insectivorous diet. Genus *Serratia* was found to be significantly more abundant in the second recapture period in Michigan. *Serratia* are known to produce chitinase which facilitates the degradation of insects' exoskeletons and is found to be abundant in insectivores such as barn swallows (Kreisinger *et al.* 2017). Similarly, an increased abundance of *Candidatus* Hamiltonella

within the first recapture period in Michigan may be a result of insect ingestion. *Hamiltonella* is a symbiotic bacteria of insects, including aphids, which comprise a portion of Kirtland's diet (Deloria-Sheffield *et al.* 2001, Dykstra *et al.* 2014). A recent comparison of diet, fecal and intestinal microbiota of bats identified an excess of bacteria associated with food materials in the fecal microbiota compared to the gut microbiota, indicating that fluctuations in the fecal microbiota are not necessarily indicative of compositional changes of colonizing bacteria that are functionally relevant to the host (Ingala *et al.* 2018). The shift in abundance of Proteobacteria, including genera *Serratia* and *Candidatus* Hamiltonella, is consistent with the insect-rich diet in Michigan, though further examination is needed to identify which bacteria are colonizing the gut in response to a changing diet and which are transient bacteria acquired from food materials.

1.5.4 Host-associated processes

Bacterial taxa presence and abundance may fluctuate in response to host requirements. Phylum Firmicutes has been linked to weight gain, increased nutrient uptake, and metabolic efficiency in birds (Angelakis and Raoult 2010, Teyssier *et al.* 2018). Firmicutes, specifically Class *Bacilli* and *Clostridia*, are abundant in migratory birds and may assist with the metabolism of carbohydrates, sugars, and fatty acids, facilitating migration and other energetically demanding activities (Grond *et al.* 2017, Cao *et al.* 2020). *Clostridia* and *Bacilli* were the most abundant classes of Firmicutes in Kirtland's Warblers. The abundance of these classes were lower in the second recapture period in Michigan than in the first recapture period or The Bahamas. Initial capture in The Bahamas occurred within the two months prior to the start of spring migration. Around this time birds begin to accumulate fat deposits to sustain them throughout long-distance migration (Fox and Walsh 2012). At the first recapture in Michigan, individuals are actively seeking and defending breeding

territories. Both activities are energetically expensive and associated with increased metabolism, potentially associated with higher abundance of Firmicutes in gut microbiota. It is also possible that the bacteria in early Michigan are residual from The Bahamas and stopover sites (Lewis *et al.* 2017). Additional sampling is needed to better identify bacterial lineages associated with specific metabolic demands of birds throughout the annual cycle.

Identifying the purpose or response of specific microbes in relation to host behaviors is essential to increasing knowledge of host-microbe interactions. The genera *Corynebacterium* and *Mycoplasma* have been found to be significantly more abundant in both migratory shorebirds and barn swallows when compared to sympatrically occurring, conspecific non-migratory populations (Risely *et al.* 2017, Turjeman *et al.* 2020). Both genera contain pathogenic bacteria that may increase in abundance during the physiological stress of migration. However, Risely et al. (2017) suggested that the abundance of Corynebacterium observed in recent migrants may be due to a possible inflammatory immune response rather than pathogen invasion. In our study, neither *Corynebacterium* nor *Mycoplasma* were significantly associated with any sampling period or host characteristic. These genera were each found in low abundances in less than 50% of birds. Within the recent arrivals to Michigan, we identified two genera of significantly higher abundance – *Bryocella*, an aerobic chemo-organotroph, and *Candidatus* Hamiltonella. Neither genera are presumed to have an increased abundance due to the physiological stress of migration, rather they are likely to have been acquired through the ingestion of food materials. We found no specific bacterial taxon to be associated with recently migrating individuals that might play a role in or be a response to migration.

Sex specific conditions, such as hormones, behaviors, and reproductive physiology may influence or be influenced by the microbiome (Pearce *et al.* 2017, Escallón *et al.* 2019). In the

breeding season, close proximity of male and female birds can lead to convergence of microbial composition resulting in reduced variation between males and females (White *et al.* 2010). We found no significant variation in overall beta diversity between sexes, although female showed slightly higher alpha diversity than males. However, our dataset is heavily skewed towards males (N_{males}=144, $N_{females}$=21) and these results could vary with the addition of more females. In Rufous-collared Sparrows (*Zonotrichia capensis*), cloacal microbiota diversity increased as males transitioned from non-breeding to breeding condition (Escallón *et al.* 2019), whereas we observed a decrease in diversity in the fecal microbiota of Kirtland's Warblers, which showed a decrease in diversity. These sparrows are non-migratory and do not experience the same extreme habitat change that the Kirtland's do, which could potentially explain the alpha diversity differences between species.

We generally found no significant compositional differences between age groups in the full dataset implying that adult age does not influence the microbiota of these birds. Variation in microbial composition between adults and chicks has been well documented (Kreisinger *et al.* 2017, Grond *et al.* 2017, Videvall *et al.* 2019) but comparisons between age classes of adult wild birds is lacking. However, we did see a difference in beta diversity between SY and ASY in the first recapture period in Michigan. Second year males often do not successfully establish and defend breeding territories against older males which in turn results in these individuals moving at larger spatial scales than territorial adults (Cooper and Marra 2020). Increased variation is also observed with increased movement in barn owls during the breeding season (Corl *et al.* 2020). The lack of an established breeding territory and subsequent floating behavior could result in those individuals being exposed to a different suite of environmental bacteria.

1.5.5 Shared Microbial Profile

Defining the species-specific shared microbial profile is a critical step in understanding the consistent components of often dynamic and complex microbial assemblages but can be hindered by lack of common parameters defining 'shared' (Risely 2020). In this study we define the species-specific shared microbial profile as ASVs found in >50% of all individuals in each of the three sampling periods. These stable components are commonly tied to biological processes within the host and their identification may lead to an increased understanding of host-microorganism interactions and dependencies (Tschöp *et al.* 2009). Identifying shared microbes can be confounded by environmentally derived, transient bacteria that are common across individuals but play no functional role within the host. By resampling the same individuals and within the same population we establish a shared microbial profile that is persistent across multiple environments and time periods, lessening the probability that transient bacteria are counted as shared. Accordingly, our results are in line with a decreased probability of including location or time period specific bacteria in that none of the bacterial groups we identified as differentially abundant at a specific time period, such as genera *Solirubrobacter* or *Serratia*, overlap with ASVs of the Kirtland's Warbler shared microbial profile.

Identification of microbial taxa that persist with the gut across multiple habitats and time periods will help identify those that may play a role in host biological function. Our analyses identified a group of microbial lineages, including several that likely play a role in digestion and nutrient uptake, as the species-specific shared microbial profile of Kirtland's Warblers. Eight ASVs in genus *Bacteroides* (Phyla Bacteroidetes*)* were identified as shared across the majority of individuals. *Bacteroides* are common gut microbes in humans that are frequently associated with food material breakdown and production of nutrients and energy (Wexler 2007). Though common

in birds, the exact functions of *Bacteroides* are unknown; however, it is thought they play a similar role in food digestion (Bennett *et al.* 2013, Waite and Taylor 2015, Grond *et al.* 2018). Family *Ruminococcaceae* (Phyla Firmicutes*),* contains numerous bacterial species that degrade cellulose (Duncan *et al.* 2007). Our sampling of Kirtland's Warblers identified seven ASVs from this family that are common throughout the population, including several ASVs from genus *Faecalibacterium*. Similarly, the Greater Sage-Grouse (*Centrocercus urophasianus)* hosts a rich diversity of Ruminococcaceae associated with seasonal variation in foliage consumption (Drovetski *et al.* 2019). These bacteria may aid in the digestion of the various fruits and berries ingested throughout the year which become a primary diet component on the wintering grounds.

Additionally, through repeated sampling of the same birds at three discrete time periods, we have documented the proportion of ASVs that individuals retain over time. Although several previous studies have described the proportion of core ASVs to total ASVs detected within their study, interpretations may vary depending on the number of birds sampled, laboratory methods and parameters defining shared bacterial taxa, and may therefore not represent the number of core ASVs in each individual (Lewis *et al.* 2016, Grond *et al.* 2017). We show that individuals sampled in triplicate retain 18-26 ASVs over time. This represents an average of 25.06% of all lineages detected per individual per sampling point, and we argue it best reflects the proportion of stable, persistent bacteria within an individual. Documenting the species-specific shared microbial profile of Kirtland's Warblers as well as temporally persistent lineages across seasons and changing environments provides model data from which we can begin to understand the extent to which birds depend on their gut microbiota.

1.6 CONCLUSION

The ability to study the same individuals and populations throughout the annual cycle greatly enhances our understanding of the consequences of changing environments and seasonal physiological stressors on gut microbiota. We demonstrate that a significant compositional shift occurs in the community structure of gut bacteria as Kirtland's Warblers migrate from The Bahamas to Michigan. Additionally, we describe a species-specific shared microbial profile and the proportion of bacterial lineages retained across three periods of the annual cycle within individuals. Though Kirtland's Warblers were recently removed from the endangered species list after recovering from near extinction, continued management and research is needed for this species to survive (Cooper, Rushing, *et al.* 2019). In species that have experienced severe population declines, such as Kirtland's Warbler, the subsequent decrease in genomic diversity may leave the species vulnerable to invading pathogens (Radwan *et al*. 2010). Gut microbiota may be critical in mitigating disease pathogenesis in these species by providing microbially mediated protection against invading pathogens (Ubeda *et al.* 2017, DeCandia *et al.* 2020). The symbiotic relationship birds form with their microbiota can confer immunological, developmental, and physiological benefits (Grond *et al.* 2018). Additionally, as anthropogenic influences continue to impact the habitat Kirtland's occupy, the microbiota of the birds may be used as a proxy for individual and population level health (Trevelline *et al.* 2019). Healthy gut microbiota should be included in the maintenance of threatened and endangered species (Allan *et al.* 2018, Roth *et al.* 2019) and this study provides model data as to how species with small population sizes and extreme habitat specialization react to changing environments.

2 Intestinal microbiota of Nearctic-Neotropical migratory birds more variable over time than across host species

2.1 ABSTRACT

Seasonal migration of Nearctic-Neotropical passerine birds may have profound effects on the diversity of host-associated microbiota. Migratory birds inherently experience environments and diets that can be highly variable over the course of the annual cycle. In this study we characterize the intestinal microbiome of four closely related species of migratory *Catharus* thrushes at three time points of their annual migratory cycle: during spring migration, on the summer breeding territories and during fall migration. Using observations replicated over three years, we find that microbial community diversity of *Catharus* thrushes is significantly different across different periods of the annual cycle, and community composition is more similar within than across years. We recovered two phyla, Cyanobacteria and Planctomycetota, that are not commonly described from birds to be in relatively high abundance in specific years. We found that few bacterial genera were consistently found across individuals, indicating the lack of a species-specific shared microbial profile. This study contributes to the growing number of observations of microbiota in wild birds throughout varying ecological conditions and reveals potential axes across which an animal's microbial phenotype flexibly adapts to novel environments throughout the annual cycle.

2.2 INTRODUCTION

Numerous species take advantage of resources that appear in seasonally fluctuating environments by completing a biannual migration, including approximately 40% of all bird species (Alerstam *et al*. 2003, Dingle and Drake 2007, Winger *et al.* 2019). To facilitate long-distance movements between breeding and non-breeding areas, migratory birds exhibit flexible phenotypes during their

life course, including in their gastrointestinal tracts. For example, internal organs vary in size and capacity across the migratory cycle, including the stomach, liver and gizzard (Piersma 1998, Battley *et al.* 2000, Bauchinger *et al.* 2005). Prior to migration, the intestinal tract of migratory birds can increase in size to facilitate increased food intake, metabolic capacity, and energy assimilation (McWilliams and Karasov 2005). Before departing, portions of the intestinal tract may be reduced in size to decrease weight and increase flight efficiency (Piersma 1998, Hedenström 2008).

Associated with these physical changes, we expect the community of host-associated microbial symbionts that occupy the gastrointestinal tract of migratory birds to shift in abundance and composition. These microbes play a pivotal role in numerous host-associated functions. Gut microbiota allow for some birds with dietary specialization to ingest food materials which may be toxic to other animals, such as for the orange-tufted sunbird (*Cinnyris osea*) which feeds on nectar containing toxic alkaloids (Gunasekaran *et al.* 2021) or the greater sage-grouse (*Centrocercus urophasianus*) which feeds on the chemically defended plant sagebrush (*Artemisia spp.*) (Kohl *et al.* 2016). Gut microbiota have also been shown to facilitate immune system maturation, impact metabolism, affect behaviors, and correlate with mate choice and breeding success (Yeoman *et al.* 2012, Apajalahti and Vienola 2016, Broom and Kogut 2018, Davidson *et al.* 2020, Slevin *et al.* 2021, Taff *et al.* 2021). These microbial communities are often dynamic, varying in response to both intrinsic and extrinsic factors, including environment, diet, or host phylogeny (Hird *et al.* 2015, Adair and Douglas 2017, Capunitan *et al.* 2020*,* Skeen *et al.* 2021). An individual bird's microbiota is shaped in large part by the ecologies, diet, and environment of the host, often more so than by the host species identity (Michel *et al.* 2018, Grond, Santo Domingo *et al.* 2019

Capunitan *et al.* 2020, Song *et al.* 2020). Additionally, differences in habitat occupancy within a single bird species affect the gut microbiota (Hird *et al.* 2014, Wu *et al.* 2018).

Several studies have examined the factors that impact the microbiome of migratory birds, such as the effects of pathogens, variable breeding habitats, and migratory period (Grond, Santo Domingo *et al.* 2019, Turjeman *et al.* 2020, Li *et al.* 2021). Composition of microbiota has been shown to vary at different stages of the migratory cycle within the same population of birds, linked to altered feeding patterns (Wu *et al.* 2018, Skeen *et al.* 2021). Additionally, both local and long-distance movement is associated with altered microbial diversity (Wu *et al*. 2018, Corl *et al.* 2020). A comparison of migrants with non-migratory conspecifics at the same time and in the same place identified bacterial taxa which may be associated with migration (Risely *et al*. 2018, Turjeman *et al.* 2020). In actively migrating birds, the host-associated microbiota may rapidly acclimate to the local environmental microbial pools, including on stopover sites (Lewis *et al*. 2016, Zhang *et al.* 2020). Increased understanding of migratory bird microbiota offers the opportunity to identify and investigate factors that impact the diversity and dynamics of microbiota when hosts are exposed to highly variable environments, changing diets, and extreme physiological stress across the annual cycle.

In this paper we assess variation in gastrointestinal microbiota across years and seasons in four migratory bird species. We predict that the physiological changes that occur in birds in preparation for and during migration, as well as the substantial change in habitat and diet, could result in significant shifts of abundance and diversity of their gastrointestinal microbiota. We characterized the intestinal microbiota of four closely related species of migratory *Catharus* thrushes during spring and fall migration over a period of three years (2017-2019), the Veery (*C. fuscescens*), Hermit Thrush (*C. guttatus*), Grey-Cheeked Thrush (*C. minimus*) and Swainson's

Thrush (*C. ustulatus*). These thrushes were collected through a collaboration between the Field Museum of Natural History in Chicago, Illinois and the Chicago Bird Collision Monitors (CBCM) volunteer program. Volunteers with CBCM collect birds throughout downtown Chicago that have died as a result of window collisions during each spring and fall migration. We also include a small sampling of thrushes collected on their breeding grounds in Minnesota (2017), Michigan (2018), and Manitoba, Canada (2019). The four species of thrushes were chosen because we could obtain sufficient sample sizes and because much is known of their ecologies and physiologies (Winker and Pruett 2006, Mack and Wong 2020).

The genus *Catharus* (Famly *Turdidae*, Order *Passeriformes*) contains 12 species, including migrants to North America and the non-migratory nightingale-thrushes of Central and South America (Clements and Principe, 2000). The breeding ranges for the species in this study span northern Canada through to the northern Midwest of the United States. The non-breeding distributions include the southern United States and may reach as far as southern Brazil and northern Argentina (Heckscher *et al.* 2020, Mack and Wong 2020). The four species in this study undergo their annual molt prior to fall migration. Additionally, they are all omnivorous, primarily consuming insects and berries or other fruits, with the proportion of insects or fruit varying throughout the annual cycle (Heckscher *et al.* 2020, Mack and Wong 2020, Whitaker *et al.* 2020). Bacteria can aid in the digestion of specific food materials. For example, strains within genera *Serratia* and *Paenibacillus* have chitinolytic capabilities to breakdown insect exoskeletons and have been found in heightened abundance in insectivorous animals (Meena *et al*. 2014, Kreisinger *et al.* 2017). Similarly frugivorous animals have increased abundance of bacteria known to aid in cellulose degradation (Karasov and Douglas 2013). Therefore, we would expect variation in specific diet-associated bacteria to change with the proportional variation of insects and fruits

throughout the annual cycle. Variation in flocking behavior throughout the annual cycle may also impact host-associated microbiota, especially as mixed-species flocks form at stopover sites (Grond *et al*. 2018). Some species, like the Hermit Thrush, have been observed in mixed species flocks with birds at stopover sites, while other species such as Veery apparently remain solitary (Moore *et al.* 1990).

We collected luminal contents of the lower intestines from birds in North America during three periods of the annual cycle: spring migration, breeding season, and fall migration and test for consistency of these patterns by replicating across three years. The microbiota of the lower intestines represents downstream mixing from the previous regions of the gastrointestinal tract and therefore can be used to assess general community composition of gut microbiota of the host (Wilkinson, Hughes *et al.* 2016, Drovetski *et al*. 2018, Yan *et al.* 2019). In previous studies, the bacteria recovered form intestinal samples are comparable to that of fecal matter with similar richness and community composition within host species (Wilkinson, Jogler *et al.* 2016, Drovetski *et al*. 2018, Videvall *et al*. 2019).

We predict if shared environmental variables drive the microbiota, similarities will be found across species and differ between seasons. For example, all four thrush species flying south through Chicago during fall migration may experience more similar habitats and food resources than birds flying north on spring migration. In that case, fall birds would have a microbial composition more similar to other fall birds than when compared to spring migratory birds, even of the same species. If physiological effects, such as increased metabolic activity associated with migration, are the primary factor impacting microbiota structure, then spring and fall birds will be more similar to each other than when compared to the summer breeding birds. Additionally, if birds acquire additional bacteria throughout the annual cycle, we would predict that older birds

have a more diverse microbiome. In our evaluations, we also consider that different bacterial groups may be impacted differentially and hence show different patterns; for example some responding to seasonal change, some to age groupings, and some being species specific.

2.3 METHODS

2.3.1 Bird collection

Throughout the spring and fall migratory periods volunteers with the CBCM organization collect birds throughout Chicago that have died as a result of window collision. The birds were collected in early morning and sent to The Field Museum where they were processed. The four thrush species in this study are primarily nocturnal migrants (Winker and Pruett 2006) and were recovered by CBCM the morning after collision with buildings. All individuals included in this study were processed or frozen within 24 hours of death. Fall migrants were aged based on skull ossification and categorized as Hatch Year (HY – birds that hatched the previous breeding season and were migrating for the first time) or After Hatch Year (AHY – birds that hatched prior to the previous summer). Sex was determined from the gonads. In some cases physical damage from the collision prevented age and/or sex determination. A total of 747 individuals were collected throughout the spring and fall migratory periods of 2017-2019. A small portion of the birds (n=60) were collected early in the breeding season in Minnesota (2017), Michigan (2018), and Manitoba, Canada (2019). The summer breeding bird samples were to assess if the microbiota of actively migrating birds differed significantly from migratory birds that were not actively migrating.

2.3.2 Intestinal sample collection

Luminal contents of the lower intestines are similar to fecal samples, which are commonly used in studies of microbiota of wild birds (Wilkinson, Hughes *et al.* 2016, Drovetski *et al.* 2018, Videvall *et al.* 2019). We collected the luminal contents of the lower intestine and stored them on Flinders Technology Associates cards (FTA Cards; GE Whattman, Maidstone, Kent, UK). Previous studies have shown that results from FTA Cards are comparable to those resulting from long term ultra-cold storage (Song *et al.* 2016, Wang *et al.* 2018). We used FTA Cards for all sample preservation, to be comparable with summer fieldwork, where accessible cold storage was unavailable. We used sterilized instruments to detach the lower intestines from the cloaca. We then expressed the contents of four to eight centimeters of the posterior end of the lower intestines. We noted food materials visible in the luminal contents, such as seed or fruit. We transferred the sample to the FTA Cards using a sterile swab. We air dried the FTA Cards and stored them in airtight containers with desiccants. Each swab was transferred to a cryotube and stored at -20°C. The spring and fall migrant specimens are housed at The Field Museum. The summer bird specimens are accessioned at the University of Michigan Museum of Zoology and Cleveland Museum of Natural History. All intestinal contents are stored at The Field Museum.

2.3.3 DNA isolation and sequencing

We transferred approximately 1 cm^2 of the FTA Cards to extraction plates. We randomized samples across extraction plates so that plates included samples from all species, seasons, and years, to ensure potential differences in microbial composition were not due to laboratory work bias. Following the manufacturer's extraction protocol, we extracted using the Qiagen DNeasy PowerSoil kit (Qiagen, Hilden, Germany). We included 16 negative controls, two per extraction plate, which included no sample or sample preservation materials, for quality control and to

account for possible contamination during extraction and PCR. We used the Earth Microbiome Project universal primers 515F/806R to amplify the V4 region of the 16S rRNA genetic Marker (Caporaso *et al.* 2011, Caporaso *et al.* 2012). We then used the Illumina MiSeq Platform to obtain paired-end 150 base pair reads (Kozich *et al.* 2013). We used four sequencing lanes and loaded 188 samples and four controls per lane. Subsampling and DNA isolation took place in the Pritzker Lab at The Field Museum using a specialized fume hood to reduce possible contamination. All subsequent sample processing and sequencing took place at the Argonne National Laboratory (Lemont, Illinois, USA).

2.3.4 Sequence analysis

We processed raw sequence data with the program QIIME2 (Quantitative Insights Into Microbial Ecology, version 2021.4; Bolyen *et al.* 2019). Following standard demultiplexing and quality filtering, we generated amplicon sequence variants (ASVs) using the Divisive Amplicon Denoising Algorithm (Callahan *et al.* 2016). Using a quality score threshold of 35 (Mohsen *et al.* 2019) we trimmed all sequences outside of base pair positions 13 and 150. We classified ASV taxonomies using the Silva reference database (version 132; Quast *et al.* 2012). After classification we removed all ASVs identified as chloroplasts and mitochondria. We aligned sequencing using MAFFT and then built a phylogenetic hypothesis for all bacterial sequences using FastTree (Price *et al.* 2010, Katoh and Standley 2013). Reads that did not align to any known bacterial phylum were blasted to confirm their non-bacterial sources and removed from the final dataset. We identified bacterial contaminants with the R package *decontam* using the prevalence-based contaminant determination (Davis *et al.* 2018). We used the 16 extraction blanks that were processed in parallel with the other samples as controls. *Decontam* identified 120 contaminant

Table 2.1 Distribution of Amplicon Sequence Variants (ASVs) found in negative controls and true samples, assessed using the R package *decontam*. The majority of ASVs recovered from all samples were not observed in any of the negative controls and 240 ASVs were found in negative controls of which 120 were identified as contaminants.

#Neg. Controls	0	1	2	3	4	5	6	9	13
#ASVs	26,649	215	13	5	4	5	2	1	1

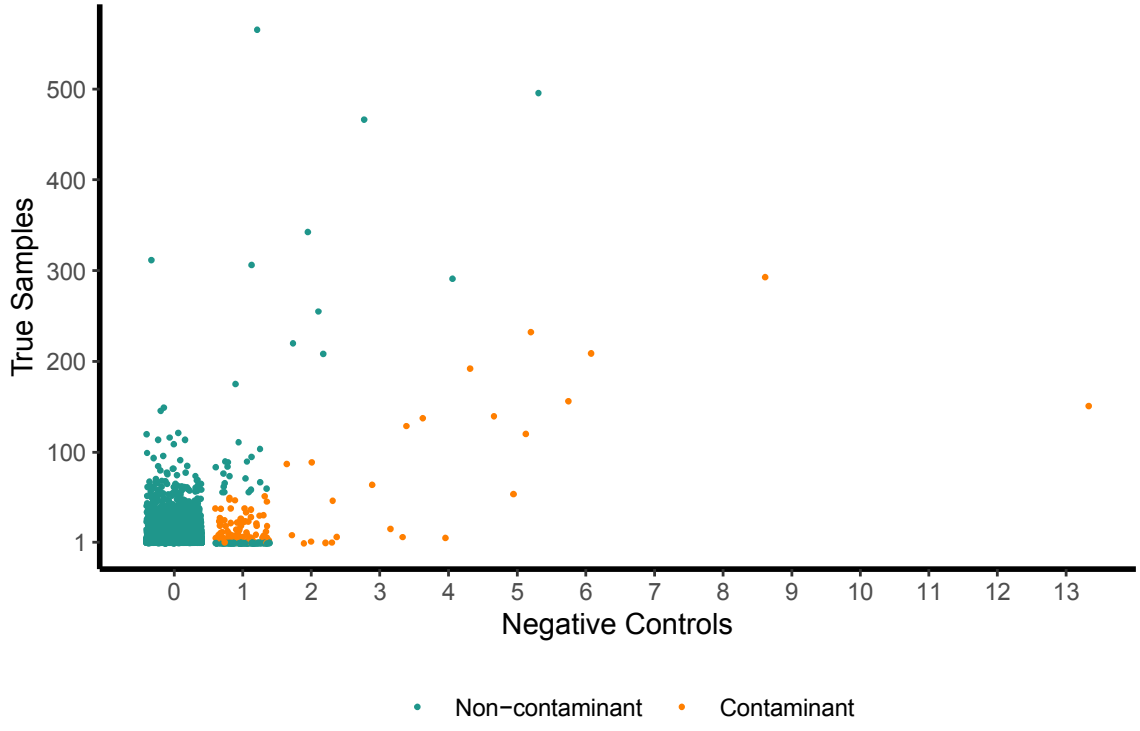


Figure 2.1 The R package *decontam* uses a prevalence-based algorithm to identify Amplicon Sequence Variants (ASVs) which are statistically likely to be contaminants. Each dot in the figure represents one ASV and all ASVs in the dataset are represented. Using the prevalence of ASVs found in negative controls compared to true samples, ASVs were considered a contaminant based on a stringency threshold of 0.5 (from a possible range of 0 to 1). A total of 240 ASVs were recovered from negative control samples, of which 120 were removed as true contaminants. The true contaminants are colored orange while the non-contaminant ASVs are colored in blue.

ASVs using a threshold of 0.5 (from a possible range of 0 to 1), which were subsequently removed from all libraries (Figure 2.1, Table 2.1).

2.3.5 Quality control

To ensure that biases were not introduced during sample collection or processing, we compared alpha and beta diversity measures of sample collector (four people), extraction plate (eight plates), MiSeq lane (four lanes), and if samples were taken from fresh birds or those that had been frozen prior to processing. Quality control measures of collector and fresh vs. frozen birds were compared within the same year so that observed differences between years did not bias results. Samples were randomized across extraction plates and therefore also MiSeq lanes so quality control measures were analyzed across the full dataset. No significant differences were observed with any of the quality control categories (Table 2.2).

2.3.6 Normalization of microbial data

We rarefied all libraries to 500 reads and 5,000 reads. This resulted in the removal of 67 libraries and 279 libraries, respectively. The majority of results were consistent across analyses at both levels of normalization. We discuss the results of the libraries normalized at 500 reads and note when results differ at 5,000 reads.

2.3.7 Alpha Diversity

We estimated alpha diversity of rarefied libraries using both richness and the Shannon Diversity Indices with the R package *phyloseq* (McMurdie and Holmes 2013). The Shannon

Table 2.2 Alpha (**2.2A**: Observed Richness, **2.2B**: Shannon Diversity Index) and Beta (**2.2C**) diversity comparisons to ensure biases were not introduced during sample collection or sequencing. We compared diversity metrics of sample collector (four people), extraction plate (eight plates), MiSeq sequencing lane (four lanes), and if samples were collected from fresh birds or those that had been frozen prior to sample collection. Quality control measures of collector and fresh vs. frozen birds were compared within the same year so that observed differences between years did not bias results. These analyses were conducted on libraries rarefied at 500 reads. *There were no frozen birds in 2017.

Table 2.2A Alpha diversity, Observed Richness

Variable	Year	Sum Sq.	Mean Sq.	F value	Pr(>F)
Collector	2017	0.65	0.325	2.328	0.102
	2018	0.498	0.2488	1.579	0.211
	2019	0.08	0.081	0.407	0.524
Fresh vs. Frozen*	2018	0.42	0.423	1.972	0.162
	2019	0.42	0.422	2.217	0.146
Extraction Plate	All years	2.03	0.0338	1.65	0.134
MiSeq Lane	All years	2.42	2.437	1.734	0.189

Table 2.2B Alpha diversity, Shannon Diversity

Variable	Year	Sum Sq.	Mean Sq.	F value	Pr(>F)
Collector	2017	0.5	0.249	0.327	0.722
	2018	1.43	0.7154	0.832	0.438
	2019	1.5	1.509	1.08	0.3
Fresh vs. Frozen*	2018	2.61	2.61	1.84	0.176
	2019	1.3	1.28	0.907	0.342
Extraction Plate	All years	5.7	0.955	0.689	0.659
MiSeq Lane	All years	3.8	3.768	2.734	0.099

Table 2.2C Beta diversity, Bray-Curtis and unweighted UniFrac dissimilarity

		Bray-Curtis		Unweighted UniFrac	
Variable	Year	Global R	*P* value	Global R	*P* value
Collector	2017	-0.067	0.842	0.067	0.177
	2018	-0.037	0.829	-0.083	0.975
	2019	0.092	0.1	0.072	0.13
Fresh vs. Frozen*	2018	0.012	0.08	0.016	0.09
	2019	0.116	0.21	0.044	0.169
Extraction Plate	All years	0.0526	0.072	0.043	0.09
MiSeq Lane	All years	0.042	0.063	0.065	0.076

Diversity Index was approximately normally distributed but we log transformed the observed richness measures to meet assumptions of normality. Due to the low level of shared ASVs across individuals (see results, including Table 5.1) and possible functional redundancies (Shade 2017, Li *et al.* 2021), we did not conduct alpha diversity analyses at the ASV level but did so at every other taxonomic level. We conducted ANOVAs (*aov* function in the *stats* R package) with post-hoc comparisons using Tukey's HSD test. We tested for and found no significant interaction between year and season, so all variables were modeled as independent factors. These variables include year (2017, 2018, 2019), season (Spring, Summer, Fall), and species (Grey-Cheeked, Hermit, Swainson's, Veery). We also compared alpha diversity of host sex (male or female) and age (HY or AHY) independently on reduced datasets, omitting samples where the host metadata was unable to be obtained and, in the case of age, only on fall birds as all spring birds are considered AHY. for age and sex variables we conducted a Kruskal-Wallace test to use as a non-parametric pairwise comparison of alpha diversity measures.

2.3.8 Beta diversity

We compared beta-diversity between years, seasons, and host species separately, using the Bray-Curtis dissimilarity and weighted UniFrac metrics (Beals 1984, Lozupone *et al*. 2011). We visualized the resulting using nMDS of weighted UniFrac distances setting the number of dimensions to four. We determined significance using analysis of similarities (ANOSIM) with 9,999 permutations (Clarke 1993). The *R* test statistic derived from the ANOSIM test compares the mean of ranked dissimilarities between and within groups. *R* values closer to 1.0 reflect increased levels of dissimilarity between groups while *R* values close to 0 reflect a distribution of ranks that is similar within each group. A significance level of $p < 0.05$ was applied to test the null

hypothesis of no differences between microbial communities of different categories. We conducted similar analyses for sex and age, on reduced datasets.

2.3.9 Differential abundance

To identify genus and phyla level taxa which differ in abundance across years, seasons, and host species, we used ANCOM-BC (analysis of composition of microbiomes with bias correction; Lin and Peddada 2020). ANCOM-BC estimates changes between groups using the log-transformed values of absolute sequence counts, therefore we used all unrarefied libraries of at least 500 reads. This method accounts for the compositional nature of microbiome data by using a linear regression framework to estimate and eliminate bias introduced by differences among sampling fractions, while controlling false discovery rate. We set a significance cutoff of $P_{adj} < 0.05$ with a Bonferroni correction.

2.3.10 Species-specific common microbes

We quantified microbial profiles common to *Catharus* and within each species as microbial ASVs and genera recovered from >50% of all individuals (Grond *et al.* 2017, Risely 2020). We quantified year and season specific lineages as being present in >50% of all individuals within the subset. We analyzed shared microbes at the ASV and genus level using unrarefied libraries of at least 500 reads using the *microbiome* R package (Lahti and Shetty 2018). Additionally, we tested for shared ASVs at lower prevalence within the full dataset to determine if and in what proportion the majority of ASVs become common across all individuals.

2.4 RESULTS

2.4.1 Microbiota community profiling

We obtained 747 libraries (Hermit, n=262; Gray-cheeked, n=89; Swainson's, n=326; Veery, n=70) throughout nine sampling periods between Spring 2017 and Fall 2019. Table 2.3 gives a breakdown of the samples by species, sampling period, age, and sex. In total 60,727,571 reads were generated, but a substantial portion (41,308,281; 68%) were from host DNA and 822,060 reads (1.35%) belonged to *Apicomplexan* pathogens. We removed host DNA contamination, *Apicomplexan* pathogens, and other non-bacterial or unknown reads for a final dataset of 17,949,438 reads with an average number of reads per library of 24,029 (±28,088 standard deviation [S.D.], range = 1-150,331, median = 11,199; Figure 2.2A). We recovered a total of 26,895 ASVs with an average of 100 ASVs per individual (± 221, range 1-1,849, median = 20; Figure 2.2B, 2.2C).

In total 46 bacterial phyla, 142 classes, 367 orders, 677 families, and 1,1735 described genera were recovered. The five most common phyla comprising 88% of all reads were Proteobacteria (28%), Planctomycetota (23%), Cyanobacteria (18%), Actinobacteriota (12%), and Firmicutes (7%) and (Figure 2.3, Table 5.2). *Planctomycetes* (22%, Phylum Planctomycetes) was the most abundant class, followed by *Cyanobacteriia* (18%, Phylum Cyanobacteria) and *Alphaproteobacteria* (16%, Phylum Proteobacteria). As discussed below, the abundance of Planctomycetota and Cyanobacteria recovered in this study is high, relative to previously published research (Dewar *et al.* 2014, Hird *et al*. 2015, Ambrosini *et al.* 2019, Trevelline *et al*. 2020). The relative abundance of phlya varied by host species, season, and year (Figure 2.4, Figure 2.5)

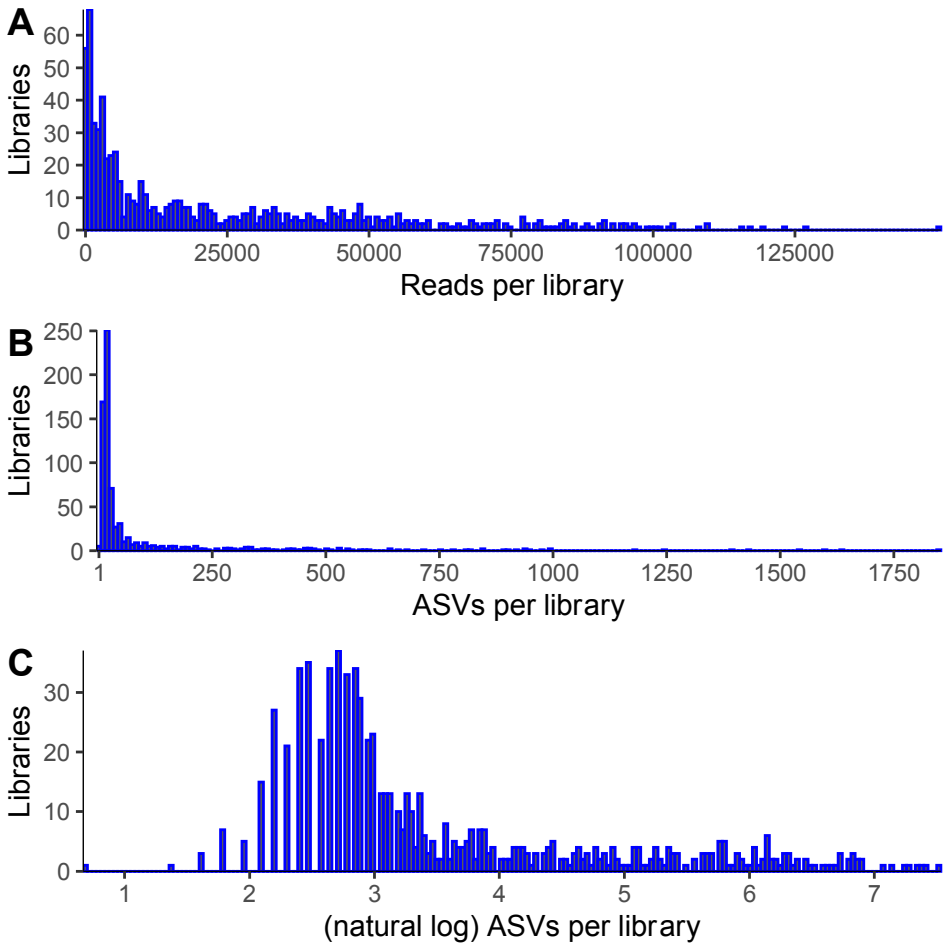


Figure 2.2 Distribution of number of reads and Amplicon Sequence Variants (ASVs) per library. **2.2A** Distribution of reads per sequenced library. After removal of contaminants and non-Bacteria reads, a total of 17, ,438 reads remained. The average number of reads per library was 24,029 (±28,088 S.D.) and individual libraries ranges from 1-150,331 reads. **2.2B** Distribution of number of ASVs per sequenced library with absolute values (top) and natural log transformed values (bottom). A total of 26,895 ASVs were recovered with an average of 100 ASVs per individual (S.D. ± 221) and ranging from 1-1,849 per library. **2.2C** Natural log transformation of ASVs per library.

Table 2.3 Breakdown of samples by species (Grey-Cheeked, Hermit, Swainson's, Veery), sampling Season (Spring, Summer, Fall), Year (2017, 2018, 2019), age (HY – Hatch Year, AHY – After Hatch Year), and sex. Age categories include Fall birds only, all spring birds are considered AHY. Table 2.3A shows the breakdown of samples in the full datasets, with 747 sequenced libraries. Table 2.3B shows the breakdown of samples when libraries are rarefied at 500 reads, resulting in the removal of 67 libraries. Table 2.3C shows the breakdown of samples when libraries are rarefied at 5,000 reads, resulting in the removal of 279 libraries.

Table 2.3A Full Dataset

		Grey-Cheeked	Hermit	Swainson's	Veery	Total
Total		89	262	326	70	**747**
Season	Spring	25	68	68	34	**195**
	Summer	0	23	26	11	**60**
	Fall	64	171	232	25	**492**
Year	2017	18	46	81	5	**150**
	2018	38	77	107	23	**245**
	2019	33	139	138	42	**352**
Sex	female	38	129	155	23	**345**
	male	48	122	154	30	**354**
	unknown	3	11	17	17	**48**
Age	AHY	12	27	36	4	**79**
	HY	51	140	186	20	**397**
	unknown	1	4	10	1	**16**

Table 2.3B Libraries rarefied at 500 reads, removal of 67 libraries

		Grey-Cheeked	Hermit	Swainson's	Veery	Total
Total		76	246	296	62	**680**
Season	Spring	24	63	61	32	**180**
	Summer	0	22	26	9	**57**
	Fall	52	161	209	21	**443**
Year	2017	16	44	70	5	**135**
	2018	31	68	91	19	**209**
	2019	29	134	135	38	**336**
Sex	female	29	119	141	21	**310**
	male	44	117	138	24	**323**
	unknown	3	10	17	17	**47**
Age	AHY	7	24	29	4	**64**
	HY	44	133	172	16	**365**
	unknown	1	4	8	1	**14**

Table 2.3C Libraries rarefied at 5,000 reads, removal of 279 libraries

		Grey-Cheeked	Hermit	Swainson's	Veery	Total
Total		56	169	208	35	**468**
Season	Spring	21	41	38	15	**115**
	Summer	0	16	19	7	**42**
	Fall	35	112	151	13	**311**
Year	2017	8	37	49	4	**98**
	2018	26	42	64	12	**144**
	2019	22	90	95	19	**226**
Sex	female	19	87	103	11	**220**
	male	35	75	91	14	**215**
	unknown	2	7	14	10	**33**
Age	AHY	4	11	23	1	**39**
	HY	30	99	123	11	**263**
	unknown	1	2	5	1	**9**

Figure 2.3 Relative abundance of bacterial phyla with libraries rarefied to 500 (**2.3A**) and 5,000 (**2.3B**) reads. Stacked bars illustrate the relative abundance of the eight most common bacterial phyla with each columns representing an individual bird, ordered by date of collection (within the respective year), and separated by season. Phyla with total abundance less than 1% are summed in the grey bar.

Figure 2.3A Relative abundance of bacterial phyla with libraries rarefied to 500 reads

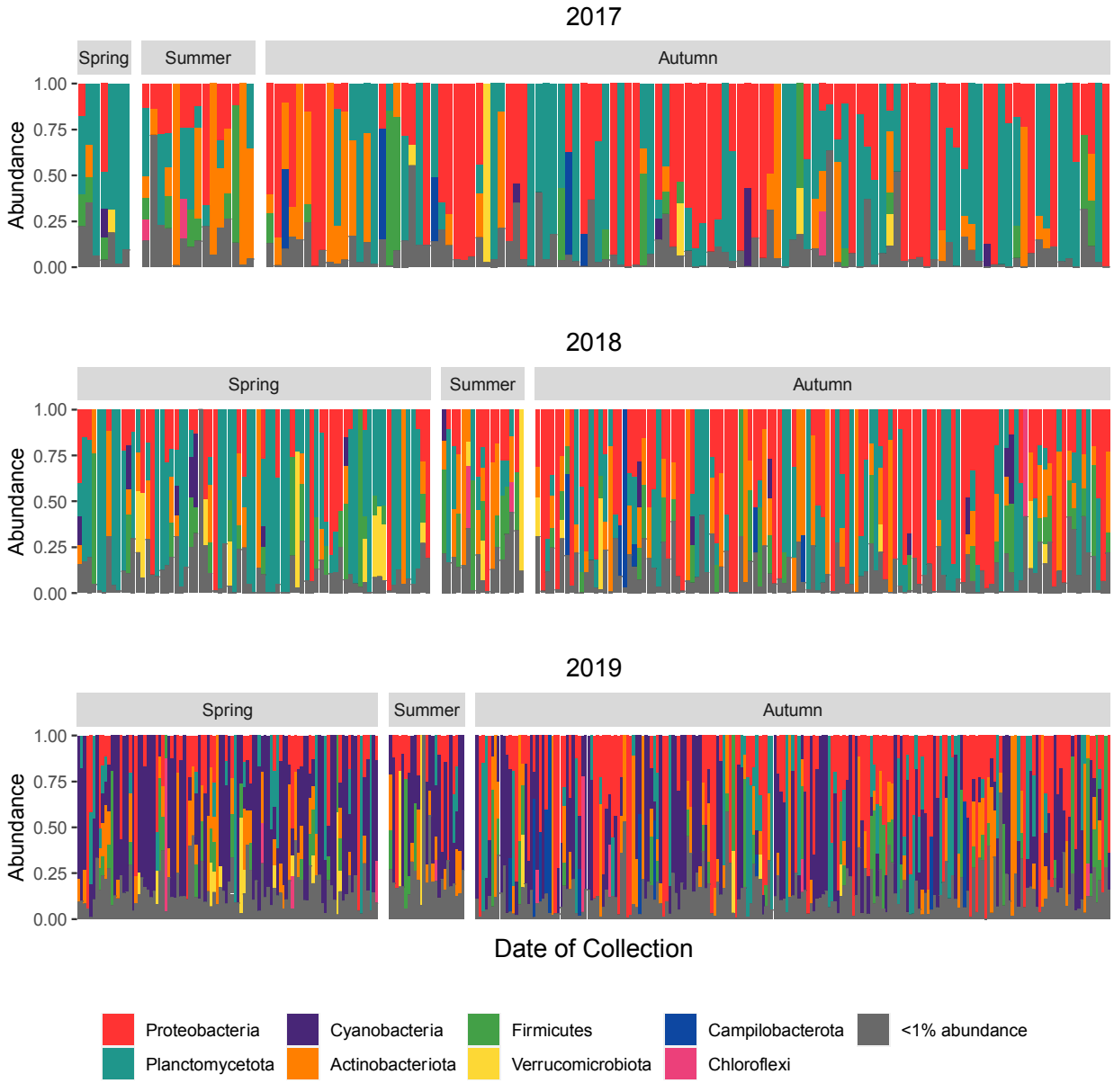


Figure 2.3B Relative abundance of bacterial phyla with libraries rarefied to 5,000 reads

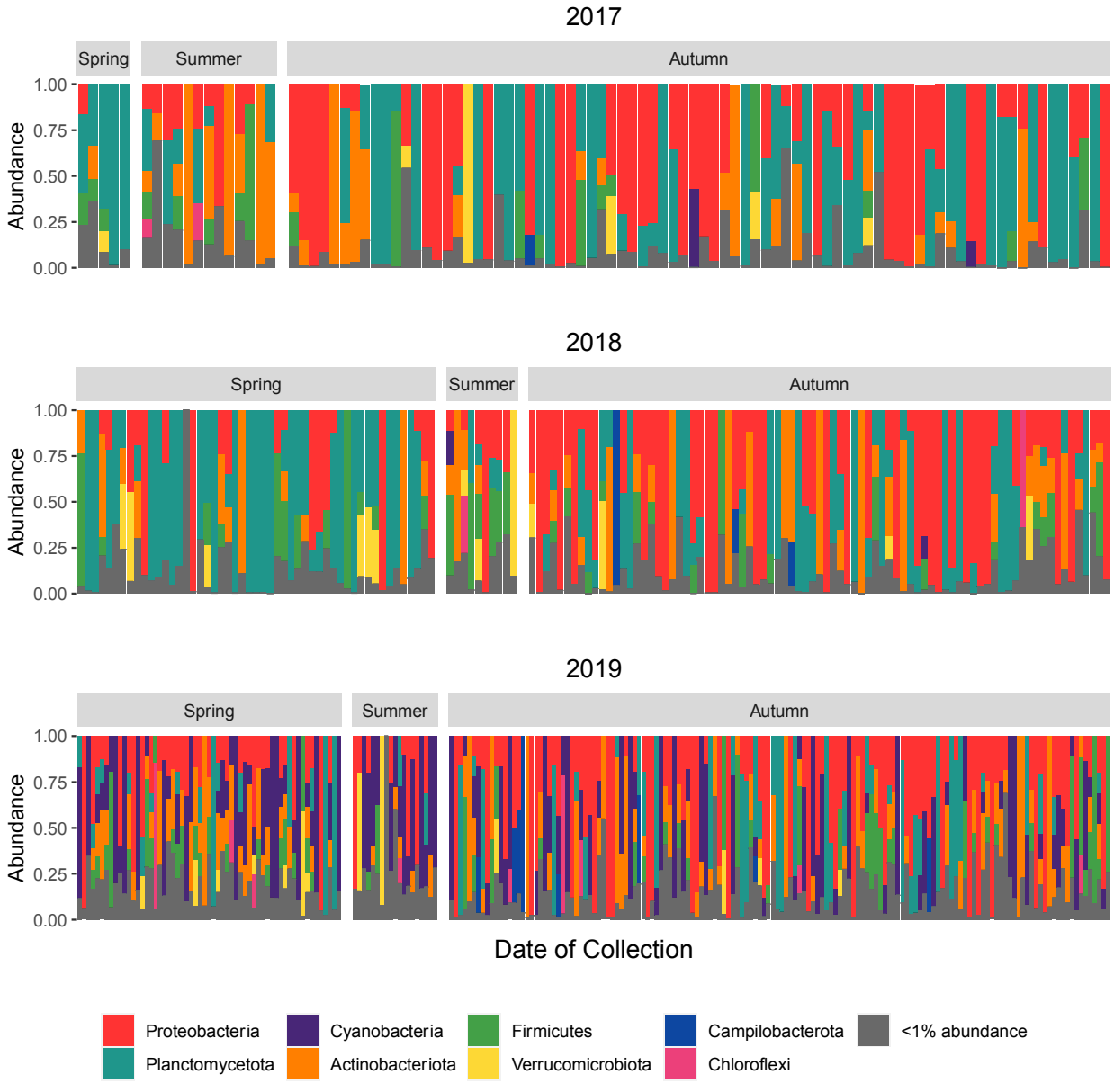


Figure 2.4 Relative abundance boxplot of most abundant phyla with libraries rarefied to 500 (**2.4A**) and 5,000 (**2.4B**) reads, representing the variation seen in relative abundance species, seasons, and year. Individual points represent the relative abundance of each phyla per individual bird. Colors of boxplots correspond to host species.

Figure 2.4A Relative abundance boxplot with libraries rarefied to 500 reads.

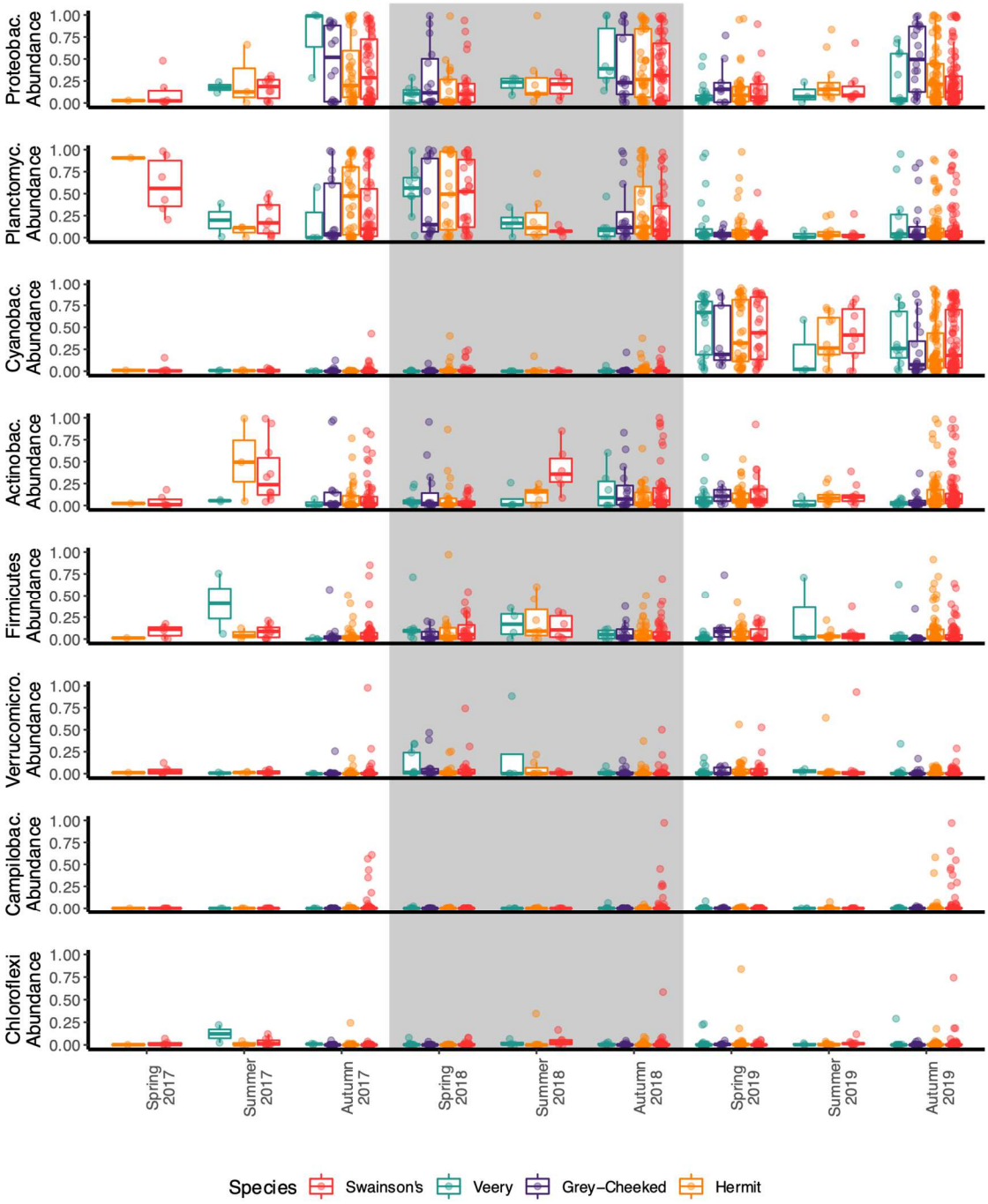
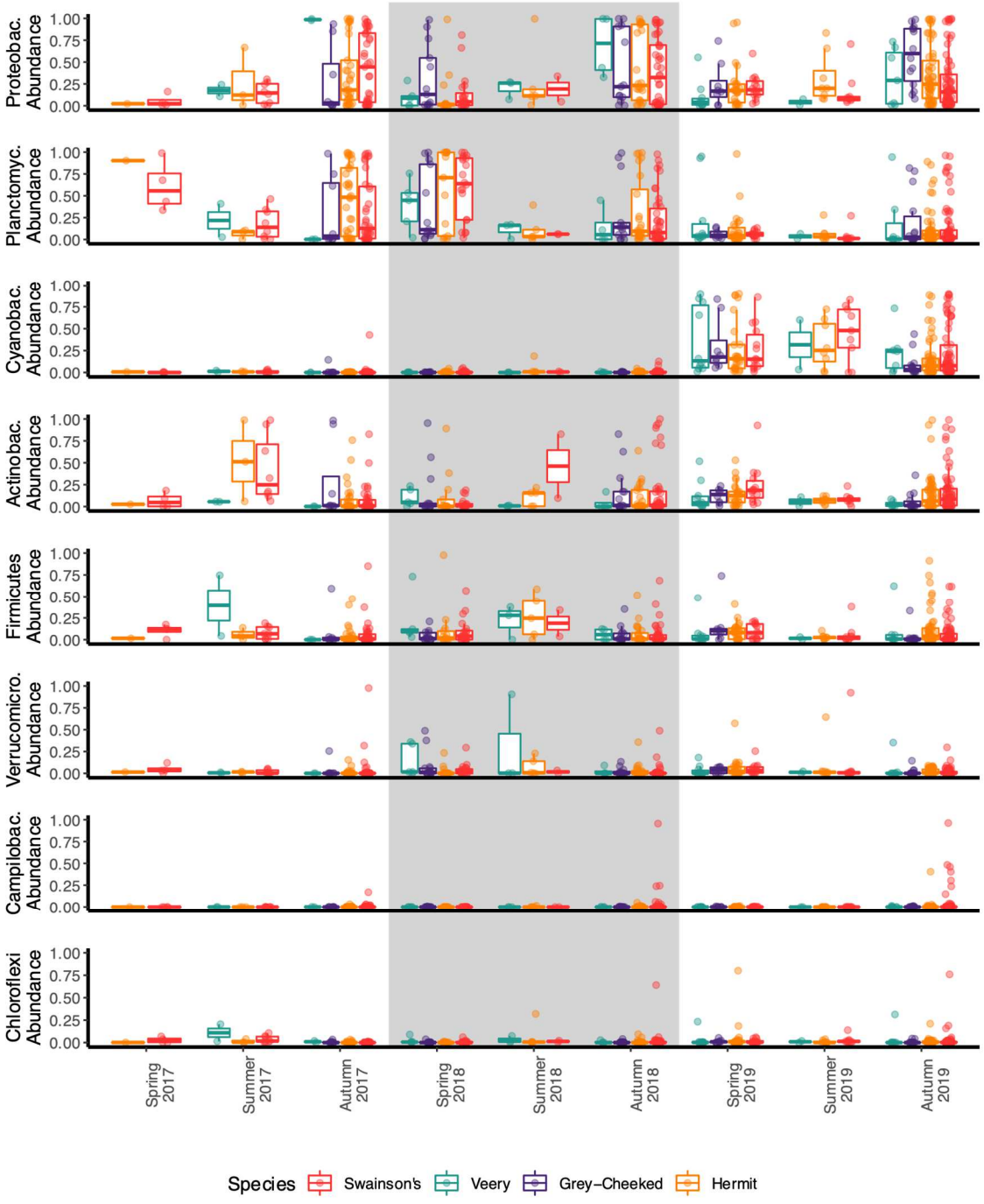


Figure 2.4B Relative abundance boxplot with libraries rarefied to 5,000 reads.



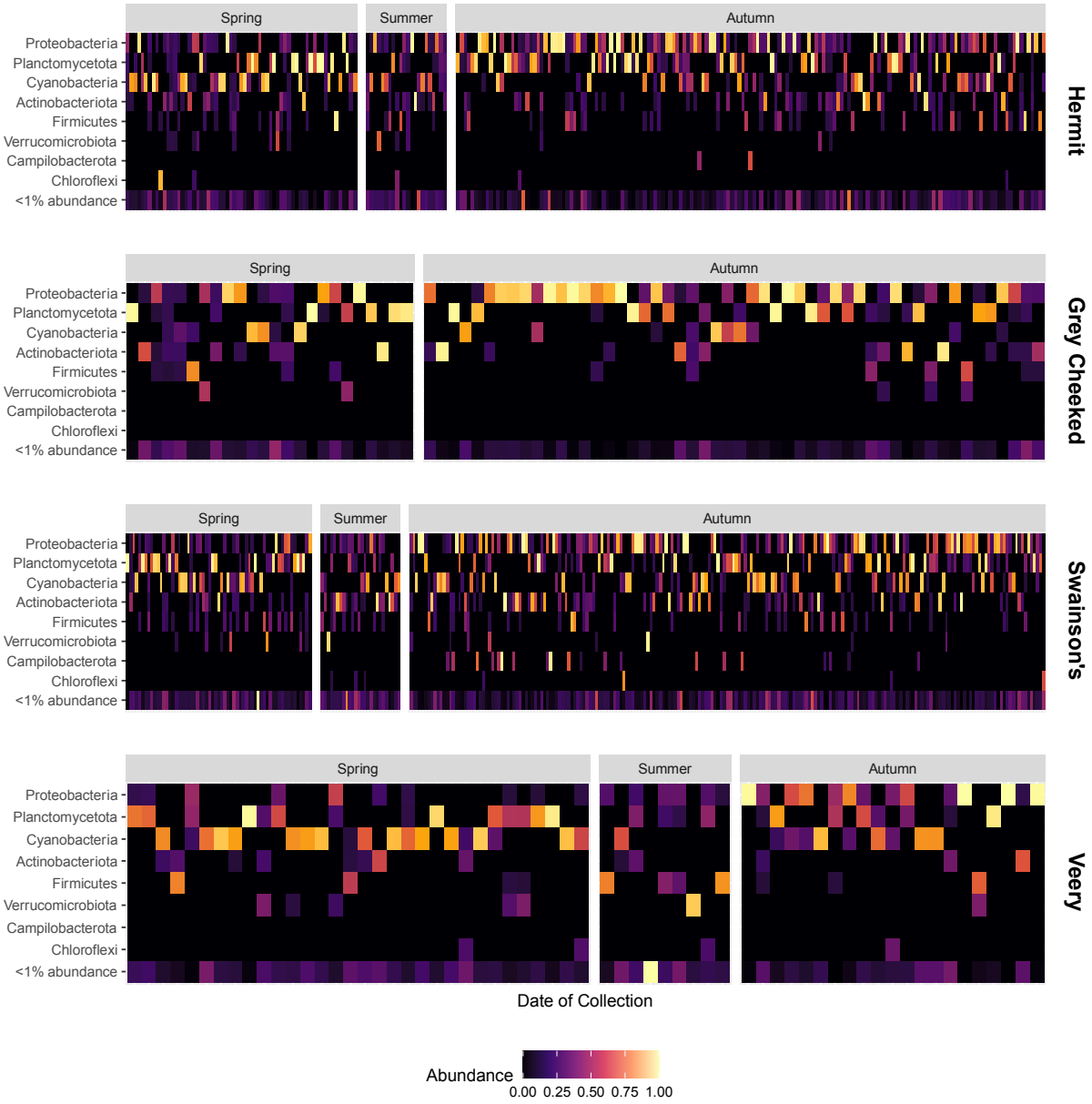


Figure 2.5 Heatmap illustrating the relative abundance of the eight most abundant bacterial phyla as well as all phyla constituting less than 1% of total reads summed together of libraries rarefied to 500 reads. Each column represents an individual sample, ordered by date of collection and separated by season and host species.

2.4.2 Differential abundance

We determined bacterial genera and phyla that were differentially abundant in host species, seasons and years using the ANCOM-BC method on unrarefied datasets of at least 500 reads. Variation between host species included four phyla and 11 genera that were differentially abundant in pairwise comparisons of host species (Figure 2.6, Table 5.3). The relative abundance of Actinobacteria was elevated in Swainson's and Grey-Cheeked Thrush compared to Hermit. Swainson's showed significant enrichment of Campilobacterota compared to Hermit and decreased enrichment of Patescibacteria compared to Grey-Cheeked. Hermit Thrush has lower relative abundance of Actinobacteroita compared to Grey-Cheeked.

Annual differences in bacterial phyla showed similar distributions when comparing 2017 and 2018. However, we found a surprising difference in relative abundance between 2019 and the previous two years. Planctomycetota and Firmicutes were significantly enriched in 2017 and 2018 than 2019. In 2019, Cyanobacteria were exceptionally abundant (Figure 2.7, Table 5.4). In addition to the seven phyla that displayed significantly different abundances between years, we identified 28 bacterial genera that were enriched in specific years. This includes *Aliterella* (Phylum Cyanobacteria) as significantly more abundant in 2019 than in 2017 or 2018.

Twelve phyla differed between seasons (Figure 2.8, Table 5.5). Myxococcota and Dependentiae had highest relative abundance in the summer, Proteobacteria and Campilobacterota in the fall, and Plactomycetota and Fibrobacterota in both the spring and the fall Our ANCOM-BC analyses identified 45 genera to be differentially abundant in across seasons (Figure 2.8, Table 5.5). Several genera containing common pathogenic microbes were significantly enriched in specific sampling periods, such as *Escherichia-Shigella* in the fall, *Neochlamydia* in the spring and *Diplorickettsiaceeae* in the summer.

Figure 2.6 Analysis of composition of microbiomes with bias-correction (ANCOM-BC) of bacterial genera (**2.6A**) and phyla (**2.6B**) that were differentially abundant in host species. An asterisk (*) indicates significantly different abundances between host species. Within each comparison, negative natural log fold change values indicate an increase in abundance with the first species listed and positive log fold change values signify an increase in abundance with the second host listed. For example, *Gaiellas* is significantly more abundant in Grey-Cheeked Thrushes when compared to Hermit Thrushes.

Figure 2.6A ANCOM-BC analysis of differential abundance in bacterial genera between host species.

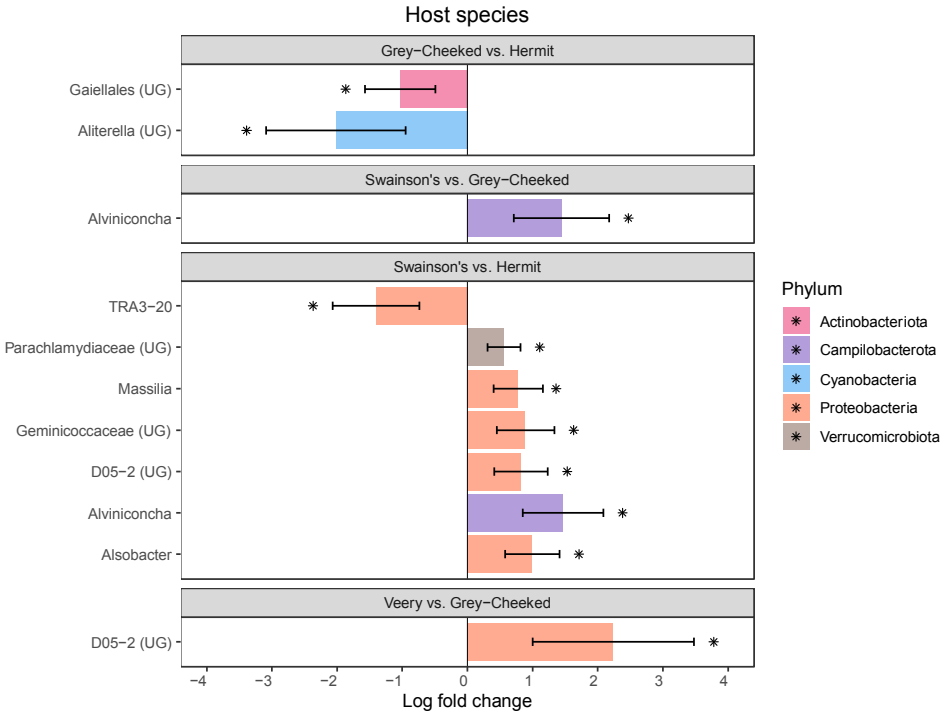


Figure 2.6B ANCOM-BC analysis of differential abundance in bacterial phyla between host species.

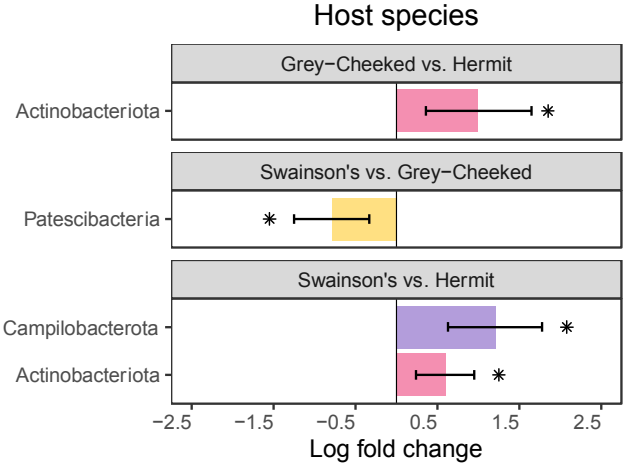


Figure 2.7 Analysis of composition of microbiomes with bias-correction (ANCOM-BC) of bacterial genera (**2.7A**) and phyla (**2.7B**) that were differentially abundant in different years. An asterisk (*) indicates significantly different abundances between years. Within each comparison, negative natural log fold change values indicate an increase in abundance with the first compared year and positive log fold change values signify an n increase in abundance with the second compared year. For example, Subgroup-17 is significantly more abundant in 2017 and 2018 when compared to 2019. There were no significant differences of phyla between 2017 and 2018.

Figure 2.7A ANCOM-BC analysis of differential abundance in bacterial genera between years.

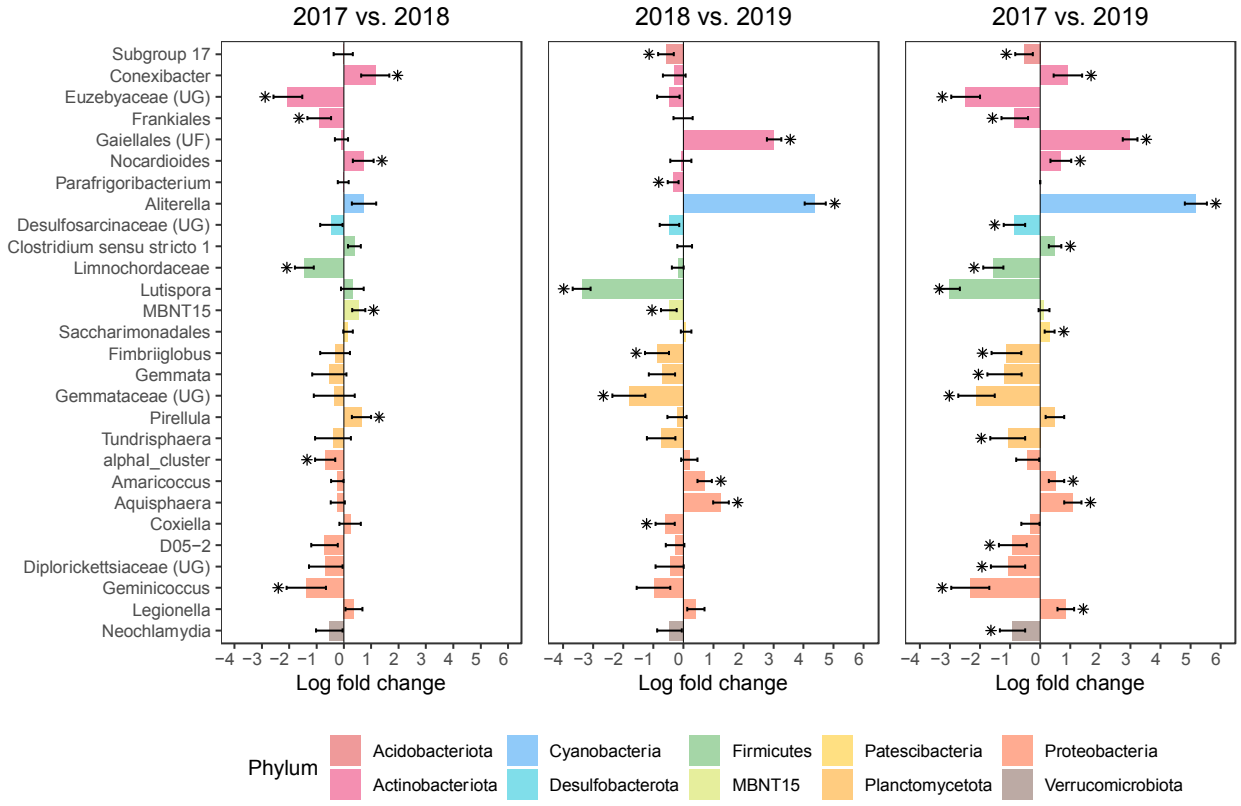


Figure 2.7B ANCOM-BC analysis of differential abundance in bacterial phyla between years.

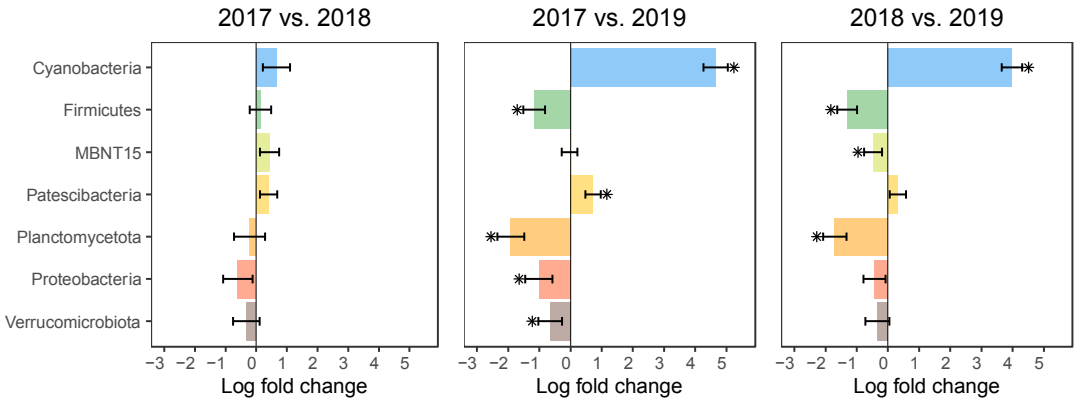


Figure 2.8 Analysis of composition of microbiomes with bias-correction (ANCOM-BC) of bacterial genera (**2.8A**) and phyla (**2.8B**) that were differentially abundant in different seasons. An asterisk (*) indicates significantly different abundances between seasons. Within each comparison, negative log fold change values indicate an increase in abundance with the first compared season and positive log fold change values signify an increase in abundance with the second compared season. For example, *Anthrobacter* is significantly more abundant in the Fall than in the Spring.

Figure 2.8A ANCOM-BC analysis of differential abundance in bacterial genera between seasons.

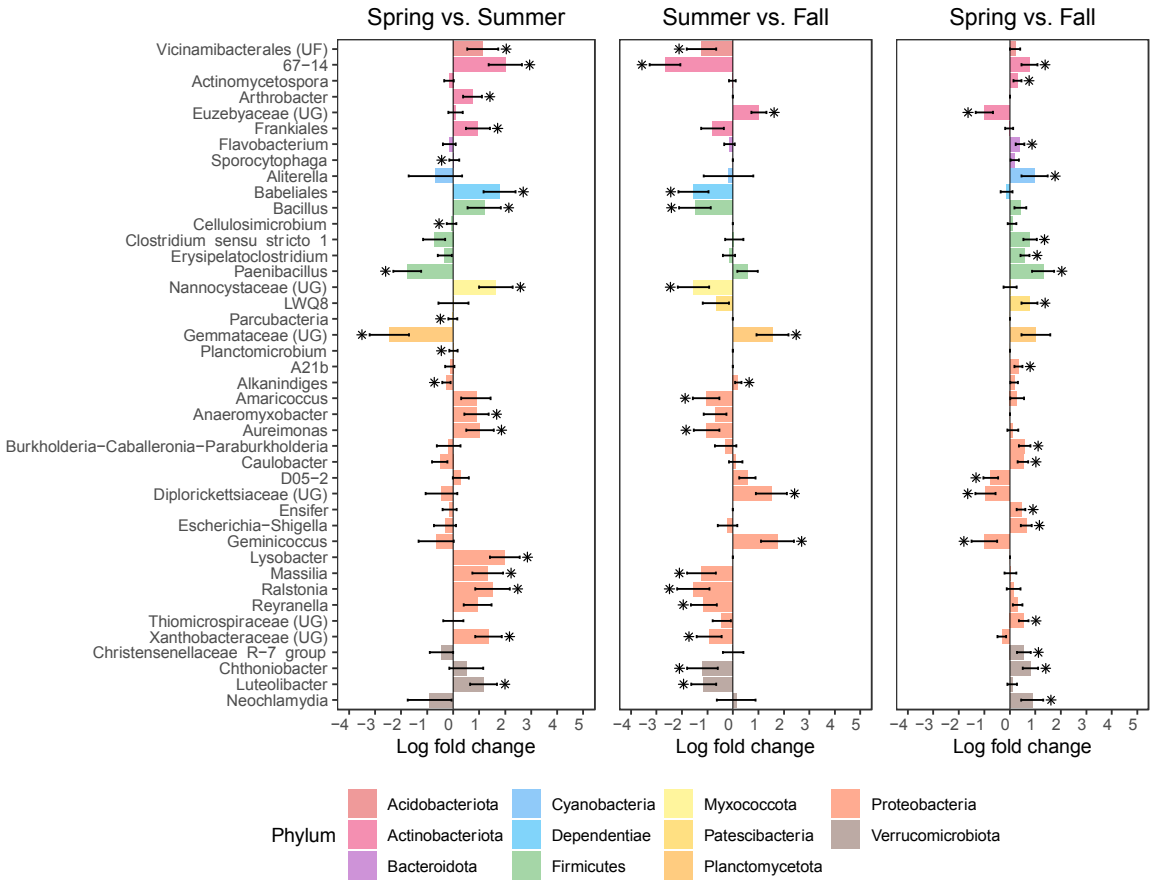
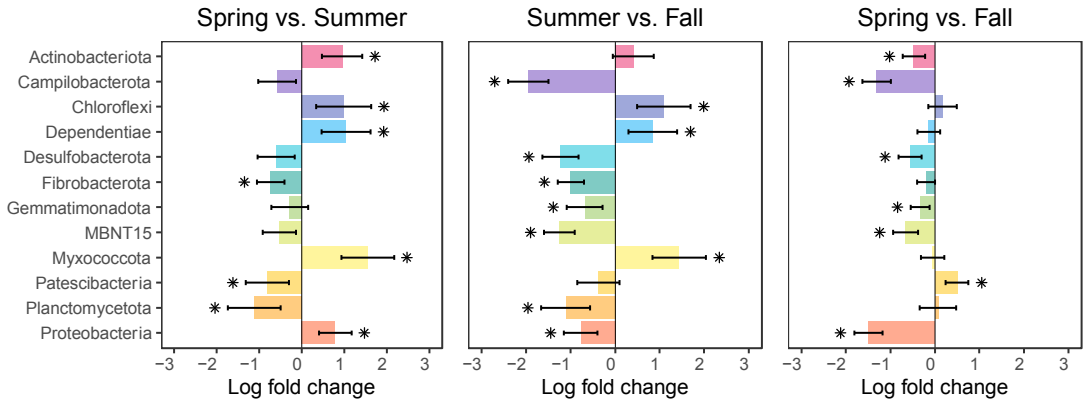


Figure 2.8B ANCOM-BC analysis of differential abundance in bacterial phyla between seasons.



2.4.3 Shared microbial profiles

Eleven genera and three ASVs within those genera were identified as present in more than 50% of all libraries (Table 5.1). Thirty-one genera were found in at least 25% of all libraries, 164 genera in at least 10% and 339 genera in at least 5%. Additionally, at the ASV level, 14 ASVs were recovered from at least 25% of all libraries 59 in at least 10% and 241 ASVs were shared by at least 5% of all individuals. The three ASVs shared by at least 50% of all libraries were from genus *Aliterella* (Phylum Cyanobacteria), an unnamed genus in the family *Gemmataceae* (Phylum Planctomycetota) and an unnamed genus in the family *Geminicoccaceae* (Phylum Proteobacteria). In addition to the 11 genera found in the majority of individuals from the full dataset, seven other genera were common in certain subsets. *Lutispora* (Phylum Firmicutes) was present in 76% of all samples in 2017 and 80% of samples from 2018. Genus *Limnochordaceae* (Phylum Firmicutes) was present in 56% of individuals collected in 2017. *Gemmata* (Phylum Planctomycetota) and *Ralstonia* (Phylum Proteobacteria) were both found in the majority of summer birds. *Aquisphaera* of Phylum Planctomycetota was common n 2019 birds only, though not restricted to a specific season. Table 5.1 lists all additional common microbes. Notably, *Aliterella* was the most common genus in the dataset, found in 77% of individuals. This prevalence was driven by a single ASV, whose species identity has not yet been described.

2.4.4 Alpha diversity

Consistently, across both levels of rarefaction, all taxonomic levels, and both diversity metrics, year and season showed significant differences in alpha diversity (Table 2.4). The differences across seasons were primarily driven by high diversity in summer compared to lower levels of diversity in spring and fall birds (Figure 2.9; Observed Richness [OR]: Spring-Summer p_{adj}=0.24,

Table 2.4 Results of analyses of alpha diversity values for natural log of Observed ASV richness and Shannon Diversity Index compared across bacterial taxonomic levels of genus through phylum. Model factors include year (2017, 2018 or 2019), season (spring, summer or fall) or host species. Alpha diversity comparisons of host sex (male or female) and host age (hatch year or after hatch year) were conducted on reduced datasets. We denoted significant with $p < 0.05$. Table 2.4A contains alpha diversity analysis results comparing natural log Observed ASV richness of libraries rarefied to 500 reads. Table 2.4B contains alpha diversity analysis results comparing Shannon Diversity Index richness of libraries rarefied to 500 reads. Table 2.4C contains alpha diversity analysis results comparing natural log Observed ASV richness of libraries rarefied to 5,000 reads. Table 2.4D contains alpha diversity analysis results comparing Shannon Diversity Index richness of libraries rarefied to 5,000 reads.

Table 2.4A Alpha diversity comparison of Observed ASV richness, libraries rarefied to 500 reads.

Taxa	Variable	Sum Sq	Mean Sq	F value	Pr(>F)
Genus	Year	5.83	2.91	15.17	**<0.001**
	Season	1.45	0.73	3.78	**0.023**
	Species	0.75	0.25	1.3	0.275
	Sex	0.04	0.04	0.18	0.668
	Age	0.18	0.18	0.87	0.351
Family	Year	6.11	3.06	17.92	**<0.001**
	Season	1.46	0.73	4.28	**0.014**
	Species	0.71	0.24	1.39	0.245
	Sex	0.02	0.02	0.11	0.742
	Age	0.19	0.19	1.04	0.307
Order	Year	4.91	2.45	17.1	**<0.001**
	Season	1.24	0.62	4.33	**0.014**
	Species	0.45	0.15	1.05	0.368
	Sex	0.01	0.01	0.05	0.829
	Age	0.17	0.17	1.06	0.304
Class	Year	2.6	1.3	15.03	**<0.001**
	Season	0.9	0.45	5.18	**0.006**
	Species	0.25	0.08	0.97	0.405
	Sex	0	0	0.03	0.852
	Age	0.11	0.11	1.21	0.272
Phylum	Year	1.96	0.98	17.95	**<0.001**
	Season	0.38	0.19	3.45	**0.032**
	Species	0.3	0.1	1.81	0.143
	Sex	0	0	0.08	0.783
	Age	0.03	0.03	0.53	0.469

Table 2.4B Alpha diversity comparison of Shannon Diversity Index richness, libraries rarefied to 500 reads.

Taxa	Variable	Sum Sq	Mean Sq	F value	Pr(>F)
Genus	Year	12.55	6.27	4.92	**0.008**
	Season	11.76	5.88	4.61	**0.01**
	Species	7.6	2.53	1.99	0.115
	Sex	0.33	0.33	0.25	0.614
	Age	2.23	2.23	1.73	0.189
Family	Year	14.4	7.2	6.54	**0.002**
	Season	12.53	6.26	5.69	**0.004**
	Species	6.37	2.12	1.93	0.124
	Sex	0.25	0.25	0.22	0.639
	Age	2.42	2.42	2.18	0.14
Order	Year	11.69	5.84	6.25	**0.002**
	Season	11.36	5.68	6.08	**0.002**
	Species	4.82	1.61	1.72	0.162
	Sex	0.12	0.12	0.13	0.72
	Age	2.21	2.21	2.36	0.125
Class	Year	8.28	4.14	7.54	**0.001**
	Season	10.5	5.25	9.56	**<0.001**
	Species	2.21	0.74	1.34	0.26
	Sex	0	0	0	0.964
	Age	1.71	1.71	3.14	0.077
Phylum	Year	7.23	3.61	10.56	**<0.001**
	Season	6.4	3.2	9.35	**<0.001**
	Species	1.5	0.5	1.46	0.223
	Sex	0.02	0.02	0.05	0.831
	Age	1.86	1.86	5.41	0.02

Table 2.4C Alpha diversity comparison of Observed ASV richness, libraries rarefied to 5,000 reads.

Taxa	Variable	Sum Sq	Mean Sq	F value	Pr(>F)
Genus	Year	6.22	3.11	13	**<0.001**
	Season	2.1	1.05	4.39	**0.013**
	Species	1.62	0.54	2.26	0.081
	Sex	0.04	0.04	0.16	0.687
	Age	0.05	0.05	0.17	0.676
Family	Year	5.7	2.85	14.34	**<0.001**
	Season	1.84	0.92	4.63	**0.01**
	Species	1.15	0.38	1.93	0.125
	Sex	0.02	0.02	0.08	0.777
	Age	0.05	0.05	0.24	0.625
Order	Year	4.14	2.07	13.71	**<0.001**
	Season	1.38	0.69	4.57	**0.011**
	Species	0.78	0.26	1.71	0.163
	Sex	0.01	0.01	0.06	0.808
	Age	0.05	0.05	0.31	0.576
Class	Year	2.13	1.06	12.93	**<0.001**
	Season	0.58	0.29	3.54	**0.03**
	Species	0.48	0.16	1.93	0.124
	Sex	0.01	0.01	0.13	0.717
	Age	0.03	0.03	0.28	0.598
Phylum	Year	1.28	0.64	15.75	**<0.001**
	Season	0.28	0.14	3.44	**0.033**
	Species	0.23	0.08	1.9	0.128
	Sex	0.01	0.01	0.15	0.698
	Age	0.01	0.01	0.11	0.736

Table 2.4D Alpha diversity comparison of Shannon Diversity Index richness, libraries rarefied to 5,000 reads.

Taxa	Variable	Sum Sq	Mean Sq	F value	Pr(>F)
Genus	Year	41.83	20.91	11.94	**<0.001**
	Season	12.13	6.06	3.46	**0.032**
	Species	12.47	4.16	2.37	0.07
	Sex	1.19	1.19	0.64	0.424
	Age	2.17	2.17	1.16	0.282
Family	Year	40.37	20.19	13.81	**<0.001**
	Season	12.02	6.01	4.11	**0.017**
	Species	9.46	3.15	2.16	0.092
	Sex	0.8	0.8	0.51	0.475
	Age	2.28	2.28	1.47	0.227
Order	Year	32.45	16.22	13.49	**<0.001**
	Season	10.79	5.4	4.49	**0.012**
	Species	6.99	2.33	1.94	0.123
	Sex	0.48	0.48	0.37	0.542
	Age	2.2	2.2	1.74	0.189
Class	Year	21.05	10.52	16.03	**<0.001**
	Season	8.3	4.15	6.32	**0.002**
	Species	2.65	0.88	1.35	0.259
	Sex	0.01	0.01	0.01	0.912
	Age	1.42	1.42	2.08	0.15
Phylum	Year	15.32	7.66	19.3	**<0.001**
	Season	5.54	2.77	6.99	**0.001**
	Species	1.3	0.43	1.09	0.353
	Sex	0.02	0.02	0.04	0.837
	Age	1.48	1.48	3.52	0.062

Figure 2.9 Alpha diversity density plots using the natural log values of observed diversity and Shannon Diversity Index of bacterial genera on libraries normalized at 500 (**2.9A**) and 5,000 (**2.9B**) reads. Density plots generated for collection year (2017, 2018, 2019), season (spring, summer, autumn), host species (Grey-Cheeked, Hermit, Swainson's, Veery), age class (Hatch Year, After Hatch Year), and host sex (male, female). Dashed lines indicate median values for the alpha diversity measure of each subgroup.

Figure 2.9A Alpha diversity of bacterial genera with libraries rarefied to 500 reads

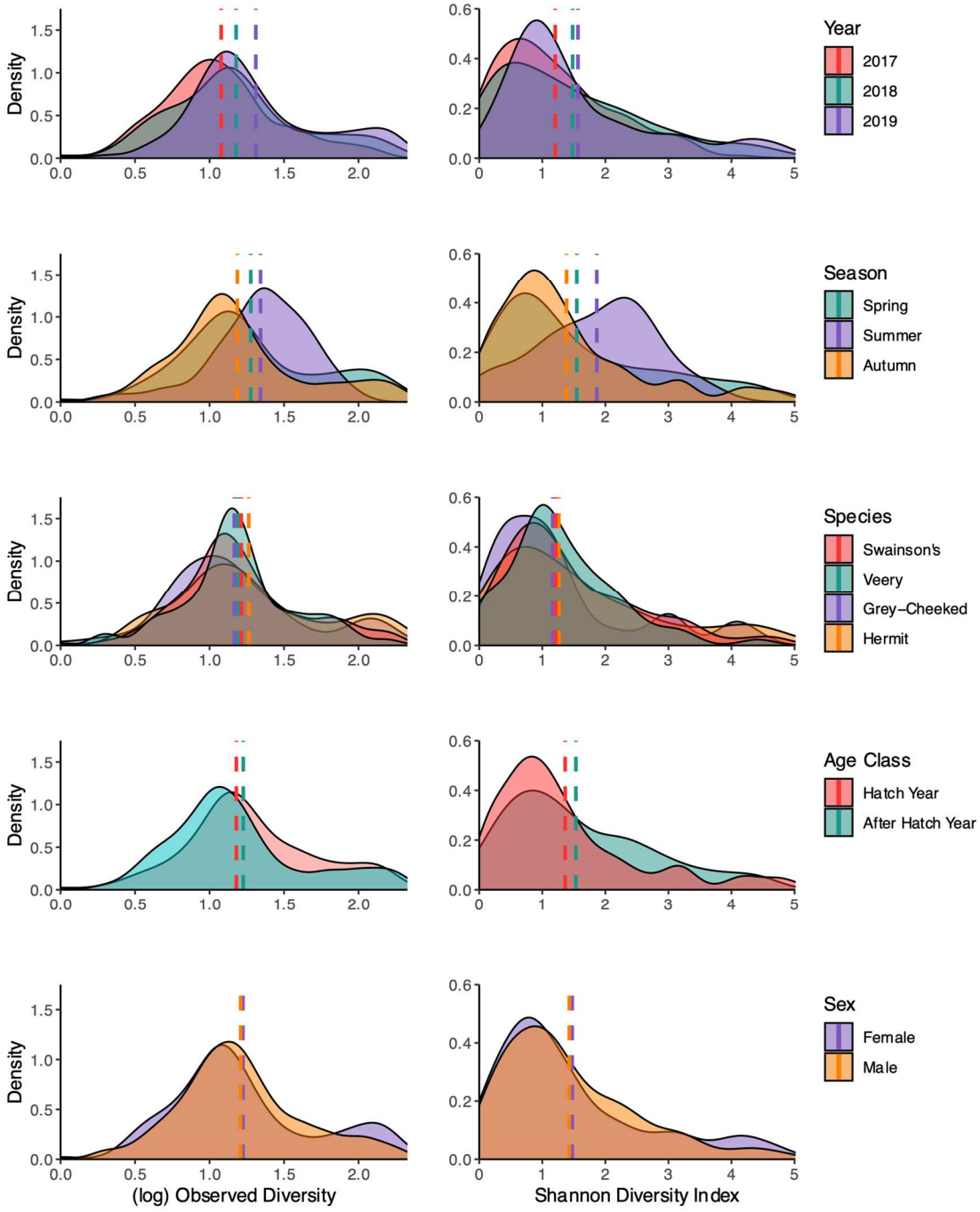
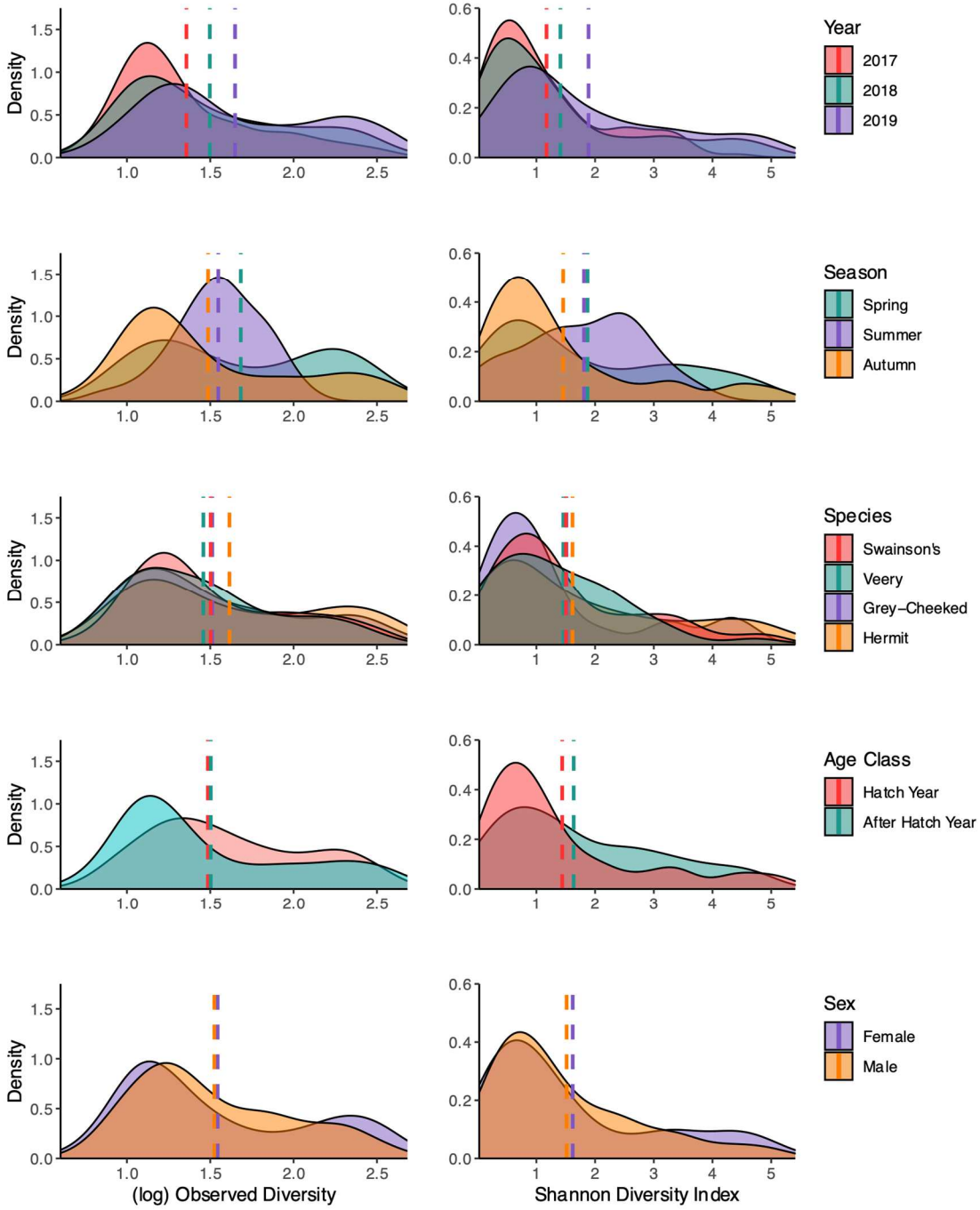


Figure 2.9B Alpha diversity of bacterial genera with libraries rarefied to 5,000 reads



Spring-Autumn p_{adj}=0.37, Summer-Autumn p_{adj}=0.03; Shannon Diversity [SD]: Spring-Summer p_{adj}=0.052, Spring-Autumn p_{adj}=0.697, Summer-Autumn p_{adj}=0.007). The average diversity of birds from 2019 was elevated compared to those from 2018 and the average alpha diversity of birds from 2017 was lowest (Figure 2.9). There were no significant differences, at either level of rarefaction, any taxonomic level, or diversity metric between host species, sex, or age, with the exception of a comparison between hatch year and after hatch year birds at the phylum level (SD: p=0.02, Kruskal-Wallace test). Older birds showed slightly elevated alpha diversity compared to younger birds. There were no significant differences in pairwise comparisons between species for either alpha diversity metric (Table 2.4).

2.4.5 Beta diversity

Community-level analysis revealed sharp distinctions of gut microbiota in birds between years with both Bray-Curtis dissimilarity (R=0.371, *P*<0.001) and weighted uniFrac distances (R=0.311, *P*<0.001) (Figure 2.10, Table 2.5). Comparisons of host species also revealed significant shifts in microbial composition, however low global R values indicate that this significance may be due to dispersion of samples, rather than true differences in community composition of microbes (Chapman and Underwood 1999) (BC: R=0.032, *P*=0.003; WU: R=0.047, *P*<0.001). Visual inspection of the ordination plot shows no clear clustering by species (Figure 2.11). No significant differences were detected in community dissimilarity based on season (BC: R=0.024, *P*=0.077; WU: R= -0.021, *P*<0.898), host age (BC: R=0.068, *P*=0.989; WU: R=-0.047, *P*=0.938) or host sex (BC: R=0.002, *P*=0.152; WU: R=0.0, *P*=0.340) (Figure 2.12).

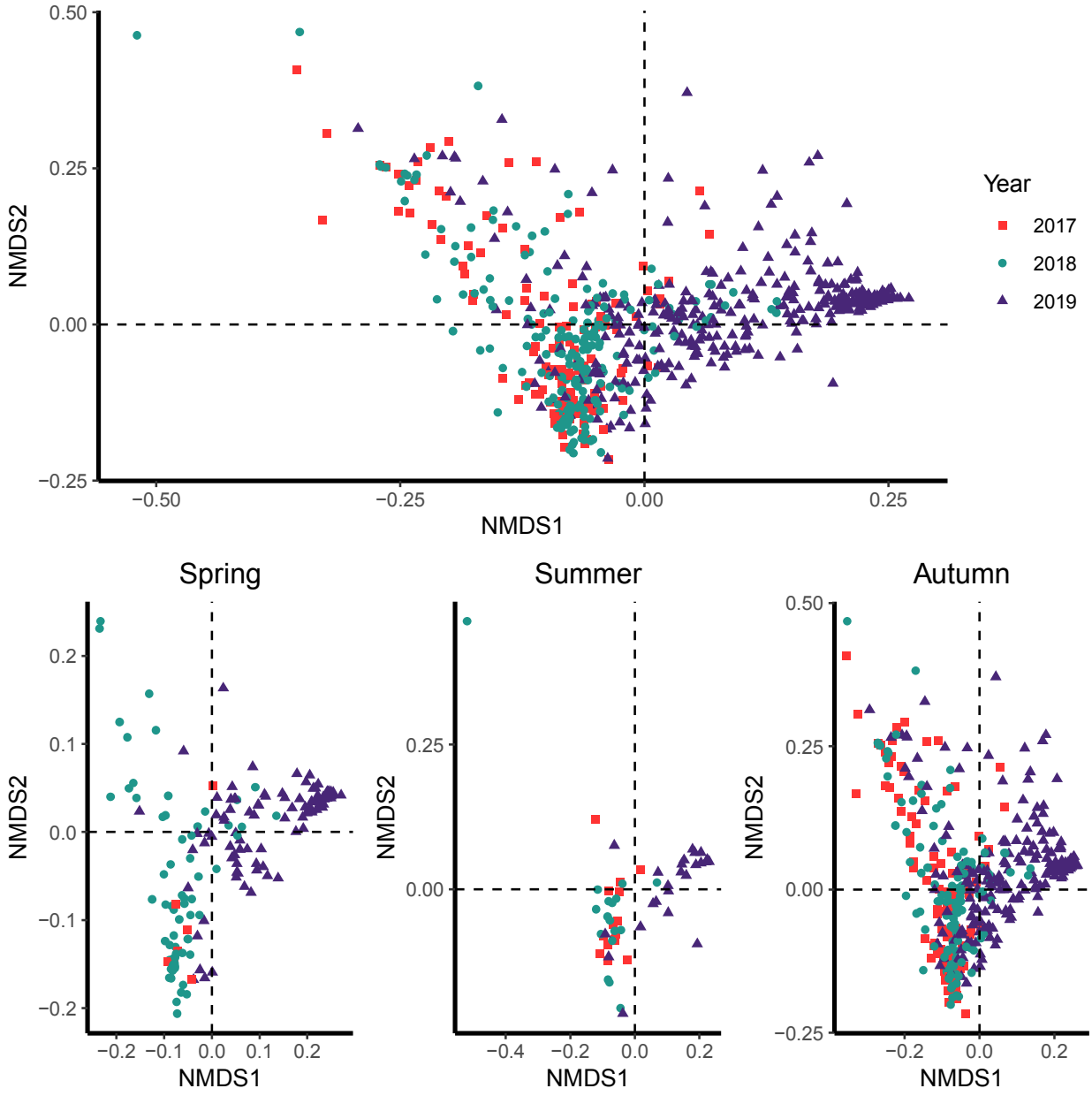


Figure 2.10 Unweighted uniFrac based non-metric multidimensional scaling (nMDS) ordination of *Catharus* intestinal microbiota by year (stress = 0.103). The bottom row contains plots within the same ordination space and subset by season.

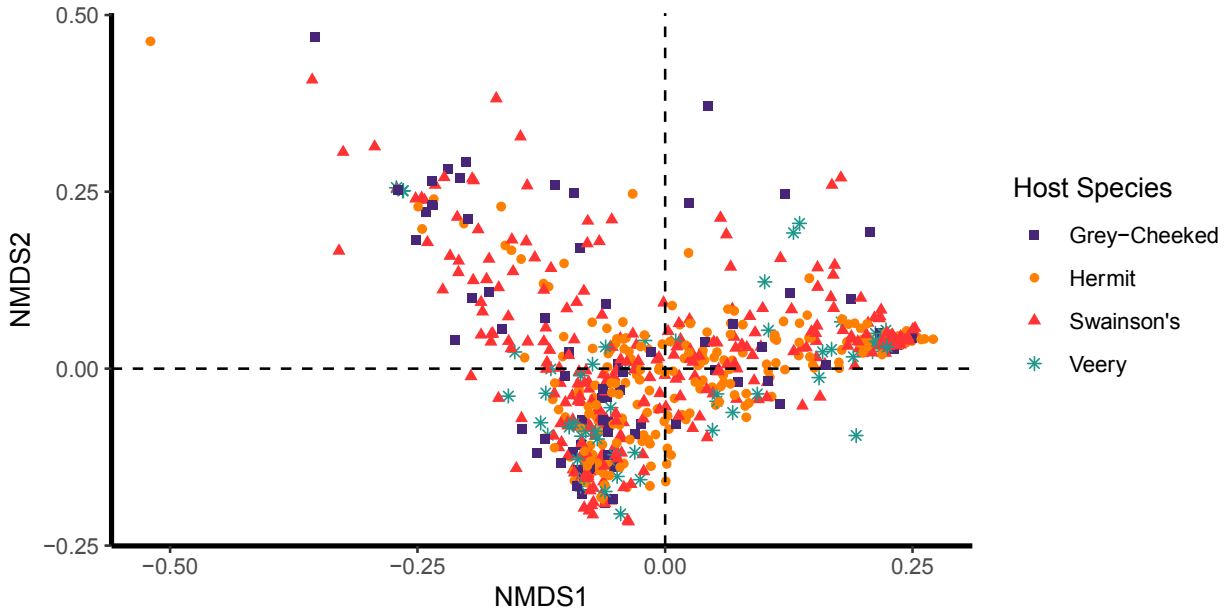


Figure 2.11 Unweighted uniFrac based non-metric multidimensional scaling (nMDS) ordination of *Catharus* intestinal microbiota by host species (stress = 0.103).

2.5 DISCUSSION

Our results highlight that the microbiome is dynamic over time, with both year and season impacting the overall composition of thrush microbiota. We find that temporal variation over years and seasons has a more observable impact to the diversity and composition of microbiota than host species, age, or sex. Migratory birds have evolved numerous physiological adaptations that enable them to complete long distance flights (Piersma 1998, Battley *et al.* 2000, Bauchinger *et al.* 2005). These adaptations, or the act of migration itself, may impact host-associated microbiota (Hedenström 2008, Song *et al.* 2020). Migratory birds inherently experience highly variable environments throughout the annual cycle. Our results indicate a strong prevalence of environmentally derived microbiota, and the lack of a consistent, shared microbial profile indicate that these environmentally derived microbiota may be transient. Finally, some of our results may be attributed directly to host-associated processes, such as annual molt. Below, we discuss how migration, host characteristics, and the environment influence the microbiota of *Catharus* thrushes.

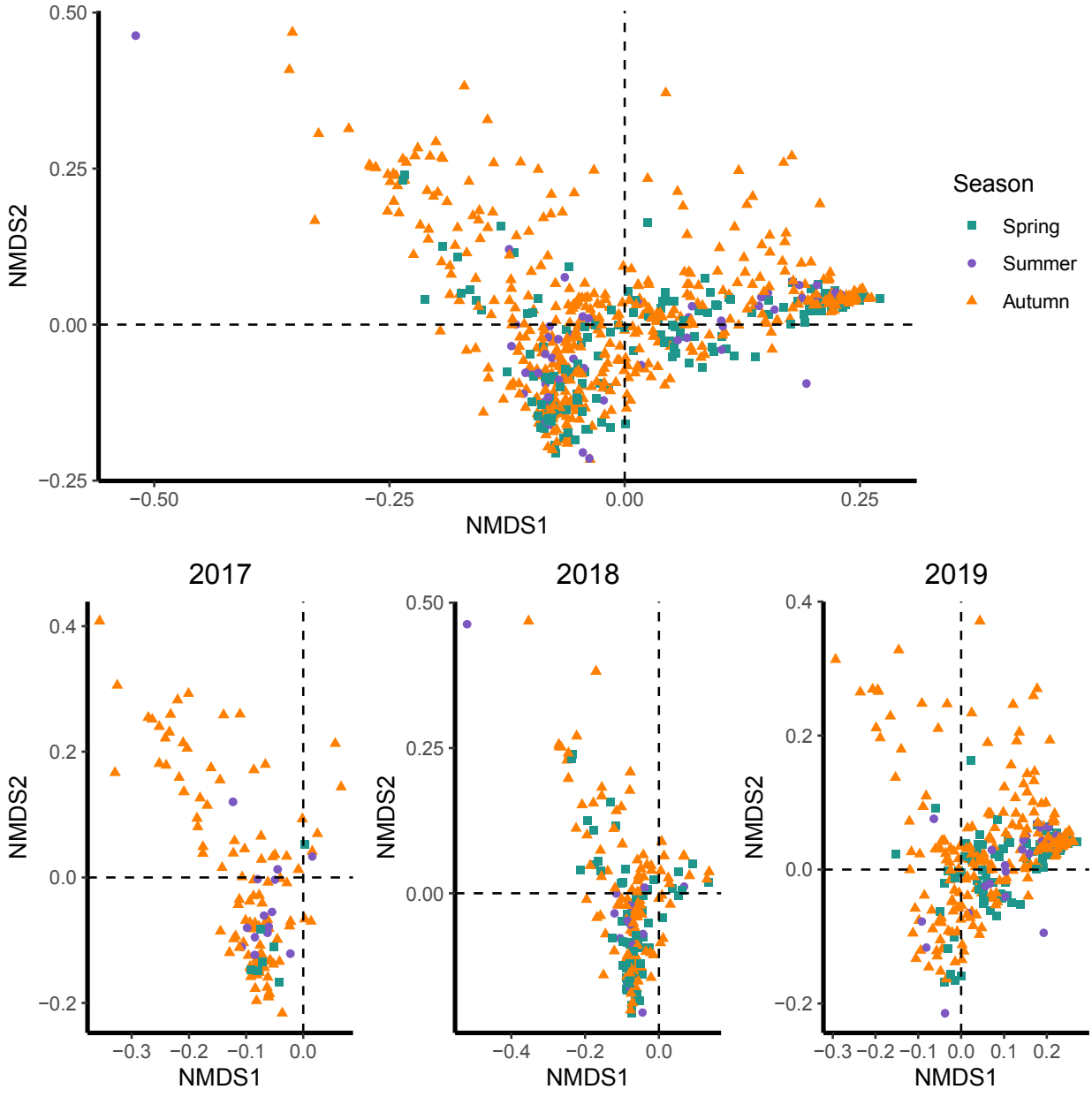


Figure 2.12 Unweighted UniFrac based non-metric multidimensional scaling (nMDS) ordination of *Catharus* intestinal microbiota by season (stress = 0.103). The bottom row contains plots within the same ordination space and subset by year.

Table 2.5A Results of analysis of similarity (ANOSIM) comparisons of beta diversity using Bray-Curtis and weighted UniFrac dissimilarity metrics across the bacterial taxonomic levels of genus through phylum with libraries rarefied to 500 reads.

		Bray-Curtis		Weighted UniFrac	
Taxa	**Variable**	**Global R**	***P* value**	**Global R**	***P* value**
Genus	Year	0.371	**<0.001**	0.311	**<0.001**
	Season	0.024	0.077	-0.021	0.898
	Species	0.032	**0.003**	0.047	**<0.001**
	Sex	0.002	0.152	0.000	0.340
	Age	-0.068	0.989	-0.047	0.938
Family	Year	0.335	**<0.001**	0.303	**<0.001**
	Season	0.018	0.140	-0.014	0.806
	Species	0.036	**0.001**	0.047	**<0.001**
	Sex	0.003	0.094	0.000	0.343
	Age	-0.076	0.994	-0.049	0.947
Order	Year	0.321	**<0.001**	0.303	**<0.001**
	Season	0.006	0.364	-0.023	0.912
	Species	0.034	**0.001**	0.046	**<0.001**
	Sex	0.003	0.068	0.001	0.302
	Age	-0.073	0.989	-0.054	0.959
Class	Year	0.244	**<0.001**	0.281	**<0.001**
	Season	0.000	0.499	-0.007	0.673
	Species	0.036	**0.001**	0.043	**<0.001**
	Sex	-0.002	0.773	0.000	0.484
	Age	-0.047	0.945	-0.026	0.815
Phylum	Year	0.211	**<0.001**	0.254	**<0.001**
	Season	0.055	**<0.001**	0.013	0.169
	Species	0.006	0.278	0.015	0.050
	Sex	-0.001	0.622	0.000	0.451
	Age	-0.018	0.722	-0.029	0.887

Table 2.5B Results of analysis of similarity (ANOSIM) comparisons of beta diversity using Bray-Curtis and weighted UniFrac dissimilarity metrics across the bacterial taxonomic levels of genus through phylum with libraries rarefied to 5,000 reads.

		Bray-Curtis		Weighted UniFrac	
Taxa	**Variable**	**Global R**	***P* value**	**Global R**	***P* value**
Genus	Year	0.319	**<0.001**	0.219	**<0.001**
	Season	0.015	0.238	-0.026	0.876
	Species	0.041	**0.001**	0.042	**0.003**
	Sex	0.004	0.119	0.000	0.347
	Age	-0.050	0.904	-0.027	0.726
Family	Year	0.268	**<0.001**	0.212	**<0.001**
	Season	0.008	0.356	-0.018	0.788
	Species	0.045	**0.001**	0.042	**0.003**
	Sex	0.005	0.079	0.001	0.311
	Age	-0.052	0.900	-0.030	0.753
Order	Year	0.253	**<0.001**	0.213	**<0.001**
	Season	0.001	0.477	-0.027	0.893
	Species	0.043	**0.002**	0.041	**0.004**
	Sex	0.004	0.100	0.001	0.287
	Age	-0.053	0.904	-0.036	0.798
Class	Year	0.174	**<0.001**	0.197	**<0.001**
	Season	-0.011	0.695	-0.012	0.705
	Species	0.038	**0.003**	0.043	**0.002**
	Sex	-0.003	0.890	-0.002	0.809
	Age	-0.027	0.754	-0.011	0.596
Phylum	Year	0.144	**<0.001**	0.168	**<0.001**
	Season	0.054	**0.004**	0.011	0.277
	Species	0.005	0.336	0.012	0.153
	Sex	-0.003	0.829	-0.003	0.838
	Age	-0.020	0.678	-0.031	0.829

2.5.1 Community composition

The high-level composition of *Catharus* intestinal microbiota is generally similar to that previously reported in numerous species of birds, with Proteobacteria, Actinobacteroita and Firmicutes representing a large portion of the overall composition (Dewar *et al.* 2014, Hird *et al.*

2015, Ambrosini *et al.* 2019, Trevelline *et al*. 2020). However, unlike these studies, Planctomycetota and Cyanobacteria represent a substantial portion of the overall microbiota. Additionally, Bacteroidota, found in relatively higher abundances in avian microbial studies which used fecal matter or cloacal swabs, was often absent or in low abundance in the intestinal samples used in this study (Hird *et al.* 2014, Videvall *et al.* 2019, Turjeman *et al.* 2020). The low relative abundance of Bacteroidota reported here, though inconsistent with several previous surveys of bird microbiota, may be a true characteristic of migratory thrushes and not an artifact of sample type, as an analysis of Swainson's Thrush microbiota on stopover in Louisiana reported similarly low abundances (Lewis *et al.* 2016, Grond *et al.* 2017, Ambrosini *et al.* 2019, Dietz *et al.* 2020).

2.5.2 Migration

Increased abundance of genus *Corynebacterium* has been correlated with migration, as it is found in heightened levels in three species of migratory birds compared to closely related, non-migratory conspecifics (Risely *et al.* 2017, Corl *et al.* 2020). It has been hypothesized that *Corynebacterium* may enable increased fat deposition or may be associated with an immune response brought on by the stress of migration (Risely *et al.* 2017, Zhang *et al.* 2021). This genus appears in less than 20% of the individuals in this study, and we found no significant enrichment of *Corynebacterium* abundance in actively migrating birds compared to those on the breeding grounds.

Rather than *Corynebacterium*, we observed increased relative abundance of several bacterial genera that contain known pathogen strains in fall or spring birds when compared to summer birds. *Neochlamydia, Esherichia-Shigella* and *Coxiella* were all significantly enriched in actively migrating birds during either spring or fall. Migration is a physically taxing endeavor

which may stress the immune system and increase pathogen susceptibility (Owen and Moore 2008, Altizer *et al*. 2011). Though several genera containing known pathogen bacteria were more prevalent in actively migrating birds compared to those that were on the summer breeding grounds, pathogenicity was not assessed in this study.

A caveat is that bacterial genera which may include well known pathogenic species are not composed solely of disease-causing strains. For example, most *Yersinia* in migratory birds in Sweden have been identified as non-pathogenic (Niskanen *et al.* 2003). One study of migratory passerines on stopover observed an increased abundance of bacterial genera which contain potentially pathogenic strains, but found no evidence of illness within the host, suggesting the genera may actually act more as commensals, possibly providing some type of benefit to the host (Lewis *et al.* 2017). For example, while *Corynebacterium* contains several known pathogenic strains (Oliveira *et al*. 2017), Risely *et al.* (2017) postulates the prevalence of this genus in healthy migratory birds may indicate the presence of a metabolic platform to increase fat deposition (Zhang *et al*. 2021). In this study, though we were limited to *post mortem* inspection, there were no obvious physical indicators of illness in the birds collected. Overall, our observation of the increased enrichment of genera such as *Neochlamydia, Esherichia-Shigella* and *Coxiella* warrant further assessment to determine if these taxa are pathogenic and in increased prevalence due to the decrease immune capacity often seen in migratory birds.

More generally, there were weak differences between the four species of thrush in this study during spring and fall migration, which became slightly more pronounced between birds on the breeding grounds. Additionally, summer birds consistently exhibited higher alpha diversity on the breeding grounds compared to fall or spring birds. These results suggest that actively migrating birds may have a reduced microbiota compared to birds that are not actively migrating. Phenotypic

flexibility associated with migration invokes numerous changes to the birds' digestive system, including atrophication of the intestinal tract (Piersma and Gill 1998, McWilliams and Karasov 2001). These changes may reduce birds' colonized gut microbiota and promote increased presence of bacteria from the local environmental pool, as suggested in a study of migratory passerines on stopover after crossing the Gulf of Mexico (Lewis *et al*. 2016). This results in the gut microbiota of different species of birds co-occurring at the same stopover sites to exhibit similar gut composition. Our results are consistent with these previous observations and support hypotheses that the migration process limits intestinal microbiome diversity and homogenizes intestinal microbiota across species.

2.5.3 Host

2.5.3.1 Host species

Overall, our results show minimal host species effects observed with weak differences in the overall community structure between species. Additionally, few bacterial phyla or genera were significantly more abundant in specific host species. These results imply that the four species of migratory thrushes do not show unique specifies-specific profiles due to differing physiologies or ecologies. Previous research has indicated that environment and diet are more influential than host genetics in shaping avian gut microbiota (Grond *et al.* 2018, Song *et al.* 2020). Host taxonomy can play a weakly significant role compared to abiotic factors (Hird *et al.* 2015, Capunitan *et al.* 2020).

2.5.3.2 Molt

Several components of the microbiome may be directly tied to host processes and characteristics. *Bacillus* is a genus which includes feather-degrading bacteria found naturally occurring on many species of birds and may play a role in the timing of the annual molt birds undergo as part of the annual cycle (Gunderson 2008). Molt occurs when old feathers are shed and replaced by new feathers. There is a strong link between the presence of feather degrading *Bacillus* and feather degradation. It has been suggested that the process of molting is an adaptation to microbial control (Burtt and Ichida 1999, Giorgio *et al*. 2018). The species of thrushes studied here all molt prior to or at the beginning of fall migration (Cherry 1985, Pyle 1997). We found the genus to be significantly more abundant in summer birds than spring or fall birds. The enrichment of *Bacillus* in summer birds may be from bacteria ingested while preening of feathers pre-molt.

2.5.3.3 Age

We observe a slight, though not significant, increase in alpha diversity in the AHY fall migrants compared to the HY fall migrants. Changes in microbial diversity and community structure between adults and chicks has been well documented (Grond *et al.* 2017, Kreisinger *et al.* 2017, Videvall *et al.* 2019), but comparisons between age classes of adult wild birds are relatively few. Recently, the microbiota of female tree swallows was assessed during the breeding season revealing that older birds had significantly higher diversity than birds in their first breeding season, possibly due to increased opportunities for mating and therefore increased contact with other birds (Hernandez *et al.* 2021). In thrushes the increased diversity in the older birds may be due to increased contact with other birds during the mating season. The increased diversity may also be a result of the older birds foraging far from the nest while rearing the hatchlings, leading

to more exposure of local environments, which has been shown to increase microbial diversity (Corl *et al.* 2020).

2.5.3.4 Diet

Variation in diet is known to influence the microbiome (Grond, Perreau *et al.* 2019, Li *et al.* 2021, Song *et al.* 2020). Many species of birds consume different food sources throughout the annual cycle. For example, Swainson's Thrush consume more insects than fruit during spring migration and breeding seasons but tend to consume more fruit during fall migration (Parrish 1997). In general, frugivory in migrants is more prevalent in fall than in spring (Bairlein 2002). However, no bacteria known to aid in the digestion of fruit materials, such as those associated with complex carbohydrate degradation, were identified as more abundant in the fall or any other period of this study. However, *Paenibacillus* was significantly more abundant in fall birds, a genus which contains several chitinolytic bacteria, indicating the birds were consuming insects (Meena *et al*. 2014).

2.5.4 Environmental effect

Annual differences in climate can affect the composition and turnover of environmental microbes (Guo *et al*. 2018, Averill *et al*. 2019, De Gruyter *et al*. 2020). A study of zebra finches (*Taeniopygia guttata*) demonstrated that birds may acquire as much as 25% of the gut microbiota from environmental sources, driving some of the variation observed between years (Chen *et al*. 2020). Our results suggest that birds acquire a higher proportion of their gut microbiota from environmental sources than previously reported. Numerous bacterial genera known to be common environmental microbes were recovered from the thrushes in increased abundance in specific

years. These genera include *Frankiales*, *Nocardioides*, and *Lutispora.* Additionally, two phyla, Cyanobacteria and Planctomycetota were significantly enriched in specific years, with Cyanobacteria dominant in 2019 and Planctomycetota relatively more abundant in 2017 and 2018. The prevalence of Cyanobacteria was primarily driven by a single ASV in genus *Aliterella*, which is a recently described genus with no described function. It is likely that *Aliterella* and other Cyanobacteria were environmentally acquired or ingested with food materials (Sun *et al*. 2019) Planctomycetes are ubiquitous in the environment (Wiegand *et al.* 2018). Seasonal and yearly variation in the environmental abundances of Cyanobacteria and Plancytomycetes may be driving the variation seen within *Catharus* over time.

At all tested taxonomic levels (genus-phylum) the birds showed a high degree of interindividual variation in terms of microbial community structure as well as the most abundant bacterial taxa. Many previous studies of wild animal microbiota have reported high variation between individuals, which likely reflects the numerous environmental and physiological factors which can influence microbial assemblages (Hird *et al*. 2014, Stothart *et al*. 2019, Capunitan *et al*. 2020). The majority of birds were sampled midway through their migration and likely originated from different areas of the breeding and non-breeding ranges. However, the variation of environmental conditions could have lessened as birds approach Chicago, as they are constrained by geographic features such as Lake Michigan. Avian microbiota often reflect perturbations, such as new environments, within 24-48 hours (Lewis *et al.* 2017, Grond, Perreau *et al.* 2019). Community composition of thrush microbiota within seasons and years was more similar than microbiota of thrushes from different seasons or years. This may suggest environmental influences from locations birds had at nearby stopover sites rather than more long-term carryover from breeding or non-breeding areas.

2.5.5 Shared microbial profile

Although some surveys identify shared taxonomic units of up to 50% (Wu *et al.* 2018), the majority of studies report a much lower percentage of ASVs recovered as shared across the majority of samples in the dataset (Grond, Santo Domingo *et al.* 2019, Escallón *et al.* 2019, Jose *et al.* 2021). In thrushes, only three of the 26,895 total ASVs were found in at least 50% of all individuals, which is exceptionally low. Those three ASVs as well as the 11 shared genera have no described functions known to be associated with host processes within the bird, such as facilitating nutrient uptake or breakdown of food materials. Additionally, common intestinal flora, such as *Faecalbacterium*, are reported as core microbes in many host species (Grond *et al.* 2019, Escallón *et al.* 2019, Skeen *et al.* 2021). The shared microbes across and within *Catharus* species contained no common intestinal flora. The functions of the shared genera of *Catharus* are generally unknown. Functional characterization of the microbiome provides a complementary view of variation in microbiota between and within groups (Cadotte *et al.* 2011, Escalas *et al.* 2019). A study of migratory sympatric overwintering birds revealed that gut microbiota functions are more conserved than bacterial diversity structure, indicating that different bacteria function in similar ways (Li *et al.* 2021).

2.6 CONCLUSION

This study adds to a growing body of literature demonstrating that the diversity and community structure of host-associated microbiota of many, but not all, migratory bird species significantly varies at throughout the annual cycle (Risely *et al.* 2017, Wu *et al.* 2018, Skeen *et al.* 2021). This has important implications for the interpretation of avian microbiota studies, denoting the importance of contextualizing the results in the time period from which the samples were

collected. We add to this by characterizing *Catharus* intestinal microbiota from spring and fall migratory birds as well as on their breeding grounds. Surprisingly our annual replicates revealed that between year variation was significantly higher than across seasons. Additionally, we describe weak species-effect impacts on the composition and diversity of the microbiota and identify specific components which likely correlate with ongoing host processes. Finally, we note that the physiological changes associated with migration may have important effects on microbiota and further research is needed in this area.

One challenge of studying wild birds under natural conditions is untangling the large number of uncontrolled variables that can influence host microbial communities. By characterizing the microbiome of four closely related *Catharus* thrushes at three separate portions of the annual cycle over three years we are able to identify components of the microbiome that vary geographically and temporally, including specific bacterial taxa and overall community composition. We highlight the necessity of temporal sampling of species to gain a fuller understanding of how the microbiome can vary over time and to better identify specific components of the microbiome that are likely to be associated with specific host processes.

3 Periodicity and density-dependent dynamics of migratory bird pathogens

3.1 Abstract

The ecological dynamics of host-pathogen systems often change over time, typified in many cases by systemic temporal variation in pathogen prevalence. Long term datasets are crucial to understanding the temporal dynamics of natural populations, allowing for a systemic approach in identifying variability of prevalence and diversity over time. In this study, we use a novel data source of data, in the form of a long-term collection of salvaged birds housed at the Field Museum of Natural History in Chicago, Illinois, USA. We document temporal variation in the prevalence of three genera of avian Haemosporidians (*Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) in 4,306 individuals from four species of migratory *Catharus* thrushes collected during spring and fall migration over a 24-year time period (1996-2019). First, we assess the accuracy of our PCR diagnostic tests using maximum likelihood estimations and determine pathogen prevalence across time periods. Second, we used statistical Bayesian modeling to infer periodicity in pathogen prevalence, identifying cycling patterns unique to each host species-pathogen genus pairing. Third, we generated a density-dependent model of migratory bird epizootics and identify parameter ranges inferred from the results of the identified cycles. We determined that avian haemosporidians exhibit distinct seasonality, generally exhibiting higher prevalence in the fall than the spring, multi-year periodicity spans 8-19 years depending on the host-pathogen pair, and that factors relating to host population size, such as net fecundity, reasonably replicate the patterns observed in the statistical model, resulting in a plausible mechanism driving periodicity of avian haemosporidians.

3.2 INTRODUCTION

Host-pathogen systems are often dynamic over time, typified in many cases by cyclical variation in pathogen prevalence (Anderson and May 1980, Earn *et al.* 1998, Kallio *et al*. 2009). Long-term datasets are crucial to understanding temporal dynamics of natural pathogen populations, allowing for a systemic approach in identifying potential variability of prevalence and diversity (Hosseini *et al.* 2004, Lloyd-Smith *et al*. 2007, Gisder *et al.* 2017). Empirical examples of disease cycles in animal populations are quite rare, largely due to logistical obstacles to collecting time series data of disease prevalence. Yet these studies provide crucial understanding of disease ecologies. High levels of temporal variation can make short-term records susceptible to errors of interpretation, especially if conditions were anomalous during the period of study. Long-term studies are particularly important with respect to episodic ecological processes or complex phenomena, and therefore play an important role in formulating and testing ecological theory (Lindenmayer and Likens 2010). Episodic processes and complex phenomena require long term studies in order to separate pattern from noise.

Environmental change resulting from human driven climatic warming is predicted to drastically alter pathogen distribution as well as the ranges of insect vectors that may transmit pathogens from host to host (Meyer Steiger *et al.* 2016, Kamal *et al.* 2018). Additionally, anthropogenic changes have altered both host and pathogen communities resulting in increased contact between pathogens and naïve host populations (Keesing *et al.* 2010, Schmeller *et al.* 2020). Therefore, it is imperative to understand how pathogen dynamics have historically varied across recent time scales to inform predictions of future pathogen prevalence and spread. However, a persistent challenge in properly assessing changing disease prevalence in long-term, real-life datasets is separating noise from pattern and dealing with unpredictable dynamics. This challenge

can be met through the use of system-appropriate modeling, sufficient data within and across time periods, and a focal study system in which there is sufficient knowledge of both host and pathogen associated characteristics.

In systems where pathogens are endemic, cyclical fluctuations in prevalence can be observed within and across years (Gulland 1992, Altizer *et al*. 2011). Vector borne pathogens, such as those that cause the disease malaria, often peak in prevalence during warm or rainy seasons, as the climatic conditions result in increased abundance of pathogen spreading insect vectors (Bacaër and Guernaoui 2006). Some airborne respiratory viruses, such as influenza, exhibit strong seasonal fluctuations in temperate regions, often peaking in the colder months (Dushoff *et al.* 2004, White *et al.* 2005). Recurrent outbreaks of avian influenza occur at two to eight year intervals in some species of North American waterfowl, theorized to be driven by the intensity of environmental transmission (Wang *et al*. 2012, Sharp *et al.* 1993). Epizootic events, defined as a significant increase in pathogen prevalence, can result from regular variation in host or parasite biology, such as waning immunity (Sydenstricker *et al.* 2005), seasonally fluctuating environments (Bacaër and Guernaoui 2006), or host movement between geographically disparate locations (Van Dijk *et al.* 2014). Determining pathogen prevalence dynamics is a key step in identifying drivers of pathogen spread, which is especially important with hosts that move between distinct environments as those animals may be exposed to numerous pathogen communities throughout the course of migration (Jourdain *et al.* 2007, Fuller *et al.* 2012).

Migratory animals are exposed to novel pathogen communities throughout the annual cycle, including on the breeding grounds, on migration, and in non-breeding areas, potentially resulting in multiple epizootic events throughout the year (Engering *et al.* 2013, Fritzsche McKay and Hoye 2016). In addition to exposure to different pathogen communities, the physiological

stress of migration may deplete immune resources, leaving the host vulnerable to infection (Reperant *et al.* 2011). This is especially evident in many species of migratory birds, which complete bi-annual migrations that may span multiple continents (Owen and Moore 2006). Birds may contract diseases at stopover sites, on the breeding grounds, or at the non-breeding winter locations (Altizer *et al.* 2011, Prosser *et al.* 2011, Pulgarin *et al.* 2019). Much research on pathogens of migratory birds focuses on how birds may spread disease across flyways, whereas few studies have focused on how pathogens act across the annual cycle. This is important to understand because migratory birds have several characteristics which may make them more vulnerable to pathogens, including exposure to novel pathogen communities and decreased immunity resulting from the physiological stress of migration or defending their nests/breeding territories, or from increased contact with vectors during nesting.

One important group of pathogens that are endemic in birds belong to the Order *Haemosporidia* (Phylum Apicomplexa) and include the causative agents of the disease malaria (Valkiūnas 2004). Of particular interest are the genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon*, each of which are vectored by different groups of insects (Haemoproteus – *Ceratopogonidae* biting midges; *Plasmodium* – *Cuculidae* mosquitoes; *Leuco*cytozoon – *Simuliidae* black flies). Haemosporidian infection often persists at low level, chronic conditions following the acute infection phase (Manwell 1934, Ortego *et al.* 2008). Haemosporidia have varying impacts on the host, spanning from being the primary driver of extinction in some species of Hawaiian honeycreeper to no apparent indicators of detrimental effect on the host (Bensch *et al.* 2007, Atkinson and LaPointe 2009). Although Haemosporidia have been widely studied in birds, few studies report time series (Bensch *et al.* 2007, Wilkinson, Handel *et al*. 2016).

In this study, we screen a set of four species of migratory *Catharus* thrushes collected over a period of 24 years (1996-2019) at the Field Museum of Natural History in Chicago, Illinois during both spring and fall migrations. The four host *Catharus* species in this study are the Veery (*C. fuscescens*), Hermit Thrush (*C. guttatus*), Grey-Cheeked Thrush (*C. minimus*) and Swainson's Thrush (*C. ustulatus*). We detail the prevalence of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* and analyze cyclical temporal variations in pathogen prevalence using a hierarchical statistical framework. We complement this analysis with a density-dependent model of migratory bird epizootics. We use these methods to determine the length and occurrences of peaks in pathogen prevalence and discuss mechanisms by which they may occur.

3.3 METHODS

3.3.1 Sample and data collection

3.3.1.1 Sample collection and storage

Starting in 1978, Field Museum of Natural History staff and volunteers from the Chicago Bird Collision Monitors non-profit organization have led a salvage program to recover birds that collide with building in Chicago. Throughout the spring and fall migratory periods, deceased birds are collected in early morning and immediately sent to the museum for processing and specimen preparation. Live but injured birds are sent to wildlife rehabilitation centers. Individuals that do not survive rehabilitation are sent to the museum for processing.

During specimen preparation, individual birds were aged based on skull ossification and categorized as Hatch Year (HY – during fall migration, birds that hatched that summer) or After

Hatch Year (AHY – all spring birds and fall birds that were at least one year old). Sex was determined through examination of the gonads. In some cases, physical damage from window collision prevented age and/or sex determination. Pectoral muscle was subsampled from each individual and frozen in liquid nitrogen for long term storage. We used subsampled pectoral muscle to screen for haemosporidian pathogens (Fecchio *et al.* 2019). All specimens used in this study are accessioned at The Field Museum.

3.3.1.2 Molecular detection of haemosporidian pathogens

Pathogen presence was detected through replicated amplification using nested PCR methods and products were sequenced to confirm infection status of the host, pathogen genus and lineage identity. We extracted genomic DNA from pectoral muscle tissue specimens and then conducted multiple nested PCRs targeting the standard 476 base pair barcoding region of the haemosporidian cytochrome b (*cytb)* genetic marker. Primers HAEMNF/HAEMNR2 and HAEMF/HAEMR2 targeted *Haemoproteus* and *Plasmodium*, with HAEMNF1/HAEMFL3 and HAEMFL/HAEMR2L amplifying *Leucocytozoon* (Hellgren *et al.* 2004, Waldenström *et al*. 2004).

We identified individuals likely positive for haemosporidian infection through visualization on gel electrophoresis, then purified and bi-directionally Sanger sequenced all inferred positive samples. We cleaned and trimmed all sequence data using Geneious Prime (Biomatters, version 2021.0.1). Finally, we confirmed host infection status as well as pathogen genus and lineage identity using the MalAvi reference database (Bensch *et al*. 2009). Lineages were identified as known with a 100% match in the MalAvi database or novel with <100% match.

We determined infection status only after successful sequencing and genus identification (Bensch *et al.* 2021). Subsequently, we marked each bird as positive for *Haemoproteus*,

Plasmodium, or *Leucocytozoon*. Co-infections occur when multiple lineages of a haemosporidian pathogen are detected. We attempted to visually resolved the sequences of co-infections. If visual resolution is not possible, those samples were determined to be unresolved co-infections. Unresolved co-infections can still be identified to pathogen genera with high confidence but lineage identification is not possible.

3.3.1.3 Accuracy of PCR diagnostic test

False negatives are a common occurrence in blood pathogen detection (Richard *et al*. 2002, Freed and Caan 2006). To reduce the possibility of a false negative, we screened each sample in triplicate with independent PCR runs with each of the primer sets (Lutz *et al.* 2015). To assess overall accuracy of PCR pathogen diagnostic tests, we estimated the probability of not detecting an infection within an individual sample, a false negative. We did so by quantifying all possible outcomes for the triplicate PCR runs per individual. These outcomes are {111}, {110}, {100} and {000}, where 1 indicates positive infection and 0 indicates no infection. For example, in the case of {110} two of the PCR runs show that the bird is positive for the haemosporidian pathogen while one PCR run shows no indication of infection. We used the frequency of {110} and {100} to estimate the proportion of {000}, birds that were actually infected but did not indicate so in any PCR test and therefore were a false negative. We assumed that if any of the three amplifications were positive, as confirmed by sequencing and lineage identification, then the individual was infected. We also assume that the probability of successful amplification is the same across all samples.

To determine the proportion of false negatives, we computed the likelihood of the data given a probability, $p_{0/1}$, of no PCR amplification when the individual is infected, and P is the

probability of being infected. For example, the likelihood for an individual with two false amplifications is $L\{100\} = 3P(p_{0/1})^2$ where the factor of 3 accounts for the possibility of the first, second or third round of PCR amplification being successful. Similar expressions apply to the other three outcomes, and the total likelihood is calculated as the product over all individuals. We conducted a grid search over a range of values for P and $p_{0/1}$ to find the maximum likelihood solution. Given the relatively high frequency of {111} outcomes in the data, we found that maximum likelihood estimated of the proportion of true infections was very close to those of the proportion observed, and hence made no corrections.

3.3.1.4 Prevalence and Confidence intervals

We define prevalence as the fraction of infected individuals compared to the total number of individuals screened. We calculated prevalence of each pathogen genus and overall infection within the entire dataset, by species, and by sampling period (Spring or Fall migratory period per year). We placed binomial 95% confidence intervals on prevalence.

3.3.2 Hierarchical inferential model

3.3.2.1 Statistical model

We developed a hierarchical Bayesian inference model to estimate length and statistical support of seasonal and multi-year oscillations in pathogen prevalence. We implemented our model within Stan (Carpenter *et al.* 2017) via the *RStan* interface (Stan development team, 2016) in R version 3.6 (R core development team, 2016). Four Hamiltonian Monte Carlo chains each with 25,000 iterations were used with the first 5% discarded as burn-in. Parameter estimates are

reported with medians as well as 80% and 95% credible intervals. We modeled fraction of individuals infected across t=1,…,n time periods with a total of 48 time periods. Spring of 1996 was modeled as the first time periods with all other time periods following in succession through Fall 2019. Input data are binomial per individual bird screened with 0 indicating no evidence of infection and 1 with a positively identified infection. We tested all host species, pathogen pairings as well as each pathogen genus from the full dataset using the following model:

$$[1] \qquad \theta_t = \theta_{bg} + \beta_{ann} cos\left(2\pi\left(\frac{t}{2} - \frac{\phi_{ann}}{2}\right)\right) + \sum_{k=1}^{K} \beta_k \, cos\left(2\pi\left(\frac{t}{\lambda_k} - \phi_k\right)\right) + \epsilon_t$$

Our model specifies:

- θ: logit transformed predictor for the prevalence
    - $\theta_{bg} \in [0,10]$: background θ, the latent prevalence variable for the full dataset
    - θ_t: prevalence predictor for time period t
- β: weighting of the periodic deviance term
    - β_{ann}: seasonal periodic deviance term
    - β_k: multi-year oscillation deviance term
- ϕ: offset term (phase shift) referring to what position of the cycle the dataset begins at
    - $\phi_{ann} \in [0,1]$: seasonal offset term
    - $\phi_k \in [0,1]$: multi-year oscillation offset term
- K: number of multi-year oscillations to infer
- $\lambda \in [1,48]$: length, in time periods, of one full oscillation
- ϵ_t: deviance/error

Our model specifies that θ is the logit transformed predictor for the prevalence with θ_{bg}, the background theta, as the latent prevalence variable for the full dataset and θ_t as the prevalence predictor for time period *t*. β refers to the weighting of the periodic deviance term with β_{ann} for the within-year periodic deviance and β_k as the deviance term for multi-year oscillations. Complementing β, ϕ is the offset term which indicates what position of the cycle the dataset begins with. The within-year offset, ϕ_{ann}, will identify if birds peak in spring or fall migratory periods while ϕ_k will identify where the cycle starts in multi-year oscillations. We directed the model to infer *k*, the number of multi-year oscillations. We tested for k=2 and failed to identify multiple multi-year oscillations and therefore ran all subsequent analysis with k=1. Finally, λ denotes the length, in time periods, of one full multi-year oscillation. We recorded two time periods per year, so the model imputed length of one cycle, in years, as $\lambda/2$. The term $\beta_{ann} cos\left(2\pi\left(\frac{t}{2} - \frac{\phi_{ann}}{2}\right)\right)$ determines if there are seasonal, within-year oscillations, where prevalence consistently peaks in spring or fall migratory birds. In the within-year oscillation term, the factor of 2 in the denominator represents the two sampling periods we record per year. The term $\sum_{k=1}^{K} \beta_k \, cos\left(2\pi\left(\frac{t}{\lambda_k} - \phi_k\right)\right)$ identifies *k* multi-year, intra-annual oscillations.

We used the following prior distributions

- θ_{bg} ~ normal(1,10)
- β_{ann} ~ exponential(0.01)
- β ~ exponential(0.01)
- ϕ_{ann} ~ beta($1e^{-6}$,$1e^{-6}$)
- ϕ ~ uniform (0,1)
- λ ~ uniform (1,48)

We used a normal distribution for background θ with a range of one to ten. For λ we summarized the prior distribution as weakly informative. uniform encompassing the full range of time periods, in this case from one through 48. This allowed for the model to assess all possible lengths of multi-year oscillations. We also summarized ϕ with a uniform distribution of zero to one and ϕ_{ann} with a strongly informative beta distribution of ($1e^{-6}$,$1e^{-6}$). We used an exponential distribution with a value of 0.01 for β and β_{ann}. We tested numerous values for β and β_{ann} between 0.001 and 1 and assessed Bayes factor support to determine best values.

Finally, we inferred τ, the model imputed fraction infected per time period *t* with the following equation:

$$[2] \qquad \tau_t = \frac{1}{1 + e^{-\theta_t}}$$

3.3.2.2 Model testing

We estimated Bayes factor support for our model and evaluated according to Kass and Rafferty (1995). Bayes factors are ratios of the marginal likelihoods between two models and are commonly used for pairwise comparisons for the relative goodness of fit between two nested models. Within our null model (M_0), we reduced the prior of seasonal and multi-year cycling parameters to zero, resulting in a model with no cycling. We compared our null model against one which had seasonal cycling only (M_1), multi-year cycling only (M_2), and a model which contained both seasonal and multi-year cycling (M_3). Analysis of Bayes Factors found strongest support for the full model, M_3 (Table 3.1). We further vetted M_3 by testing a range of prior distribution values

for β and β_{ann} from which we identified an exponential distribution = 0.01 to be the optimal value for both priors.

3.3.3 Migratory bird pathogen epizootic model

In this model, we investigate epizootic events on the breeding and wintering territories and the impact these events have on pathogen prevalence dynamics. We use the results from the hierarchical inferential model to inform expected behaviors of modeled pathogen prevalence over time. Given the widespread distribution of haemosporidians, we assume all birds have equal probability of exposure to pathogens through vectors. To describe the dynamics of pathogen

Table 3.1 Model comparison results using Bayes Factors. Within our null model (M_0), we reduced the prior of seasonal and multi-year cycling parameters to zero, resulting in a model with no cycling. We compared our null model against one which had seasonal cycling only (M_1), multi-year cycling only (M_2), and a model which contained seasonal and multi-year cycling (M_3). Table **3.1A** reports log likelihood values of each model and Table **3.1B** lists comparison values between the null model (M_0) and each of the three other models (M_1, M_2, M_3).

Table 3.1A Model values

Pathogen	Host	M_0	M_1	M_2	M_3
Haemoproteus	All Birds	-1146.34	-1151.37	-1149.48	-1149.43
	Hermit	-336.54	-340.83	-342.35	-355.95
	Grey-Cheeked	-159.31	-164.61	-164.15	-178.80
	Swainson's	-520.31	-537.30	-524.03	-537.47
	Veery	-113.87	-119.04	-119.57	-133.82
Plasmodium	All Birds	-2484.85	-2482.84	-2477.04	-2490.20
	Hermit	-1150.79	-1156.42	-1155.60	-1173.57
	Grey-Cheeked	-274.20	-279.85	-280.12	-294.73
	Swainson's	-877.81	-875.79	-877.54	-890.51
	Veery	-171.91	-176.67	-174.04	-187.93
Leucocytozoon	All Birds	-2493.69	-2479.18	-2509.20	-2467.43
	Hermit	-801.06	-805.86	-803.40	-815.52
	Grey-Cheeked	-399.40	-404.86	-406.12	-420.73
	Swainson's	-1045.30	-1054.15	-1050.05	-1066.55
	Veery	-189.98	-195.25	-196.40	-210.57

Table 3.1B Comparison of model values

Pathogen	Host	M_0:M_1	M_0:M_2	M_0:M_3
Haemoproteus	All Birds	**5.03**	3.14	3.09
	Hermit	4.29	5.81	**19.41**
	Grey-Cheeked	5.3	4.84	**19.49**
	Swainson's	16.99	3.72	**17.16**
	Veery	5.17	5.7	**19.95**
Plasmodium	All Birds	-2.01	-7.81	**5.35**
	Hermit	5.63	4.81	**22.78**
	Grey-Cheeked	5.65	5.92	**20.53**
	Swainson's	-2.02	-0.27	**12.7**
	Veery	4.76	2.13	**16.02**
Leucocytozoon	All Birds	-14.51	**15.51**	-26.26
	Hermit	4.8	2.34	**14.46**
	Grey-Cheeked	5.46	6.72	**21.33**
	Swainson's	8.85	4.75	**21.25**
	Veery	5.27	6.42	**20.59**

prevalence of migratory birds experiencing two epizootic events per annual cycle, during the reproductive periods and during the overwintering period, we construct a density dependent vectored disease model. This model describes disease transmission between birds (B) and the insect vectors (V). In equations 4 and 5, β is the transmission rate to birds from the insect vectors. The rate at which birds cease to be infectious is represented by γ_B. Because the insect vectors have short life spans, we do not include a similar recovery term for the vector. In equations 6 and 7, β is the transmission rate to the insect vectors from the birds.

[4] $$\frac{dS_B}{dt} = -\beta_{BV} S_B I_V$$

[5] $$\frac{dI_B}{dt} = \beta_{BV} S_B I_V - \gamma_B I_B$$

[6] $$\frac{dS_V}{dt} = -\beta_{VB} S_V I_B$$

[7] $$\frac{dI_V}{dt} = \beta_{VB} S_V I_B$$

The initial conditions of *S* and *I* are determined with the following equations:

[8] $$S_{B,1}(0) = N_n \qquad S_{B,2}(0) = \widetilde{N_n}$$

$$I_{B,1}(0) = Z_n \qquad I_{B,2}(0) = \widetilde{Z_n}$$

where N_n represents the uninfected host density in generation *n* of fall migratory birds following the reproductive period and $\widetilde{N_n}$ represents the uninfected host density of spring migratory birds occurring just prior to the reproductive period. Additionally, Z_n represents the infected host density (carriers) in generation *n* of fall migratory birds following the reproductive period and $\widetilde{Z_n}$ represents the infected host density of spring migratory birds occurring before the reproductive period.

We define two epizootic events which occurs during the reproductive period (represented by i_1) and the non-breeding time spent on the wintering territories (represented by i_2). We connect the epizootic periods and inter-epizootic periods by defining:

[9] $$i_1(N_n, Z_n) = 1 - \frac{S_B(T)}{S_B(0)}$$

$$i_2\left(\widetilde{N_n}, \widetilde{Z_n}\right) = 1 - \frac{S_B(T)}{S_B(0)}$$

Here i_1 is the cumulative fraction of birds infected during the epizootic on the breeding grounds, i_2 is the cumulative fractions of birds infected while overwinters, and T represents the end of the epizootic.

Finally, we complete our model by nesting the ordinary differential equations within the difference equations to produce the following equations:

[10] $$N_{n+1} = \lambda_1\left(1 - i_2\left(\widetilde{N_n}, \widetilde{Z_n}\right) + \theta i_2\left(\widetilde{N_n}, \widetilde{Z_n}\right)\right)\widetilde{N_n} + \lambda_2\psi(1-\theta)i_2\left(\widetilde{N_n}, \widetilde{Z_n}\right)\widetilde{N_n} + \omega N_n$$

$$Z_{n+1} = \phi\widetilde{N_n}i_2\left(\widetilde{N_n}, \widetilde{Z_n}\right)(1-\psi)$$

[11] $$\widetilde{N_n} = \left(1 - i_1(N_n, Z_n)\right)N_n + \theta i_1(N_n, Z_n)N_n$$

$$\widetilde{Z_n} = (1-\theta)i_1(N_n, Z_n)N_n$$

In equations [10] and [11] λ_1 represents the fecundity of naïve birds and λ_2 represents the fecundity of birds that were previously infected but no longer actively transmitting pathogens birds in units of uninfected offspring. The fraction of birds infected in the epizootic event that eventually clear infection is denoted by θ. The fraction of birds that survive their offspring are represented by ψ and ω refers to the fraction of birds that survive until the following time period. ϕ represents the fecundity of infected hosts in units of infected offspring; when chronically infected hosts give rise to acutely ill offspring.

3.4 RESULTS

3.4.1 Basic prevalence numbers

In total we screened 4,306 individuals (Grey-cheeked n=596, Hermit n=1,786, Swainson's n=1,578, Veery n=386) originally collected between 1996 and 2019 (Figure 3.1, Figure 6.1). We screened 3,314 birds collected during fall migration and 992 birds collected throughout the spring migratory period. Individuals screened per year varied between 30 individuals in 1997 and 434 individuals in 2019 (Table 6.2). In total, 2,240 birds (52%) were infected with at least one haemosporidian genus (Table 3.2). *Plasmodium* (Figure 6.1A) infections were detected in 26.87% of all birds and we recovered a total of 80 unique *cytochrome b* lineages, with one common lineage constituting 67% of the total. We detected *Haemoproteus* (Figure 6.1B) infections in 7.52% of birds and recovered 72 lineages of which three were common, representing 63% of all lineages. *Leucocytozoon* was the most diverse pathogen with 187 lineages recovered from 27% of all birds. Four lineages of *Leucocytozoon* were designated as common and represented 65% of all lineages recovered (Figure 6.1C, Table 6.1). Prevalence varied from 0%-100% depending on the time period, host species, and pathogen genus (Figure 3.2, Figure 6.2

Table 3.2 Overall prevalence values of host-pathogen pairs, derived from data collected from migratory bird specimens.

	Grey-Cheeked	Hermit	Swainson's	Veery	All Birds
Plasmodium	16.39%	35.13%	24.30%	15.27%	26.83%
Haemoproteus	6.62%	4.57%	10.90%	7.89%	7.54%
Leucocytozoon	36.09%	16.56%	37.95%	18.58%	27.24%
All Pathogens	50.00%	48.78%	60.31%	32.06%	52.02%

3.4.2 Accuracy of PCR diagnostic test

For the primer set that amplified *Haemoproteus* and *Plasmodium*, we saw 2709 samples with no PCR positive for the pathogen (outcome = {000}) in the three independent nested PCR diagnostic test, 614 samples with one positive ({100}), 366 samples with two positives ({110}), and 645 samples with positives from all three PCR diagnostic tests ({111}). These results indicate that each individual PCR diagnostic had an average probability of getting a false negative result of 19.0%. The possibility of getting a false negative per sample, taking into account the three independent PCR diagnostic tests, was 0.69% (=0.19^3). The results for the primer set amplifying *Leucocytozoon* DNA are as follows: {000}=2919, {100}=398, {110}=313, and {111}=704. The per PCR possibility of false negative is 16.0% and the per sample probability of recording a false negative is 0.41%.

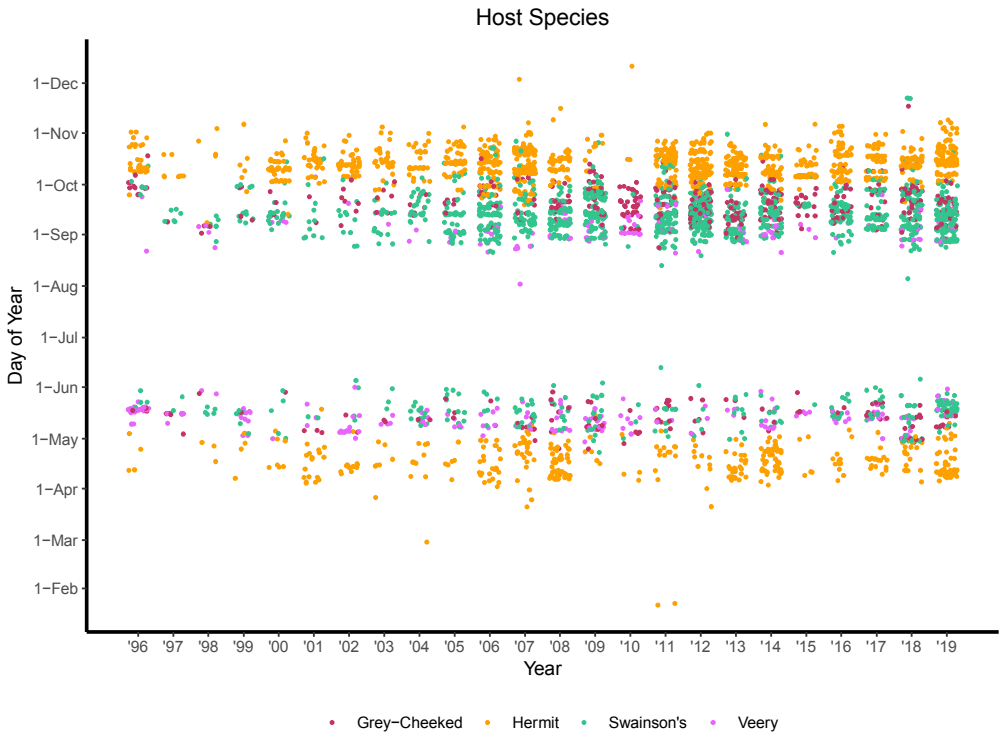


Figure 3.1 Distribution of host species over time, spanning spring and fall migratory periods of 1996-2019. Each dot represents a single bird and is colored according to host species. Fall migratory birds are represented in the upper band, spring migratory birds are represented in the lower band.

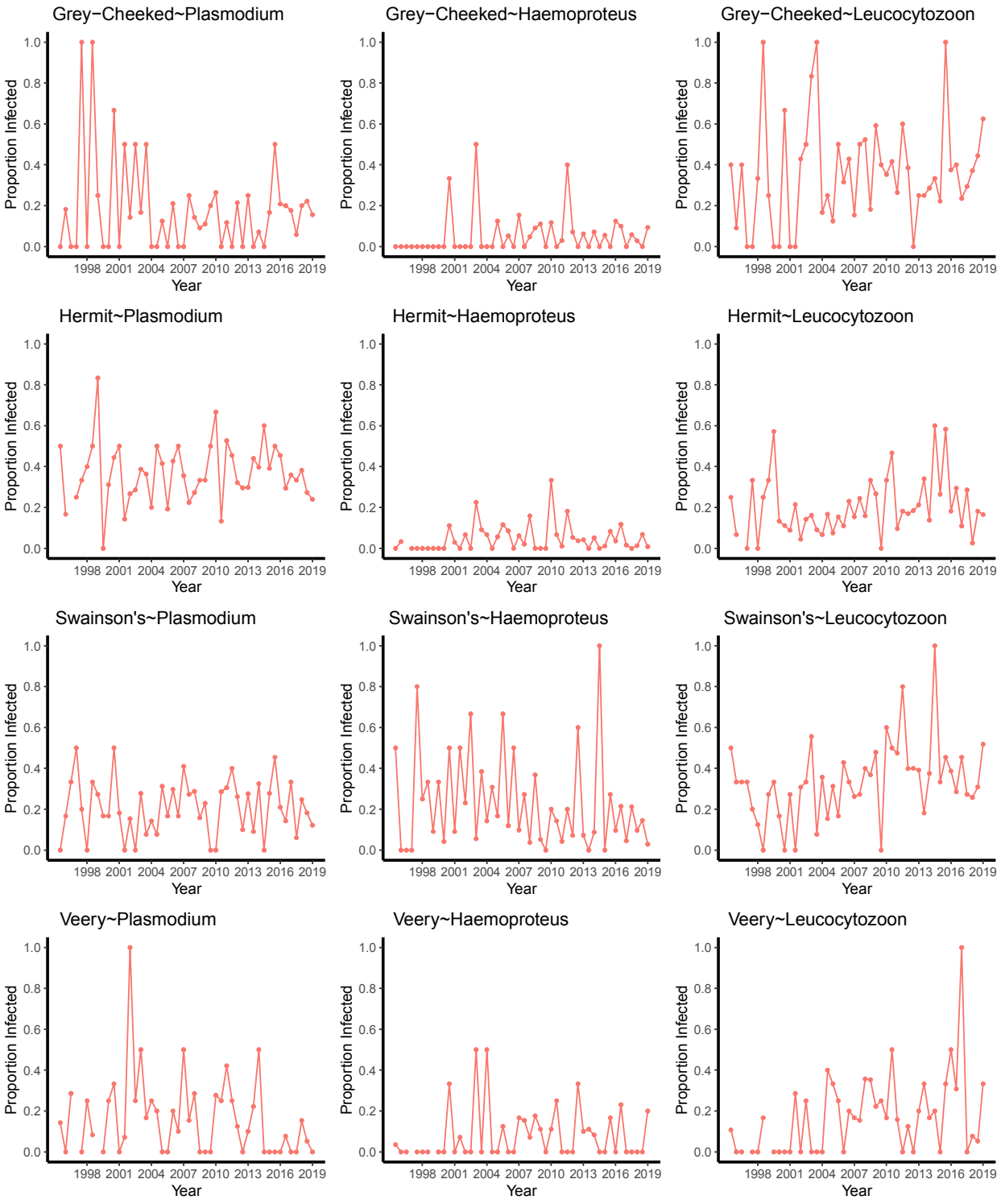


Figure 3.2 Observed proportion infected of all host-pathogen pairs. The observed proportion infected per migratory period is noted with a point and successive migratory periods are connected by with a line. There were no birds sampled and therefore no data for one migratory period with Hermits and two time periods with Veerys.

3.4.3 Hierarchical inferential model

3.4.3.1 Seasonal variation

Across the full dataset, all host-pathogen pairs displayed evidence of seasonality. *Plasmodium* exhibited distinct seasonality in all host species, with prevalence consistently higher in the fall migratory period than spring. Seasonality of *Haemoproteus* showed slightly weaker seasonal signals than *Plasmodium*, with prevalence peaking more in the fall than spring, except in Swainson's Thrush which was higher in the spring. *Leucocytozoon* shows a strong signal of

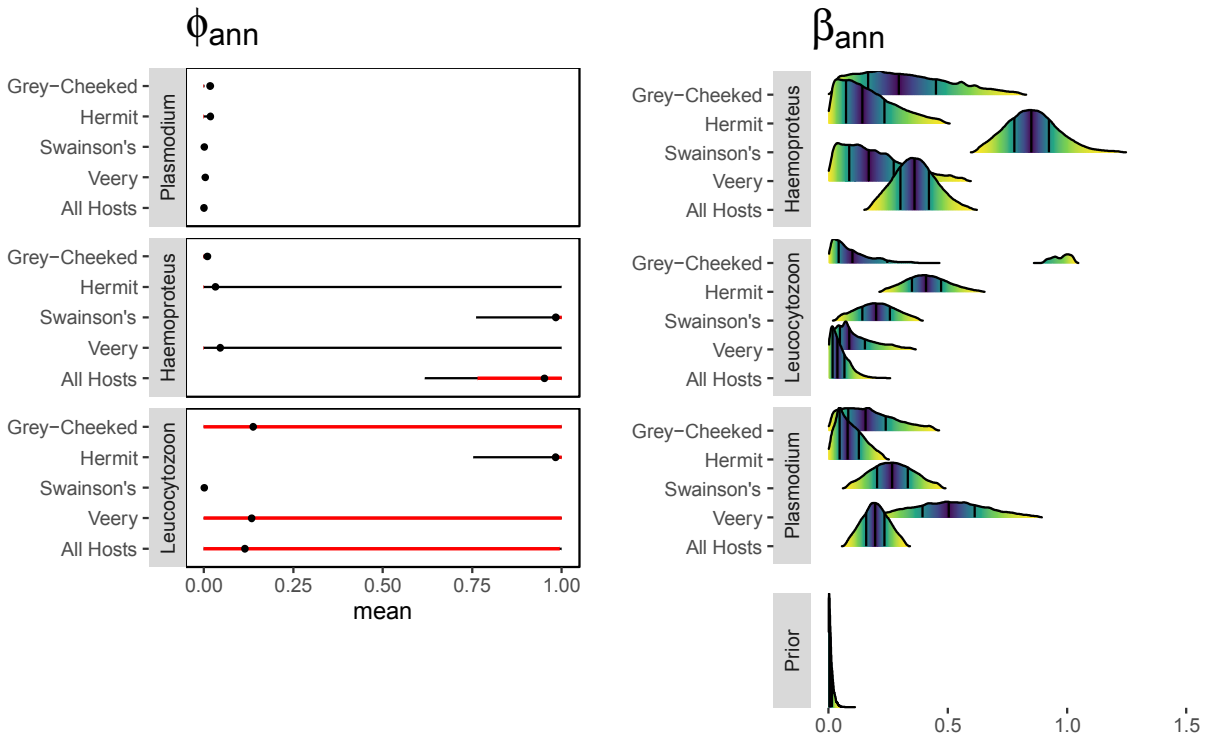


Figure 3.3 The posterior probability distribution of the seasonal offset term (ϕ_{ann}) and the magnitude of the seasonal effect (β_{ann}). The seasonal offset term indicates whether the prevalence of the pathogen consistently peaks in the spring (mean value near 1.0) or fall migratory period (mean value near 0). The mean value of the posterior distribution is represented with a black dot while the 80% credible intervals (CIs) or 95% CIs are represented by red and black bars, respectively. The magnitude of the seasonal offset is visualized with through density plots with the outer black vertical bars within each density plot representing the 80% credible intervals and the middle vertical bar representing the median value of the posterior. The expected prior distribution, set at exponential (0.01), is represented in the bottom panel.

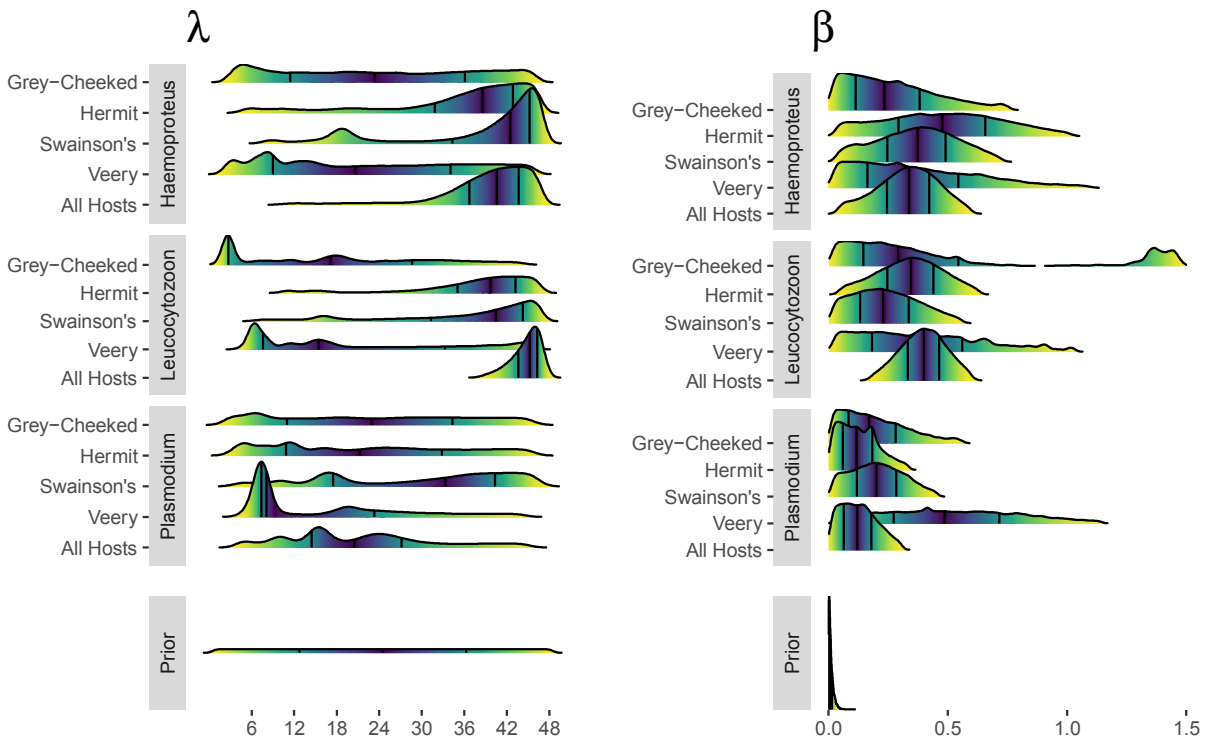


Figure 3.4 The posterior probability distribution of multi-year oscillation length (λ) and the magnitude of the multi-year oscillation (β). The posterior distributions for each parameter are visualized as density plots, with the outer black vertical bars representing the 80% credible intervals and the middle vertical bar representing the mean value of the posterior. The expected prior distributions (for λ: uniform(1,48); for β: exponential(0.01)) are represented in the bottom panels.

peaking in the fall in Swainson's Thrush, with slightly weaker signals for fall peaks in Grey-Cheeked and Veery, and Hermit thrushes had higher prevalence levels of *Leucocytozoon* in the spring (Figure 3.3, Table 6.1). When analyzing seasonality of pathogen genus across all host species, *Plasmodium* and *Leucocytozoon* displayed higher prevalence in the fall migratory period while *Haemoproteus* was consistently highest in the spring.

3.4.3.2 Multi-year oscillations

Multi-year oscillations spanned approximately 8-18 years depending on the host pathogen pair (Figure 3.4, Figure 3.6, Table 6.2). In no subset did the 95% credible intervals span to either 1 or 48. The shortest length of prevalence cycling in prevalence occurred with *Plasmodium* in Veerys, which spanned approximately 8 years (λ= 16.31, 95% Credible Interval [C.I.] = 3.68, 45.41). *Leucocytozoon* in Grey-Cheeked thrushes peaked approximately every nine years (λ= 17.82, 95% C.I. = 2.0, 45.31). The longest oscillation in pathogen prevalence occurred in Hermits infected with *Leucocytozoon* (λ= 18.8, 95% C.I. = 24.17, 46.41). With all three pathogen genera, Veerys generally displayed the shortest oscillations while Swainson's displayed the longest (Figure 3.5, Table 6.2). Cycles in *Leucocytozoon* (λ= 22.5) and *Haemoproteus* (λ= 19.5) in all hosts were longer than compared to cycle length in a specific host species, while *Plasmodium* (λ= 10.2) occurred between the upper and lower bounds of what occurred in individual species (Figure 3.5).

Migratory bird density-dependent epizootic model

In this model we assessed epizootics that occurred on breeding and non-breeding periods of the annual cycle. We used the results of the hierarchical statistical model to inform expected behaviors, and therefore model variables (Equation 1). This model accurately reproduces host population dynamics when influenced by the spread of a density dependent pathogen (Figure 3.6, Table 3.3). We found that four variables in particular influenced the length of a population cycle: $\lambda_1, \lambda_2, \phi,$ and ω. Regardless of cycle length, the net fecundity of uninfected birds (λ_1) was higher that of infected birds (λ_2), which is consistent with previous research into the effects of haemosporidian infection on reproductive success (Asghar *et* al. 2015, Bosholn *et al.* 2016).

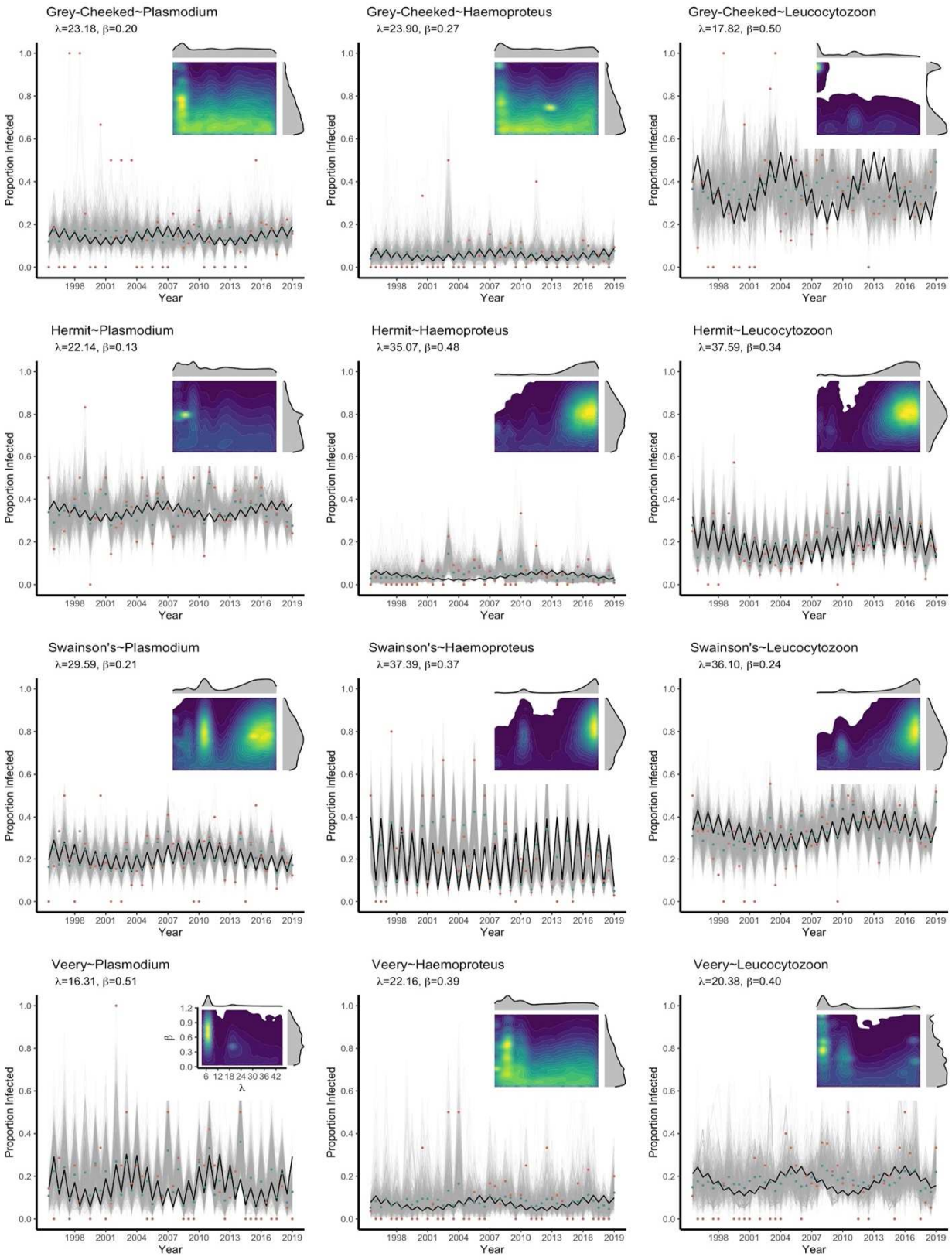


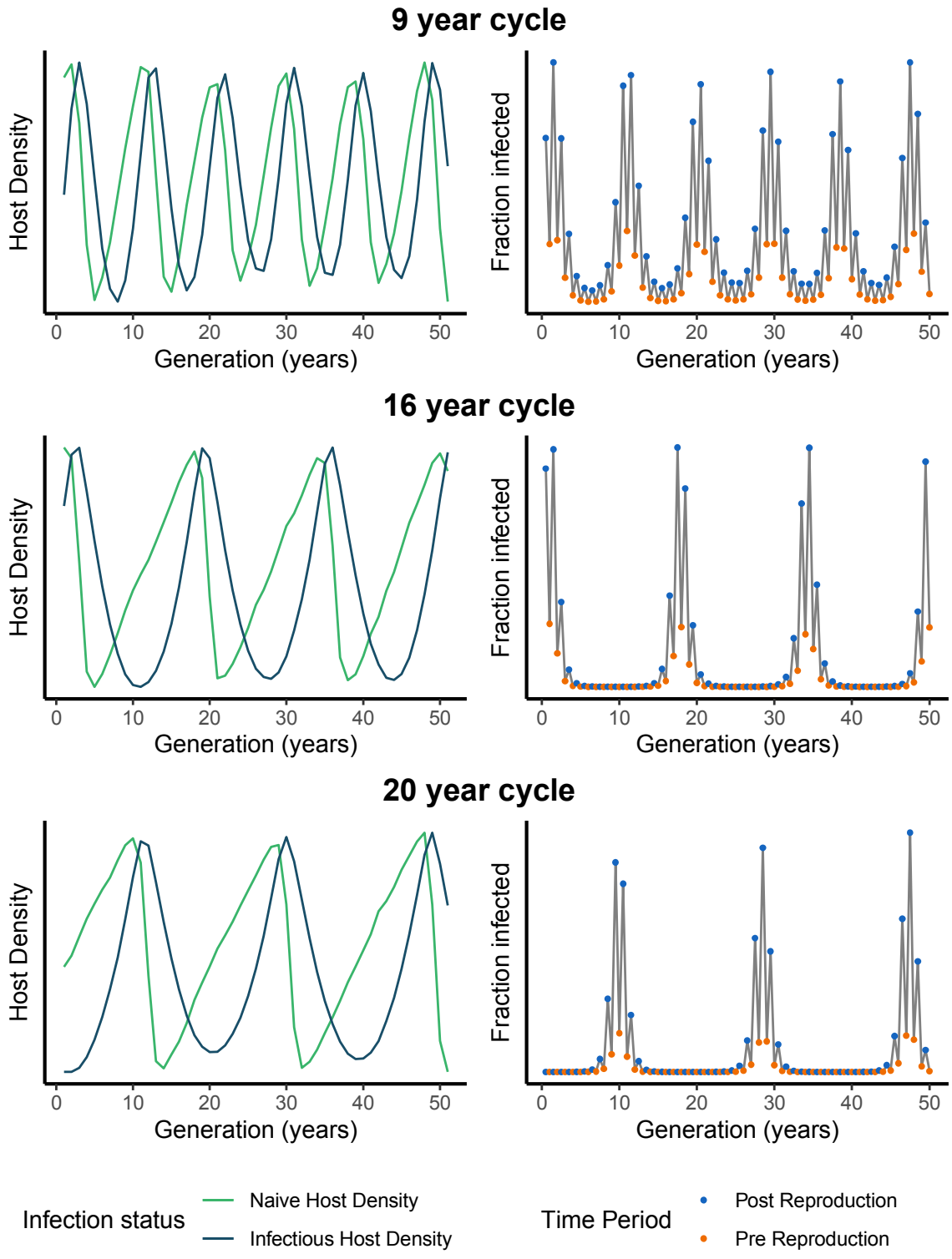
Figure 3.5 Hierarchical statistical model output for each host-pathogen pair. The observed prevalence is represented by the red points, the model inferred underlying prevalence per time

Figure 3.5 (continued) period is represented by cyan points, the median model fit is shown with the black lines throughout with 1000 random samples of the model fit shown in grey. The upper right inset panel shows the posterior distributions for the length of multi-year oscillations (λ) and magnitude of the oscillations (β).

Table 3.3 Example parameter values for which the migratory bird epizootic model results in 9, 16, and 20 year cycles.

Parameter	9-year cycle	16-year cycle	20-year cycle	Parameter explanation
γ	0.5	0.5	0.5	proportion of birds not transmitting pathogen
β_{BV}	0.1	0.1	0.1	transmission rate to birds from vectors
β_{VB}	0.001	0.001	0.001	transmission rate to vectors from birds
λ_1	1.15	1.01	1.0001	fecundity of naïve birds
λ_2	0.5	0.1	0.1	fecundity of infected but not transmitting birds
ϕ	25	50	100	fecundity of hosts in units of infected offspring
ω	0.15	0.145	0.125	fractions of birds surviving to next time period
ψ	1	1	1	fraction of birds that survive offspring

Figure 3.6 Density dependent migratory bird epizootic model dynamics modeled on 9, 16, and 20 year cycles. The left panels visualize naïve and infectious host density over time. The right panels represent the fraction of infected hosts over time with two time periods per generation (year), the post reproduction period and pre reproduction period.



3.5 DISCUSSION

Understanding how pathogens spread across host populations is a key aim in epidemiology. Time series data are essential to elucidating patterns of variation in prevalence and identifying mechanisms which influence these patterns. In this study we use an extensive collection of migratory bird specimens to survey haemosporidian pathogens in spring and fall migrants over a 24-year time span. Our analysis of avian haemosporidian pathogens in migratory *Catharus* thrushes suggests that pathogens peak in prevalence every eight to 18 years, exhibit distinct seasonality, and we show how a density-dependent transmission dynamics model can replicate features of the observed data. Additionally, pathogen periodicity varies depending on the host species, indicating mechanisms likely specific to host-pathogen pairs. We discuss our results and possible influencing factors in relation to seasonality, multi-year oscillations, and heterogeneous periodicity of migratory bird pathogens.

3.5.1 Seasonal variation

Vector borne pathogens are among those most likely to exhibit seasonal variation (Altizer *et al.* 2006) and avian haemosporidians appear to be no exception based on this study and previous work (Bensch *et al.* 2007, Hellgren *et al.* 2013, Pulgarin *et al.* 2019). The majority of host-pathogen pairs in this study exhibit consistently higher prevalence during fall migration than in the spring, with the exception of *Haemoproteus* in Swainson's thrush and *Leucocytozoon* in Hermit thrush. We also replicate seasonally fluctuating behaviors in the density-dependent epizootic model. Previous work on haemosporidians in North America migratory birds revealed within year prevalence profiles which peak during or shortly after the breeding period (Pulgarin *et al.* 2019). Relatively higher prevalence of haemosporidians has been observed in the Garden Warbler (*Sylvia borin)*, a migrant to sub-Saharan Africa, at the breeding grounds in Sweden when compared to

other portions of the migratory cycle, suggesting the patterns observed in this study may be relevant beyond Nearctic-Neotropical migrants (Hellgren *et al.* 2013).

Migratory birds are exposed to different pathogen communities throughout the annual cycle and may be especially susceptible to infection due to decreased immune capacity induced by the stress of migration (Owen and Moore 2006, Reperant *et al.* 2011). Additionally, haemosporidian pathogens are ubiquitous in most avian populations worldwide (Valkiūnas 2004). Therefore, one might hypothesize that migratory birds have a similar rate of susceptibility to infection throughout the annual cycle. However, given the seasonal differences observed in this study as well as in other species of birds (Hellgren *et al.* 2013, Ferreira Junior *et al.* 2017, Rivero de Aguilar *et al.* 2018, Pulgarin *et al.* 2019), we hypothesize that ecological and evolutionary factors, such as pathogen adaptations for increased infectiousness within local host communities and barriers to dispersal likely inhibit the interchange of haemosporidians across geographically separated areas and between birds of disparate taxonomic groups. A comparison of over-wintering migratory birds and permanent residents in the Caribbean revealed that each group housed distinct haemosporidian pathogen communities with minimal overlap (Soares *et al.* 2020), indicating minimal levels of transmission between resident and non-resident hosts. Accordingly, few lineages commonly found in Neotropical birds were recovered from the thrushes. Therefore, we suggest that geographical and host specificity of avian haemosporidians contribute to the seasonality observed with reduced transmission on stopover and overwintering sites by lineages that are common in neotropical but rare in temperate habitats.

3.5.2 Multi-year oscillations

Few studies of avian haemosporidians have spanned a time period long enough to confidently identify prevalence cycles of eight years or longer. When investigating *Haemoproteus* and *Plasmodium* in great reed warblers (*Acrocephalus arundinaceus*), Bensch *et al.* (2007) identified a periodicity of 3-4 years in a study that occurred on the summer breeding territories between 1987 to 2003 using autoregression analysis. The periodicity observed in great reed warblers is substantially smaller than observed in *Catharus* thrushes here. However, haemosporidian transmission appeared to not occur in great reed warblers on the breeding grounds, but likely only occurs at overwintering sites in sub-Saharan Africa (Bensch *et al*. 2007). The lack of transmission on the breeding grounds could influence the density dependent dynamics of haemosporidians in these birds in comparison to *Catharus* thrushes. This further supports the view that host specific differences influence prevalence and transmission dynamics of avian haemosporidians. A survey of *Plasmodium* in boreal resident species of Alaska collected during the non-breeding period over ten years (2001-2010) recorded one noticeable peak in prevalence over the course of the survey (Wilkinson, Handel *et al.* 2018). Due to the length of the study, it is impossible to tell if this is a recurrent or singular phenomenon, yet a single noticeable peak in prevalence over a period of ten years is not inconsistent with the cycles of at least eight years observed in this study.

Our models imply that pathogen prevalence peaks every eight to 18 years. In an attempt to understand mechanisms driving this apparent cyclical variation, we built a density-dependent disease transmission model. In this model, the periodicity of pathogen prevalence is impacted most by four parameters relating to host population size, including host net fecundity. The net fecundity of naïve (λ_1) and infected but not transmitting (λ_2) birds could be impacted by local climactic conditions, abundance of food sources, or predator populations (Ghalambor and Martin 2001,

Jenouvrier *et al.* 2005, Nagy and Holmes 2005). Chronic infections by avian haemosporidians were long thought to be relatively innocuous in the majority of bird species (Bensch *et al.* 2007). However, we now know that survival rate (ω) can be reduced by pathogen infection (Altizer *et al.* 2011) and recent work has shown that the effect of chronic haemosporidian infection can reduce an individual bird's reproductive capabilities by impacting both mating capabilities and fledgling success (Merino *et al.* 2000, Knowles *et al.* 2010, Bosholn *et al*. 2016). Additionally, the negative effects of haemosporidian infection may be passed on to the next generation, as Asghar *et al.* (2015) found when comparing the telomere lengths, an indicator of longevity, in chicks from infected and infected mothers. The results of our study model the effects of haemosporidian infections over time and demonstrate that an increase in host population size will lead to a subsequent increase in infected host density, followed by a population decrease of both infected and naïve host population density. Our results are consistent with the proposition that haemosporidian infections do indeed have a negative impact on the overall host population. As infection prevalence increases, the effects will eventually lead to a reduction in population density, possibly due to decreased individual and net fecundity.

3.5.3 Heterogeneous periodicity

Heterogeneity in periodicity across host-pathogen pairs may be due to host characteristics, such as genetic or behavioral, or environmental conditions including variation in microclimates across habitats (Scordato and Kardish 2014, Barrow *et al.* 2019, McNew *et al.* 2021). Different lineages of avian haemosporidians exhibit varying levels of host specificity (Galen and Witt 2014), with some evidential support that different lineages generate different patterns of periodicity (Bensch *et al.* 2007, Wilkinson, Handel *et al*. 2018). In our study, the maximum likelihood

solutions differed across most host-pathogen pairs, suggesting oscillations at different lengths of time and those that did have similar periodicity lengths were not synchronized. Although thrushes in this study are closely related with similar ecologies, certain species-specific characteristics may be driving the differences. For example, Veerys exhibited shorter cycling patterns (8-10 years) than Swainson's (14.8-18.8 years). Veerys are primarily ground nesters and feeders while Swainson's are near ground foragers, generally around 1m off the ground, and nest in the understory. Several previous studies have shown that nest height and foraging strata impact likelihood of haemosporidian infection (Fecchio *et al.* 2013, Lutz *et al.* 2015). Birds occupying different strata of their habitat encounter different insect populations which may be driving some of the variation in cycling length between the species.

The birds included in this study were collected across a relatively small area of Chicago, but the areas in which they originated from span broad geographic regions. While most of these thrushes spent the breeding period in what can broadly be categorized as boreal forests, variations in local temperature and precipitation likely influence vector abundance and therefore promote or hinder spread of pathogens (McNew *et al.* 2021). Habitat types of the overwintering sites are numerous and include savannah, rain forest, and mangroves. Haemosporidian prevalence and diversity is heavily impacted by local climatic conditions (Wilkinson, Handel *et al*. 2018, McNew *et al.* 2021). This introduces an important caveat in that the results we observe here are an aggregate of phenomena occurring within the many habitats in which the birds exist throughout their annual cycle.

3.6 CONCLUSION

Our time series data has allowed us to gain insight into the temporal dynamics and plausible transmission mechanisms of avian haemosporidians in migratory *Catharus* thrushes. We used a novel source of data in the form of curated museum specimens as a resource for investigating disease ecology and we developed an adaptable inferential statistical model to assess data driven periodicity in pathogen prevalence. Additionally, we used the results of the statistical model to inform a theoretical disease model, which accurately assumes density-dependent behaviors of disease transmission. In conclusion, we demonstrate the value of natural history collections and biorepositories, given their potential importance for accurate prediction of disease dynamics in wild populations of animals.

4 Supplemental Tables, Chapter 1

Table 4.1 Host associated metadata including sampling date and location for each individual per sampling period, including age (SY = second year, ASY = after second year) and sex (M = male, F = female).
†Denotes non-tagged birds from the 2018 first recapture period.
‡For samples included in the comparison of alpha diversity over time in the first recapture period in Michigan individual's date of arrival is included.

			Cat Island, The Bahamas			First Recapture in Michigan				Second Recap. in Michigan		
Host ID	**Age**	**Sex**	**Date of Capt.**	**Lat.**	**Long.**	**Arrival in MI‡**	**Date of Cap.**	**Lat.**	**Long.**	**Date of Cap.**	**Lat.**	**Long.**
5	SY	M				27-5-18	1-6-18	44.45	-84.30			
6	ASY	F	24-3-18	24.62	-75.63					1-7-18	44.59	-83.99
7	SY	M	23-3-18	24.66	-75.74							
8	SY	M	25-3-18	24.45	-75.54							
9	ASY	F	26-3-18	24.58	-75.64							
11	ASY	M	30-3-18	24.59	-75.64							
13	SY	F	26-3-18	24.44	-75.54							
14	SY	M	30-3-18	24.60	-75.63							
15	SY	M	25-3-18	24.44	-75.54		12-6-18	44.57	-84.27			
16	SY	M	30-3-18	24.61	-75.63	15-5-18	20-5-18	44.54	-84.84	11-7-18	44.54	-84.84
17	SY	M	28-3-18	24.54	-75.62		6-6-18	44.58	-84.02	1-7-18	44.58	-84.02
18	SY	F	3-4-18	24.65	-75.64							
19	SY	M	27-3-18	24.61	-75.64							
20	SY	M	30-3-18	24.56	-75.64	15-5-18	20-5-18	44.45	-84.30			
21	SY	M	29-3-18	24.66	-75.74							
22	SY	M					NA					
23	SY	F	29-3-18	24.47	-75.55							
24	SY	M	4-4-18	24.48	-75.57	17-5-18	20-5-18	44.59	-84.55			
26	SY	M	31-3-18	24.44	-75.53		25-6-18	44.50	-84.33			

Table 4.1 continued

Host ID	Age	Sex	Cat Island, The Bahamas			First Recapture in Michigan				Second Recap. in Michigan		
			Date of Capt.	Lat.	Long.	Arrival in MI‡	Date of Cap.	Lat.	Long.	Date of Cap.	Lat.	Long.
27	SY	F	4-4-18	24.61	-75.63					4-7-18	44.51	-84.21
28	SY	M	30-3-18	24.57	-75.62	17-5-18	20-5-18	44.55	-84.41			
29	SY	M	30-3-18	24.60	-75.64					6-7-18	44.55	-84.41
30	SY	M	2-4-18	24.52	-75.57							
32	SY	F	21-4-18	24.47	-75.55							
33	ASY	M	4-4-18	24.49	-75.57	14-5-18	20-5-18	44.55	-84.40			
35	SY	F	12-4-18	24.64	-75.64		26-6-18	44.33	-83.60			
38	SY	F	31-4-18	24.46	-75.55							
39	SY	F	1-4-18	24.44	-75.54							
40	SY	M	13-4-18	24.64	-75.64							
41	SY	M	8-4-18	24.66	-75.74	28-5-18	6-6-18	44.42	-83.68			
42	SY	M					1-6-18	44.65	-83.87			
43	SY	M	12-4-18	24.64	-75.64	17-5-18	23-5-18	44.50	-83.57	2-7-18	44.50	-83.57
44	SY	M	14-4-18	24.56	-75.64					4-7-18	44.46	-84.31
45	SY	M	13-4-18	24.64	-75.65	16-5-18	22-5-18	44.66	-83.95			
46	ASY	M	7-4-18	24.56	-75.64	14-5-18	17-5-18	44.36	-83.57			
47	ASY	M	4-4-18	24.49	-75.57							
48	SY	M	6-4-18	24.66	-75.65	14-5-18	17-5-18	44.50	-84.30			
50	SY	F	6-4-18	24.66	-75.65							
51	SY	F	5-4-18	24.44	-75.54							
54	SY	F	22-4-18	24.65	-75.64							
55	ASY	F	9-4-18	24.67	-75.76							
56	ASY	M	12-4-18	24.64	-75.65							
58	SY	M	11-4-18	24.59	-75.64	16-5-18	20-5-18	44.56	-84.40			
59	SY	M	10-4-18	24.65	-75.64		6-6-18	44.41	-83.69			
61	SY	M	12-4-18	24.66	-75.65	13-5-18	13-5-18	45.16	-84.19	10-7-18	45.16	-84.19
66	ASY	M	17-4-18	24.54	-75.63							
71	ASY	M	10-4-18	24.64	-75.64	9-5-18	14-5-18	44.55	-84.36			

Table 4.1 continued

Host ID	Age	Sex	Cat Island, The Bahamas			First Recapture in Michigan				Second Recap. in Michigan		
			Date of Capt.	Lat.	Long.	Arrival in MI‡	Date of Cap.	Lat.	Long.	Date of Cap.	Lat.	Long.
86	ASY	M	13-4-18	24.64	-75.64							
87	ASY	M								11-7-18	44.49	-84.29
91	ASY	M	13-4-18	24.64	-75.64	24-5-18	1-6-18	44.86	-84.39			
98	SY	M	24-4-18	24.62	-75.63							
99	ASY	M	8-4-18	24.66	-75.74		13-6-18	45.21	-84.13	10-7-18	45.21	-84.13
100	ASY	M	2-4-18	24.47	-75.55							
101	SY	M	1-4-18	24.44	-75.54							
103	ASY	M				21-5-18	23-5-18	44.56	-83.56			
104	SY	F	31-4-18	24.62	-75.63							
45219	SY	M	14-4-17	24.66	-75.69							
45220	ASY	M	13-4-17	24.45	-75.55	18-5-17	22-5-17	44.48	-84.28			
45221	SY	M	13-4-17	24.45	-75.55							
45222	SY	M	12-4-17	24.54	-75.59							
45223	SY	M	9-4-17	24.66	-75.74							
45224	ASY	M	9-4-17	24.66	-75.74							
45225	ASY	M	8-4-17	24.58	-75.64	16-5-17	24-5-17	44.55	-84.41	7-7-17	44.55	-84.41
45226	ASY	M	7-4-17	24.56	-75.63	17-5-17	20-5-17	44.36	-84.37	9-7-17	44.36	-84.37
45227	ASY	M	6-4-17	24.57	-75.62							
45228	SY	M	6-4-17	24.57	-75.63	27-5-17	28-5-17	44.55	-84.37			
45230	ASY	M	5-4-17	24.66	-75.65							
45231	ASY	M	5-4-17	24.66	-75.65					2-7-17	44.62	-84.64
45232	ASY	M	4-4-17	24.66	-75.65							
45233	SY	M	4-4-17	24.65	-75.64							
45234	SY	M	3-4-17	24.38	-75.51							
45235	ASY	M	2-4-17	24.61	-75.64							
45236	ASY	M	1-4-17	24.62	-75.63							
45237	ASY	M	1-4-17	24.62	-75.63	22-5-17	23-5-17	45.21	-84.20	8-7-17	45.21	-84.20
45238	ASY	M	31-3-17	24.58	-75.64							

Table 4.1 continued

Host ID	Age	Sex	Cat Island, The Bahamas			First Recapture in Michigan				Second Recap. in Michigan		
			Date of Capt.	Lat.	Long.	Arrival in MI‡	Date of Cap.	Lat.	Long.	Date of Cap.	Lat.	Long.
45239	ASY	M	30-3-17	24.61	-75.63							
45241	SY	F	29-3-17	24.61	-75.63							
45242	NA	F	29-3-17	24.60	-75.64							
45411	ASY	M	29-3-17	24.44	-75.54	2-6-17	5-6-17	44.41	-83.67			
45412	SY	M	29-3-17	24.44	-75.54							
45413	ASY	M	30-3-17	24.45	-75.54	23-5-17	25-5-17	44.48	-84.28			
45416	SY	M	31-3-17	24.45	-75.54		29-5-17	44.58	-84.54	10-7-17	44.58	-84.54
45418	SY	M					31-5-17	24.45	-75.54			
45421	ASY	M	1-4-17	24.52	-75.58							
45422	SY	M				16-5-17	22-5-17	24.52	-75.58	3-7-17	44.48	-84.28
45423	SY	M	2-4-17	24.52	-75.57	21-5-17	23-5-17	45.21	-84.20			
45425	SY	M	3-4-17	24.61	-75.64							
45426	ASY	M	4-4-17	24.65	-75.64							
45427	ASY	M				23-5-17	29-5-17	44.54	-84.27			
45428	SY	M				18-5-17	26-5-17	44.51	-83.53			
45429	SY	M	6-4-17	24.66	-75.65							
45431	SY	M	7-4-17	24.51	-75.58							
45432	ASY	M	7-4-17	24.54	-75.63	17-5-17	26-5-17	45.15	-84.18			
45435	ASY	M	8-4-17	24.47	-75.55							
45437	SY	M	9-4-17	24.46	-75.55	3-6-17	6-6-17	44.47	-84.35			
45438	ASY	M	1-4-17	44.40	-84.39	18-5-17	22-5-17	44.40	-84.39			
45441	SY	M	12-4-17	24.44	-75.53					7-7-17	44.55	-84.36
45443	ASY	M	13-4-17	24.19	-75.31							
45445	SY	F	13-4-17	24.19	-75.31							
45446	ASY	M	14-4-17	24.13	-75.48		28-5-17	44.35	-83.61			
45447	SY	M	16-4-17	24.59	-75.64	22-5-17	24-5-17	44.55	-84.42			
45448	SY	M				18-5-17	23-5-17	45.15	-84.18			
45472	SY	M								10-7-17		

Table 4.1 continued

Host ID	Age	Sex	Cat Island, The Bahamas			First Recapture in Michigan				Second Recap. in Michigan		
			Date of Capt.	Lat.	Long.	Arrival in MI‡	Date of Cap.	Lat.	Long.	Date of Cap.	Lat.	Long.
62868	NA	U					20-5-18					
62881†	SY	M					13-5-18					
62882†	ASY	M					13-5-18					
62883†	ASY	M					14-5-18					
62886†	SY	M					15-5-18					
62887†	ASY	M					15-5-18					
62888†	SY	M					15-5-18					
62889†	SY	M					15-5-18					
62890†	ASY	M					15-5-18					
62891†	SY	M					16-5-18					
62892†	SY	M					16-5-18					
62894†	ASY	M					17-5-18					
62895†	ASY	M					17-5-18					

Table 4.2 Relative abundance of each bacterial phyla (highlighted in gray) and classes with the standard deviation listed in parentheses. Phyla and classes are listed in order of most abundant in the full dataset. Relative abundances were calculated for the full dataset and within individual sampling periods.

Phylum	Class	All Samples	The Bahamas	First Recapture in Michigan	Second Recapture in Michigan
Firmicutes		**38.08% (±15.41%)**	**39.82% (±13.87%)**	**38.12% (±16.41%)**	**29.02% (±17.38%)**
	Clostridia	31.98% (±15.41%)	34.51% (±14.24%)	30.26% (±15.15%)	24.43% (±19.34%)
	Bacilli	5.06% (±9.92%)	4.15% (±6.66%)	6.93% (±14.47%)	3.93% (±4.89%)
	Erysipelotrichia	0.63% (±1.29%)	0.83% (±1.45%)	0.47% (±1.11%)	0.17% (±0.64%)
	Negativicutes	0.39% (±0.74%)	0.33% (±0.49%)	0.47% (±0.94%)	0.49% (±1.05%)
Proteobacteria		**30.76% (±20.48%)**	**23.84% (±13.85%)**	**36.87% (±22.25%)**	**47.07% (±27.90%)**
	Gammaproteobac.	21.09% (±18.40%)	14.99% (±11.55%)	25.97% (±20.95%)	37.39% (±24.39%)
	Alphaproteobac.	8.96% (±11.62%)	7.92% (±7.03%)	10.54% (±16.35%)	9.33% (±12.92%)
	Deltaproteobac.	0.68% (±1.34%)	0.90% (±1.52%)	0.44% (±1.12%)	0.35% (±0.67%)
Bacteroidetes		**17.10% (±10.03%)**	**17.77% (±9.41%)**	**16.75% (±10.54%)**	**14.80% (±11.60%)**
	Bacteroidia	17.09% (±10.03%)	17.75% (±9.41%)	16.73% (±10.55%)	14.80% (±11.60%)
	Ignavibacteria	0.01% (±0.08%)	0.01% (±0.05%)	0.02% (±0.13%)	0.00% (±0.00%)
	Rhodothermia	0.01% (±0.06%)	0.01% (±0.08%)	0.00% (±0.00%)	0.00% (±0.00%)
Actinobacteria		**7.34% (±5.92%)**	**9.72% (±6.66%)**	**4.63% (±2.82%)**	**3.66% (±2.86%)**
	Actinobacteria	4.92% (±3.94%)	6.28% (±4.33%)	3.40% (±2.61%)	2.68% (±2.39%)
	Coriobacteriia	1.49% (±1.56%)	1.84% (±1.88%)	1.10% (±0.88%)	0.96% (±0.83%)
	Thermoleophilia	0.70% (±1.45%)	1.19% (±1.79%)	0.12% (±0.23%)	0.01% (±0.03%)
	Acidimicrobiia	0.12% (±0.37%)	0.21% (±0.48%)	0.01% (±0.04%)	0.01% (±0.02%)
	Rubrobacteria	0.08% (±0.21%)	0.14% (±0.27%)	<0.01% (±0.01%)	0.00% (±0.00%)
	Nitriliruptoria	0.01% (±0.08%)	0.02% (±0.11%)	0.00% (±0.00%)	0.00% (±0.00%)
	MB-A2-108	0.01% (±0.07%)	0.02% (±0.09%)	0.00% (±0.00%)	0.00% (±0.00%)
	0319-7L14	0.01% (±0.08%)	0.01% (±0.11%)	0.00% (±0.00%)	0.00% (±0.00%)

Table 4.2 continued

Phylum	Class	All Samples	The Bahamas	First Recapture in Michigan	Second Recapture in Michigan
Cyanobacteria		**1.09% (±4.50%)**	**1.91% (±5.93%)**	**0.07% (±0.29%)**	**0.05% (±0.23%)**
	Oxyphotobacteria	1.09% (±4.50%)	1.91% (±5.93%)	0.07% (±0.29%)	0.05% (±0.23%)
	Melainabacteria	<0.01% (±0.01%)	<0.01% (±0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
	Sericytochromatia	<0.01% (±<0.01%)	<0.01% (±<0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
Acidobacteria		**0.54% (±1.79%)**	**0.36% (±0.53%)**	**0.96% (±2.98%)**	**0.13% (±0.24%)**
	Acidobacteriia	0.39% (±1.78%)	0.11% (±0.27%)	0.93% (±2.98%)	0.13% (±0.24%)
	Blastocatellia	0.08% (±0.25%)	0.13% (±0.33%)	0.01% (±0.06%)	<0.01% (±<0.01%)
	Subgroup 6	0.04% (±0.14%)	0.07% (±0.17%)	0.02% (±0.09%)	0.01% (±0.03%)
	Subgroup 25	0.01% (±0.09%)	0.02% (±0.12%)	0.00% (±0.00%)	0.00% (±0.00%)
	Thermoanaerobac.	0.01% (±0.05%)	0.01% (±0.07%)	0.00% (±0.00%)	0.00% (±0.00%)
	Subgroup 17	<0.01% (±0.04%)	0.01% (±0.05%)	0.00% (±0.00%)	0.00% (±0.00%)
	Holophagae	<0.01% (±0.03%)	0.01% (±0.04%)	0.00% (±0.00%)	0.00% (±0.00%)
	Subgroup 5	<0.01% (±0.02%)	<0.01% (±0.02%)	0.00% (±0.00%)	0.00% (±0.00%)
	FFCH5909	<0.01% (±0.01%)	0.00% (±0.00%)	<0.01% (±0.01%)	0.00% (±0.00%)
	Subgroup 22	<0.01% (±<0.01%)	<0.01% (±<0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
Synergistetes		**0.33% (±2.30%)**	**0.17% (±0.78%)**	**0.63% (±3.83%)**	**0.19% (±0.33%)**
	Synergistia	0.33% (±2.30%)	0.17% (±0.78%)	0.63% (±3.83%)	0.19% (±0.33%)
Planctomycetes		**0.31% (±0.59%)**	**0.47% (±0.72%)**	**0.12% (±0.26%)**	**0.06% (±0.12%)**
	Planctomycetacia	0.27% (±0.55%)	0.42% (±0.67%)	0.11% (±0.26%)	0.06% (±0.12%)
	Phycisphaerae	0.02% (±0.11%)	0.03% (±0.14%)	0.01% (±0.05%)	0.00% (±0.00%)
	vadinHA49	0.01% (±0.12%)	0.02% (±0.16%)	0.00% (±0.00%)	0.00% (±0.00%)
	OM190	<0.01% (±0.04%)	0.01% (±0.05%)	0.00% (±0.00%)	0.00% (±0.00%)
Tenericutes		**0.27% (±0.72%)**	**0.38% (±0.92%)**	**0.09% (±0.24%)**	**0.28% (±0.48%)**
	Mollicutes	0.27% (±0.72%)	0.38% (±0.92%)	0.09% (±0.24%)	0.28% (±0.48%)
Verrucomicrobia		**0.21% (±0.53%)**	**0.27% (±0.64%)**	**0.15% (±0.36%)**	**0.04% (±0.11%)**

Table 4.2 continued

Phylum	Class	All Samples	The Bahamas	First Recapture in Michigan	Second Recapture in Michigan
	Verrucomicrobiae	0.21% (±0.53%)	0.27% (±0.64%)	0.15% (±0.36%)	0.04% (±0.11%)
Chloroflexi		**0.18% (±0.43%)**	**0.29% (±0.51%)**	**0.05% (±0.25%)**	**0.04% (±0.15%)**
	Chloroflexia	0.08% (±0.20%)	0.13% (±0.25%)	<0.01% (±0.02%)	0.04% (±0.15%)
	TK10	0.04% (±0.14%)	0.07% (±0.18%)	0.00% (±0.00%)	<0.01% (±0.01%)
	Anaerolineae	0.03% (±0.17%)	0.03% (±0.15%)	0.03% (±0.23%)	0.00% (±0.00%)
	Gitt-GS-136	0.02% (±0.09%)	0.03% (±0.12%)	0.00% (±0.00%)	0.00% (±0.00%)
	Ktedonobacteria	0.01% (±0.14%)	0.02% (±0.19%)	0.00% (±0.00%)	<0.01% (±0.01%)
	KD4-96	0.01% (±0.05%)	0.01% (±0.04%)	0.01% (±0.07%)	0.00% (±0.00%)
	JG30-KF-CM66	<0.01% (±0.02%)	<0.01% (±0.03%)	0.00% (±0.00%)	0.00% (±0.00%)
	AD3	<0.01% (±0.02%)	0.00% (±0.00%)	<0.01% (±0.03%)	0.00% (±0.00%)
	OLB14	<0.01% (±0.01%)	<0.01% (±0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
	Dehalococcoidia	<0.01% (±<0.01%)	<0.01% (±<0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
RsaHF231		**0.13% (±1.53%)**	**0.24% (±2.06%)**	**<0.01% (±<0.01%)**	**0.00% (±0.00%)**
Euryarchaeota		**0.12% (±0.42%)**	**0.20% (±0.54%)**	**0.02% (±0.09%)**	**0.05% (±0.22%)**
	Methanobacteria	0.11% (±0.41%)	0.18% (±0.52%)	0.02% (±0.09%)	0.05% (±0.22%)
	Thermoplasmata	0.01% (±0.06%)	0.02% (±0.07%)	0.00% (±0.00%)	0.00% (±0.00%)
	Methanomicrobia	<0.01% (±0.01%)	<0.01% (±0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
	Halobacteria	<0.01% (±0.01%)	<0.01% (±0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
Chlamydiae		**0.07% (±0.56%)**	**0.01% (±0.03%)**	**0.05% (±0.21%)**	**0.40% (±1.65%)**
	Chlamydiae	0.07% (±0.56%)	0.01% (±0.03%)	0.05% (±0.21%)	0.40% (±1.65%)
Gemmatimonadetes		**0.06% (±0.17%)**	**0.10% (±0.22%)**	**0.01% (±0.09%)**	**0.01% (±0.06%)**
	Gemmatimonadetes	0.03% (±0.13%)	0.06% (±0.16%)	0.00% (±0.00%)	0.01% (±0.06%)
	Longimicrobia	0.02% (±0.10%)	0.03% (±0.11%)	0.01% (±0.09%)	0.00% (±0.00%)
	AKAU4049	<0.01% (±0.02%)	<0.01% (±0.02%)	0.00% (±0.00%)	0.00% (±0.00%)
	S0134 terr. grp	<0.01% (±0.01%)	<0.01% (±0.01%)	0.00% (±0.00%)	0.00% (±0.00%)

Table 4.2 continued

Phylum	Class	All Samples	The Bahamas	First Recapture in Michigan	Second Recapture in Michigan
Spirochaetes		**0.05% (±0.27%)**	**0.07% (±0.33%)**	**<0.01% (±0.02%)**	**0.08% (±0.33%)**
	Spirochaetia	0.05% (±0.27%)	0.07% (±0.33%)	<0.01% (±0.02%)	0.08% (±0.33%)
	Leptospirae	<0.01% (±<0.01%)	<0.01% (±<0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
Armatimonadetes		**0.05% (±0.21%)**	**0.05% (±0.24%)**	**0.04% (±0.18%)**	**<0.01% (±<0.01%)**
	Fimbriimonadia	0.02% (±0.17%)	0.04% (±0.23%)	0.01% (±0.04%)	0.00% (±0.00%)
	Armatimonadia	0.02% (±0.11%)	0.01% (±0.05%)	0.03% (±0.18%)	<0.01% (±<0.01%)
	Chthonomonadetes	<0.01% (±0.02%)	<0.01% (±0.03%)	0.00% (±0.00%)	0.00% (±0.00%)
Thaumarchaeota		**0.04% (±0.16%)**	**0.07% (±0.20%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
	Nitrososphaeria	0.04% (±0.16%)	0.07% (±0.20%)	0.00% (±0.00%)	0.00% (±0.00%)
Fusobacteria		**0.03% (±0.14%)**	**0.03% (±0.15%)**	**0.03% (±0.15%)**	**<0.01% (±0.01%)**
	Fusobacteriia	0.03% (±0.14%)	0.03% (±0.15%)	0.03% (±0.15%)	<0.01% (±0.01%)
Patescibacteria		**0.03% (±0.09%)**	**0.04% (±0.12%)**	**<0.01% (±0.02%)**	**0.02% (±0.07%)**
	Saccharimonadia	0.02% (±0.06%)	0.02% (±0.07%)	<0.01% (±0.01%)	0.01% (±0.04%)
	Microgenomatia	0.01% (±0.07%)	0.01% (±0.09%)	<0.01% (±0.01%)	0.01% (±0.06%)
	Gracilibacteria	<0.01% (±0.01%)	<0.01% (±0.02%)	0.00% (±0.00%)	0.00% (±0.00%)
	Parcubacteria	<0.01% (±0.01%)	<0.01% (±0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
	WWE3	<0.01% (±<0.01%)	0.00% (±0.00%)	<0.01% (±<0.01%)	0.00% (±0.00%)
Epsilonbacteraeota		**0.02% (±0.22%)**	**0.03% (±0.26%)**	**0.03% (±0.19%)**	**0.00% (±0.00%)**
	Campylobacteria	0.02% (±0.22%)	0.03% (±0.26%)	0.03% (±0.19%)	0.00% (±0.00%)
Deinococcus-Thermus		**0.02% (±0.13%)**	**0.04% (±0.17%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
	Deinococci	0.02% (±0.13%)	0.04% (±0.17%)	0.00% (±0.00%)	0.00% (±0.00%)
Rokubacteria		**0.02% (±0.15%)**	**0.04% (±0.20%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
	NC10	0.02% (±0.15%)	0.04% (±0.20%)	0.00% (±0.00%)	0.00% (±0.00%)
FBP		**0.01% (±0.07%)**	**0.02% (±0.10%)**	**<0.01% (±0.02%)**	**0.00% (±0.00%)**
Entotheonellaeota		**0.01% (±0.06%)**	**0.01% (±0.08%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**

Table 4.2 continued

Phylum	Class	All Samples	The Bahamas	First Recapture in Michigan	Second Recapture in Michigan
	Entotheonellia	0.01% (±0.06%)	0.01% (±0.08%)	0.00% (±0.00%)	0.00% (±0.00%)
Lentisphaerae		**<0.01% (±0.04%)**	**0.01% (±0.05%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
	Lentisphaeria	<0.01% (±0.04%)	0.01% (±0.05%)	0.00% (±0.00%)	0.00% (±0.00%)
WPS-2		**<0.01% (±0.06%)**	**0.00% (±0.00%)**	**0.01% (±0.10%)**	**<0.01% (±<0.01%)**
Nitrospirae		**<0.01% (±0.03%)**	**0.01% (±0.04%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
	Nitrospira	<0.01% (±0.03%)	0.01% (±0.04%)	0.00% (±0.00%)	0.00% (±0.00%)
Calditrichaeota		**<0.01% (±0.03%)**	**<0.01% (±0.05%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
	Calditrichia	<0.01% (±0.03%)	<0.01% (±0.05%)	0.00% (±0.00%)	0.00% (±0.00%)
WS2		**<0.01% (±0.03%)**	**<0.01% (±0.04%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
Kiritimatiellaeota		**<0.01% (±0.01%)**	**<0.01% (±0.02%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
	Kiritimatiellae	<0.01% (±0.01%)	<0.01% (±0.02%)	0.00% (±0.00%)	0.00% (±0.00%)
Latescibacteria		**<0.01% (±0.01%)**	**0.00% (±0.00%)**	**<0.01% (±0.02%)**	**0.00% (±0.00%)**
WS4		**<0.01% (±0.01%)**	**<0.01% (±0.01%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
Deferribacteres		**<0.01% (±0.01%)**	**<0.01% (±0.01%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
	Deferribacteres	<0.01% (±0.01%)	<0.01% (±0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
BRC1		**<0.01% (±0.01%)**	**<0.01% (±0.01%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
Fibrobacteres		**<0.01% (±<0.01%)**	**<0.01% (±<0.01%)**	**0.00% (±0.00%)**	**0.00% (±0.00%)**
	Chitinivibrionia	<0.01% (±<0.01%)	<0.01% (±<0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
	Fibrobacteria	<0.01% (±<0.01%)	<0.01% (±<0.01%)	0.00% (±0.00%)	0.00% (±0.00%)
Unk.		**3.31% (±6.82%)**	**4.33% (±8.04%)**	**1.39% (±2.42%)**	**4.09% (±8.20%)**

Table 4.3 Results of ANCOM-BC analysis including log fold change, standard error, confidence limits and p-values, comparing bacterial abundance in The Bahamas to the first recapture in Michigan (**4.3A**) and bacterial abundances in The Bahamas to the second recapture in Michigan (**4.3B**)

Table 4.3A ANCOM-BC results comparing bacterial abundance in The Bahamas to the first recapture in Michigan.

Phylum	Genus	Log fold change	Standard Error	Lower C.I.	Upper C.I.	adjusted p-value
Acidobacteria	*Bryocella*	0.962	0.272	0.429	1.496	0.05
Actinobacteria	*Actinomycetospora*	-0.739	0.179	-1.090	-0.388	<0.001
Actinobacteria	*Aeromicrobium*	-0.789	0.206	-1.192	-0.386	0.01
Actinobacteria	*Nocardioides*	-1.216	0.239	-1.684	-0.748	<0.001
Actinobacteria	*Pseudonocardia*	-0.818	0.198	-1.206	-0.431	<0.001
Actinobacteria	*Rubrobacter*	-1.007	0.187	-1.373	-0.641	<0.001
Actinobacteria	*Solirubrobacter*	-1.655	0.250	-2.145	-1.166	<0.001
Proteobacteria	*Aureimonas*	-1.127	0.310	-1.736	-0.519	0.03
Proteobacteria	*Beijerinckiaceae (UG)*	-0.863	0.201	-1.257	-0.468	<0.001
Proteobacteria	*Candidatus Hamiltonella*	1.518	0.366	0.801	2.236	<0.001

Table 4.3B ANCOM-BC results comparing bacterial abundance in The Bahamas to the second recapture in Michigan.

Phylum	Genus	Log fold change	Standard Error	Lower C.I.	Upper C.I.	adjusted p-value
Actinobacteria	*Blastococcus*	-0.036	0.154	-0.338	0.266	<0.001
Actinobacteria	*Cellulomonas*	-0.034	0.154	-0.335	0.268	<0.001
Actinobacteria	*Geodermatophilus*	-0.090	0.149	-0.383	0.203	<0.001
Actinobacteria	*Kineococcus*	-0.085	0.157	-0.393	0.223	<0.001
Actinobacteria	*Nocardioides*	-1.086	0.240	-1.556	-0.616	<0.001
Actinobacteria	*Rubrobacter*	-0.769	0.188	-1.137	-0.400	<0.001
Actinobacteria	*Solirubrobacter*	-1.519	0.227	-1.963	-1.074	<0.001
Actinobacteria	*Streptomyces*	0.074	0.141	-0.202	0.350	<0.001
Firmicutes	*Candidatus Soleaferrea*	-0.685	0.242	-1.160	-0.211	<0.001
Firmicutes	*Christensenellaceae* (UG)	-1.159	0.276	-1.699	-0.619	<0.001
Firmicutes	*Clostridiales* (UG)	-0.336	0.195	-0.718	0.046	<0.001
Firmicutes	*Clostridium sensu stricto 1*	-0.009	0.157	-0.317	0.300	<0.001
Firmicutes	*Ruminococcaceae* UCG-004	-0.017	0.151	-0.312	0.279	<0.001
Proteobacteria	*Aureimonas*	-1.256	0.274	-1.793	-0.719	<0.001
Proteobacteria	*Beijerinckiaceae* (UG)	-0.628	0.197	-1.013	-0.242	<0.001
Proteobacteria	*Lysobacter*	-0.335	0.201	-0.729	0.060	<0.001
Proteobacteria	*Serratia*	2.597	0.721	1.183	4.011	0.04

Table 4.4 Taxonomic classifications of ASVs identified as shared throughout all sampling periods.

Phylum	Class	Order	Family	Genus	#ASVs
Actinobacteria	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	1
Actinobacteria	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*	1
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	8
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	1
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Eubacterium hallii group*	2
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Fusicatenibacter*	1
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Roseburia*	2
Firmicutes	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	3
Firmicutes	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Subdoligranulum*	2
Firmicutes	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unclassified	2
Proteobacteria	*Gammaproteobacteria*	*Betaproteobacteriales*	*Burkholderiaceae*	*Ralstonia*	1
Proteobacteria	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia-Shigella*	1
Proteobacteria	*Gammaproteobacteria*	*Pseudomonadales*	*Moraxellaceae*	*Alkanindiges*	1

5 Supplemental Tables, Chapter 2

Table 5.1 Genera and Amplicon Sequence Variants (ASVs) found in at least 50% of all libraries within the full dataset or specific subsets of the dataset. Table includes shared genera, indicated by an X, or shared ASVs, indicated by an asterisk (*). Uncultured and unnamed genera are noted with (UG) and the closest named taxonomic level is reported. Table **5.1A** lists shared microbes across the full dataset, Table **5.1B** lists shared microbes within individual host species (Grey-Cheeked, Hermit, Swainson's, Veery), Table **5.1C** lists shared microbes of specific seasons (Spring, Summer, Fall), Table **5.1D** lists shared microbes of specific years (2017, 2018, 2019).

Table 5.1A shared microbes across the full dataset

Phylum	Genus	Entire Dataset
Actinobacteriota	*Nocardioides*	X
Actinobacteriota	*Gaiellales* (UG)	X
Actinobacteriota	*Conexibacter*	X
Actinobacteriota	67-14	X
Cyanobacteria	*Aliterella*	X*
Planctomycetota	*Fimbriiglobus*	X
Planctomycetota	*Gemmataceae* (UG)	X*
Proteobacteria	*Geminicoccus*	X*
Proteobacteria	*Diplorickettsiaceae (UG)*	X
Proteobacteria	*Legionella*	X
Verrucomicrobiota	*Neochlamydia*	X

Table 5.1B shared microbes within host species

Phylum	Genus	Grey-Cheeked	Hermit	Swainson's	Veery
Actinobacteriota	*Nocardioides*	X	X	X	
Actinobacteriota	*Gaiellales* (UG)		X	X	X*
Actinobacteriota	*Conexibacter*	X*	X	X	X
Actinobacteriota	*67-14*		X		
Cyanobacteria	*Aliterella*	X*	X*	X*	X*
Planctomycetota	*Fimbriiglobus*	X	X	X	X
Planctomycetota	*Gemmataceae* (UG)	X*	X*	X*	X*
Proteobacteria	*Geminicoccus*	X*	X*	X*	X*
Proteobacteria	*Diplorickettsiaceae* (UG)	X	X*	X	X
Proteobacteria	*Legionella*	X	X		
Verrucomicrobiota	*Neochlamydia*		X	X	X

Table 5.1C shared microbes of specific seasons

Phylum	Genus	Spring	Summer	Fall
Actinobacteriota	*Nocardioides*	X	X	X
Actinobacteriota	*Gaiellales (UG)*	X*		X
Actinobacteriota	*Conexibacter*	X	X	X
Actinobacteriota	*67-14*	X	X*	
Cyanobacteria	*Aliterella*	X*	X*	X*
Planctomycetota	*Gemmata*		X	
Planctomycetota	*Fimbriiglobus*	X	X	X
Planctomycetota	*Gemmataceae (UG)*	X*	X*	X*
Proteobacteria	*Geminicoccus*	X*		X*
Proteobacteria	*Ralstonia*		X	
Proteobacteria	*Diplorickettsiaceae (UG)*	X		X*
Proteobacteria	*Legionella*	X	X	X
Verrucomicrobiota	*Neochlamydia*	X		X

Table 5.1D shared microbes of specific years

Phylum	Genus	2017	2018	2019
Actinobacteriota	*Euzebyales (UG)*	X*		
Actinobacteriota	*Nocardioides*		X	X
Actinobacteriota	*Gaiellales (UG)*			X*
Actinobacteriota	*Conexibacter*		X	X
Actinobacteriota	*67-14*		X	X
Cyanobacteria	*Aliterella*		X*	X*
Firmicutes	*Lutispora*	X*	X*	
Firmicutes	*Limnochordaceae*	X		
Planctomycetota	*Fimbriiglobus*	X	X	X
Planctomycetota	*Gemmataceae (UG)*		X*	X*
Planctomycetota	*Aquisphaera*			X
Proteobacteria	*Geminicoccus*	X*	X*	X*
Proteobacteria	*Diplorickettsiaceae (UG)*	X*	X	X
Proteobacteria	*Legionella*			X
Verrucomicrobiota	*Neochlamydia*			X

Table 5.2 Relative abundances of each bacterial phyla (highlighted in grey) and classes with the standard deviation listed in parentheses. Phyla and classes are listed in order of most abundant in the full dataset. Relative abundances were calculated for the full dataset with libraries of at least 500 reads, as well as when libraries were rarefied to 500 or 5,000 reads.

Phylum	Class	Libraries with at least 500 reads, unrarefied	Libraries rarefied at 500 reads	Libraries rarefied at 5,000 reads
Proteobacteria		**28.74% (±31.62%)**	**28.68% (±31.7%)**	**31.08% (±33.55%)**
	Alphaproteobacteria	16.45% (±25.84%)	16.43% (±25.89%)	17.96% (±27.96%)
	Gammaproteobacteria	12.28% (±23.17%)	12.24% (±23.19%)	13.11% (±24.91%)
Planctomycetota		**22.61% (±31.30%)**	**22.57% (±31.27%)**	**24.07% (±32.73%)**
	Planctomycetes	22.46% (±31.36%)	22.41% (±31.32%)	23.88% (±32.8%)
	BD7-11	0.10% (±0.98%)	0.11% (±1.03%)	0.13% (±1.13%)
	Phycisphaerae	0.02% (±0.10%)	0.02% (±0.13%)	0.03% (±0.1%)
	OM190	0.01% (±0.09%)	0.01% (±0.13%)	0.01% (±0.03%)
	Pla4 lineage	0.01% (±0.04%)	0.01% (±0.05%)	0.01% (±0.05%)
	Pla3 lineage	<0.01% (±0.02%)	<0.01% (±0.02%)	<0.01% (±0.02%)
	vadinHA49	<0.01% (±0.01%)	<0.01% (±0.04%)	<0.01% (±0.01%)
Cyanobacteria		**18.36% (±28.72%)**	**18.33% (±28.76%)**	**11.54% (±22.34%)**
	Cyanobacteriia	18.22% (±28.74%)	18.20% (±28.78%)	11.33% (±22.32%)
	Vampirivibrionia	0.14% (±1.85%)	0.13% (±1.79%)	0.2% (±2.21%)
	Sericytochromatia	0.01% (±0.03%)	<0.01% (±0.03%)	0.01% (±0.04%)
Actinobacteriota		**12.46% (±20.13%)**	**12.54% (±20.2%)**	**13.53% (±22.18%)**
	Thermoleophilia	6.04% (±15.53%)	6.06% (±15.55%)	6.67% (±18.12%)
	Actinobacteria	5.71% (±13.32%)	5.75% (±13.39%)	6.06% (±13.74%)
	Acidimicrobiia	0.40% (±1.22%)	0.41% (±1.24%)	0.46% (±1.22%)
	MB-A2-108	0.18% (±0.70%)	0.18% (±0.75%)	0.16% (±0.47%)
	Rubrobacteria	0.07% (±0.81%)	0.07% (±0.76%)	0.1% (±0.99%)
	Coriobacteriia	0.05% (±0.26%)	0.06% (±0.28%)	0.07% (±0.3%)

Table 5.2 continued

Phylum	Class	Libraries with at least 500 reads, unrarefied	Libraries rarefied at 500 reads	Libraries rarefied at 5,000 reads
Firmicutes		**7.42% (±13.94%)**	**7.41% (±13.97%)**	**8.27% (±15.22%)**
	Bacilli	4.13% (±11.37%)	4.14% (±11.37%)	5.15% (±12.92%)
	Clostridia	2.72% (±6.21%)	2.72% (±6.23%)	2.53% (±6.04%)
	Negativicutes	0.19% (±2.53%)	0.18% (±2.53%)	0.09% (±0.29%)
	Syntrophomonadia	0.18% (±3.14%)	0.18% (±3.16%)	0.26% (±3.82%)
	Limnochordia	0.15% (±1.40%)	0.14% (±1.46%)	0.16% (±1.65%)
	Desulfitobacteriia	0.01% (±0.09%)	0.01% (±0.1%)	0.02% (±0.12%)
	Desulfotomaculia	0.01% (±0.05%)	0.01% (±0.06%)	0.01% (±0.04%)
	Thermacetogenia	<0.01% (±0.03%)	<0.01% (±0.04%)	0.01% (±0.04%)
	Thermoanaerobacteria	<0.01% (±0.03%)	<0.01% (±0.03%)	<0.01% (±0.03%)
	Dethiobacteria	<0.01% (±0.03%)	<0.01% (±0.03%)	0.01% (±0.04%)
	Incertae Sedis	<0.01% (±0.02%)	<0.01% (±0.03%)	<0.01% (±0.02%)
	Symbiobacteriia	<0.01% (±0.02%)	<0.01% (±0.04%)	<0.01% (±0.02%)
	Moorellia	<0.01% (±0.02%)	<0.01% (±0.03%)	<0.01% (±0.02%)
	D8A-2	<0.01% (±0.02%)	<0.01% (±0.01%)	<0.01% (±0.02%)
	Thermaerobacteria	<0.01% (±0.02%)	<0.01% (±0.02%)	<0.01% (±0.01%)
	Thermovenabulia	<0.01% (±0.02%)	<0.01% (±0.01%)	<0.01% (±0.02%)
	BRH-c20a	<0.01% (±<0.01%)	<0.01% (±0.01%)	<0.01% (±<0.01%)
	TTA-B61	<0.01% (±<0.01%)	Not present	<0.01% (±<0.01%)
	Sulfobacillia	<0.01% (±<0.01%)	<0.01% (±0.01%)	Not present
Verrucomicrobiota		**2.93% (±9.44%)**	**2.92% (±9.38%)**	**3.59% (±10.35%)**
	Chlamydiae	2.20% (±8.98%)	2.17% (±8.89%)	2.59% (±9.79%)
	Verrucomicrobiae	0.74% (±2.85%)	0.75% (±2.94%)	1.01% (±3.37%)
	Omnitrophia	<0.01% (±0.01%)	<0.01% (±0.01%)	<0.01% (±0.01%)
	Kiritimatiellae	<0.01% (±<0.01%)	<0.01% (±0.01%)	<0.01% (±<0.01%)

Table 5.2 continued

Phylum	Class	Libraries with at least 500 reads, unrarefied	Libraries rarefied at 500 reads	Libraries rarefied at 5,000 reads
	Lentisphaeria	<0.01% (±<0.01%)	<0.01% (±0.01%)	<0.01% (±<0.01%)
Campilobacterota	**Campylobacteria**	**1.65% (±8.69%)**	**1.66% (±8.71%)**	**1.27% (±7.84%)**
Chloroflexi		**1.31% (±5.71%)**	**1.31% (±5.73%)**	**1.61% (±6.68%)**
	Gitt-GS-136	0.41% (±4.16%)	0.4% (±4.05%)	0.54% (±4.97%)
	TK10	0.33% (±3.54%)	0.33% (±3.67%)	0.38% (±4.11%)
	Anaerolineae	0.31% (±1.56%)	0.31% (±1.61%)	0.41% (±1.84%)
	Chloroflexia	0.11% (±0.46%)	0.23% (±0.49%)	0.12% (±0.45%)
	KD4-96	0.07% (±0.27%)	0.07% (±0.26%)	0.07% (±0.22%)
	Dehalococcoidia	0.05% (±0.67%)	0.05% (±0.7%)	0.04% (±0.41%)
	JG30-KF-CM66	0.02% (±0.09%)	0.02% (±0.13%)	0.02% (±0.06%)
	Ktedonobacteria	0.01% (±0.06%)	0.01% (±0.07%)	0.01% (±0.05%)
	OLB14	<0.01% (±0.03%)	<0.01% (±0.04%)	<0.01% (±0.03%)
	AD3	<0.01% (±0.03%)	<0.01% (±0.02%)	<0.01% (±0.04%)
	SHA-26	<0.01% (±<0.01%)	<0.01% (±0.01%)	<0.01% (±<0.01%)
	P2-11E	<0.01% (±<0.01%)	<0.01% (±0.01%)	<0.01% (±<0.01%)
Myxococcota		**0.91% (±2.61%)**	**0.94% (±2.73%)**	**1.01% (±2.67%)**
	Polyangia	0.75% (±2.39%)	0.78% (±2.5%)	0.83% (±2.53%)
	Myxococcia	0.15% (±0.86%)	0.15% (±0.89%)	0.15% (±0.39%)
	bacteriap25	0.01% (±0.15%)	0.02% (±0.15%)	0.02% (±0.17%)
Bacteroidota		**0.75% (±4.29%)**	**0.77% (±4.39%)**	**0.94% (±5.05%)**
	Bacteroidia	0.75% (±4.29%)	0.77% (±4.39%)	0.94% (±5.05%)
	SJA-28	<0.01% (±0.07%)	0.01% (±0.07%)	<0.01% (±0.02%)
Acidobacteriota		**0.64% (±3.02%)**	**0.63% (±2.97%)**	**0.72% (±3.42%)**
	Vicinamibacteria	0.38% (±2.69%)	0.37% (±2.65%)	0.46% (±3.14%)
	Blastocatellia	0.15% (±1.21%)	0.26% (±1.18%)	0.11% (±1.13%)

Table 5.2 continued

Phylum	Class	Libraries with at least 500 reads, unrarefied	Libraries rarefied at 500 reads	Libraries rarefied at 5,000 reads
	Acidobacteriae	0.06% (±0.28%)	0.06% (±0.26%)	0.08% (±0.32%)
	Subgroup 25	0.02% (±0.30%)	0.02% (±0.34%)	0.03% (±0.37%)
	Subgroup 5	0.02% (±0.13%)	0.02% (±0.11%)	0.02% (±0.15%)
	Thermoanaerobaculia	0.01% (±0.08%)	0.01% (±0.11%)	0.01% (±0.09%)
	Subgroup 22	<0.01% (±0.01%)	<0.01% (±0.03%)	<0.01% (±0.02%)
	Holophagae	<0.01% (±0.01%)	<0.01% (±0.01%)	<0.01% (±0.01%)
	Subgroup 11	<0.01% (±0.01%)	<0.01% (±0.01%)	<0.01% (±<0.01%)
	Subgroup 18	<0.01% (±<0.01%)	Not present	<0.01% (±<0.01%)
	AT-s3-28	<0.01% (±<0.01%)	Not present	<0.01% (±<0.01%)
Bdellovibrionota		**0.61% (±2.31%)**	**0.61% (±2.33%)**	**0.63% (±2.52%)**
	Bdellovibrionia	0.19% (±0.58%)	0.19% (±0.6%)	0.24% (±0.64%)
	Oligoflexia	0.42% (±2.21%)	0.42% (±2.23%)	0.39% (±2.4%)
Desulfobacterota		**0.58% (±4.33%)**	**0.6% (±4.4%)**	**0.77% (±5.16%)**
	Desulfobacteria	0.407% (±4.32%)	0.49% (±4.38%)	0.63% (±5.15%)
	Desulfovibrionia	0.05% (±0.34%)	0.06% (±0.43%)	0.07% (±0.38%)
	Desulfuromonadia	0.02% (±0.11%)	0.02% (±0.12%)	0.02% (±0.12%)
	Syntrophobacteria	<0.01% (±0.01%)	<0.01% (±0.02%)	<0.01% (±0.01%)
	Desulfobulbia	<0.01% (±0.01%)	<0.01% (±0.01%)	<0.01% (±0.01%)
	Desulfarculia	<0.01% (±<0.01%)	Not present	<0.01% (±<0.01%)
	Desulfomonilia	<0.01% (±<0.01%)	Not present	<0.01% (±<0.01%)
	Syntrophia	<0.01% (±<0.01%)	<0.01% (±0.01%)	Not present
Patescibacteria		**0.34% (±0.78%)**	**0.34% (±0.82%)**	**0.32% (±0.7%)**
	Saccharimonadia	0.31% (±0.76%)	0.32% (±0.81%)	0.29% (±0.67%)
	Parcubacteria	0.02% (±0.08%)	0.01% (±0.07%)	0.03% (±0.1%)
	Microgenomatia	<0.01% (±0.03%)	<0.01% (±0.03%)	0.01% (±0.03%)

Table 5.2 continued

Phylum	Class	Libraries with at least 500 reads, unrarefied	Libraries rarefied at 500 reads	Libraries rarefied at 5,000 reads
	Gracilibacteria	<0.01% (±0.02%)	<0.01% (±0.02%)	<0.01% (±0.02%)
	Dojkabacteria	<0.01% (±<0.01%)	Not present	Not present
	WWE3	<0.01% (±<0.01%)	Not present	<0.01% (±<0.01%)
	Berkelbacteria	<0.01% (±<0.01%)	Not present	<0.01% (±<0.01%)
Dependentiae	**Babeliae**	**0.32% (±1.07%)**	**0.32% (±1.02%)**	**0.25% (±0.65%)**
MBNT15	**MBNT15**	**0.10% (±0.49%)**	**0.1% (±0.5%)**	**0.05% (±0.25%)**
Gemmatimonadota		**0.06% (±0.32%)**	**0.06% (±0.33%)**	**0.07% (±0.37%)**
	Longimicrobia	0.03% (±0.28%)	0.04% (±0.29%)	0.04% (±0.35%)
	Gemmatimonadetes	0.02% (±0.15%)	0.02% (±0.13%)	0.02% (±0.09%)
	BD2-11 terrestrial grp.	<0.01% (±0.03%)	<0.01% (±0.04%)	<0.01% (±0.03%)
	S0134 terrestrial group	<0.01% (±0.02%)	<0.01% (±0.03%)	<0.01% (±0.02%)
	AKAU4049	<0.01% (±<0.01%)	<0.01% (±0.01%)	<0.01% (±0.01%)
Deinococcota	**Deinococci**	**0.05% (±0.52%)**	**0.05% (±0.62%)**	**0.06% (±0.61%)**
Elusimicrobiota		**0.04% (±1.10%)**	**0.04% (±1.04%)**	**0.06% (±1.35%)**
	Lineage IIa	0.04% (±1.10%)	0.04% (±1.04%)	0.06% (±1.35%)
	Lineage IIb	<0.01% (±<0.01%)	Not present	<0.01% (±<0.01%)
	Elusimicrobia	<0.01% (±<0.01%)	<0.01% (±0.01%)	<0.01% (±0.01%)
Fibrobacterota	**Fibrobacteria**	**0.04% (±0.15%)**	**0.04% (±0.18%)**	**0.06% (±0.18%)**
Abditibacteriota	**Abditibacteria**	**0.01% (±0.03%)**	**<0.01% (±0.05%)**	**0.01% (±0.04%)**
Entotheonellaeota	**Entotheonellia**	**0.01% (±0.11%)**	**0.01% (±0.13%)**	**0.02% (±0.13%)**
Fusobacteriota	**Fusobacteriia**	**0.01% (±0.04%)**	**0.01% (±0.04%)**	**0.01% (±0.05%)**
Methylomirabilota	**Methylomirabilia**	**0.01% (±0.08%)**	**0.01% (±0.09%)**	**0.01% (±0.03%)**
NB1-j	**NB1-j**	**0.01% (±0.05%)**	**0.01% (±0.06%)**	**0.02% (±0.06%)**
RCP2-54	**RCP2-54**	**0.01% (±0.06%)**	**0.01% (±0.07%)**	**0.01% (±0.07%)**
SAR324 clade	**SAR324 clade**	**0.01% (±0.06%)**	**0.01% (±0.08%)**	**0.01% (±0.07%)**

Table 5.2 continued

Phylum	Class	Libraries with at least 500 reads, unrarefied	Libraries rarefied at 500 reads	Libraries rarefied at 5,000 reads
Sumerlaeota	**Sumerlaeia**	**0.01% (±0.03%)**	**<0.01% (±0.04%)**	**0.01% (±0.04%)**
Synergistota	**Synergistia**	**0.01% (±0.05%)**	**<0.01% (±0.06%)**	**<0.01% (±0.04%)**
Armatimonadota		**<0.01% (±0.03%)**	**<0.01% (±0.05%)**	**0.01% (±0.04%)**
	Armatimonadia	<0.01% (±0.03%)	<0.01% (±0.04%)	<0.01% (±0.03%)
	Chthonomonadetes	<0.01% (±0.01%)	Not present	<0.01% (±0.01%)
	Fimbriimonadia	<0.01% (±0.01%)	Not present	<0.01% (±0.01%)
Nitrospirota		**<0.01% (±0.03%)**	**<0.01% (±0.03%)**	**<0.01% (±0.04%)**
	Nitrospiria	<0.01% (±0.03%)	<0.01% (±0.03%)	<0.01% (±0.04%)
	Thermodesulfovibrionia	<0.01% (±0.01%)	Not present	<0.01% (±0.01%)
Spirochaetota		**<0.01% (±0.07%)**	**<0.01% (±0.06%)**	**<0.01% (±0.01%)**
	Spirochaetia	<0.01% (±0.07%)	<0.01% (±0.06%)	<0.01% (±0.01%)
	Brachyspirae	<0.01% (±<0.01%)	Not present	<0.01% (±<0.01%)
10bav-F6	**10bav-F6**	**<0.01% (±<0.01%)**	**Not present**	**<0.01% (±<0.01%)**
Dadabacteria	**Dadabacteriia**	**<0.01% (±<0.01%)**	**Not present**	**Not present**
Deferribacterota		**<0.01% (±<0.01%)**	**<0.01% (±0.01%)**	**<0.01% (±<0.01%)**
	Deferribacteres	<0.01% (±<0.01%)	<0.01% (±0.01%)	<0.01% (±<0.01%)
	Defferrisomatia	<0.01% (±0.01%)	<0.01% (±0.01%)	<0.01% (±0.01%)
FCPU426	**FCPU426**	**<0.01% (±0.01%)**	**<0.01% (±0.01%)**	**<0.01% (±0.02%)**
Halanaerobiaeota	**Halanaerobiia**	**<0.01% (±0.02%)**	**<0.01% (±0.02%)**	**<0.01% (±0.02%)**
Hydrogenedentes	**Hydrogenedentia**	**<0.01% (±<0.01%)**	**Not present**	**Not present**
Latescibacterota	**Latescibacterota**	**<0.01% (±<0.01%)**	**Not present**	**<0.01% (±<0.01%)**
Marinimicrobia	**Marinimicrobia**	**<0.01% (±<0.01%)**	**<0.01% (±0.01%)**	**Not present**
Nitrospinota	**P9X2b3D02**	**<0.01% (±0.02%)**	**<0.01% (±0.01%)**	**<0.01% (±0.02%)**
Rs-K70	**Rs-K70 termite group**	**<0.01% (±0.08%)**	**0.01% (±0.12%)**	**0.01% (±0.1%)**
WPS-2	**WPS-2**	**<0.01% (±0.01%)**	**Not present**	**<0.01% (±0.02%)**

Table 5.2 continued

Phylum	Class	Libraries with at least 500 reads, unrarefied	Libraries rarefied at 500 reads	Libraries rarefied at 5,000 reads
WS1	**WS1**	**<0.01% (±<0.01%)**	**Not present**	**Not present**
WS4	**WS4**	**<0.01% (±<0.01%)**	**Not present**	**<0.01% (±<0.01%)**
Unknown	**Unknown**	**0.03% (±0.16%)**	**0.03% (±0.17%)**	**0.04% (±0.19%)**

Table 5.3 Results of ANCOM-BC analysis identifying significant differences in pair-wise comparisons between host species, including natural log fold change, standard errors, confidence limits and adjusted p-values, with a $p_{adj} < 0.05$ denoting significance. Positive log fold change values indicates that the first host species listed was significantly enriched for the bacterial phylum or genus listed while a negative log fold change value indicated that the second host species listed was significantly enriched. For example, Veerys were significantly enriched in an uncultured genus from family compared to Grey-Cheeked Thrush. Table **5.3A** contains the results of the ANCOM-BC analysis of differences in bacterial genera between host species. Table **5.3B** contains the results of the ANCOM-BC analysis of differences in bacterial phlya between host species.

Table 5.3A ANCOM-BC analysis of bacterial genera between host species

Host Species	Phylum	*Genus*	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Veery vs. Grey-Cheeked	Proteobacteria	*D05-2* (UG)	2.24	0.63	1.00	3.48	**0.05**
Swainson's vs. Hermit	Verrucomicrobiota	*Parachlamydiaceae*	0.56	0.13	0.31	0.81	**0.00**
Swainson's vs. Hermit	Proteobacteria	*TRA3-20*	-1.40	0.34	-2.07	-0.74	**0.01**
Swainson's vs. Hermit	Proteobacteria	*Massilia*	0.78	0.19	0.40	1.16	**0.01**
Swainson's vs. Hermit	Proteobacteria	*Geminicoccaceae* (UG)	0.89	0.23	0.45	1.34	**0.01**
Swainson's vs. Hermit	Proteobacteria	*D05-2* (UG)	0.82	0.21	0.41	1.23	**0.02**
Swainson's vs. Hermit	Proteobacteria	*Alsobacter*	0.99	0.21	0.58	1.41	**0.00**
Swainson's vs. Hermit	Campilobacterota	*Alviniconcha*	1.47	0.31	0.85	2.09	**0.00**
Swainson's vs. Grey-Cheeked	Campilobacterota	*Alviniconcha*	1.44	0.37	0.71	2.17	**0.01**
Grey-Cheeked vs. Hermit	Cyanobacteria	*Aliterella*	-2.02	0.55	-3.09	-0.95	**0.04**
Grey-Cheeked vs. Hermit	Actinobacteriota	*Gaiellales* (UG)	-1.03	0.28	-1.57	-0.49	**0.04**

Table 5.3B ANCOM-BC analysis of bacterial phyla between host species

Host Species	Phylum	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Grey-Cheeked vs. Hermit	Actinobacteriota	1.00	0.33	0.36	1.65	**0.04**
Swainson's vs. Grey-Cheeked	Patescibacteria	-0.79	0.23	-1.25	-0.33	**0.01**
Swainson's vs. Hermit	Actinobacteriota	0.60	0.18	0.24	0.95	**0.02**
Swainson's vs. Hermit	Campilobacterota	1.21	0.29	0.63	1.78	**0.00**

Table 5.4 Results of ANCOM-BC analysis identifying significant differences in pair-wise comparisons between years, including natural log fold change, standard errors, confidence limits and adjusted p-values, with a p_{adj} <0.05 denoting significance. Positive log fold change values indicates that the first year was significantly enriched for the bacterial phylum or genus while a negative log fold change value indicated that the second year was significantly enriched. For example, *Conexibacter* was significantly enriched in 2017 when compared against 2018. Uncultured and unnamed genera and families are noted with (UG) or (UF) respectively and the closest named taxonomic level is reported. Table **5.4A** contains the results of the ANCOM-BC analysis of differential abundance in bacterial genera between 2017 and 2018. Table **5.4B** contains the results of the ANCOM-BC analysis of differential abundance in bacterial genera between 2018 and 2019. Table **5.4C** contains the results of the ANCOM-BC analysis of differential abundance in bacterial genera between 2017 and 2019. Table **5.4D** contains the results of the ANCOM-BC analysis of differential abundance in bacterial phlya between 2017 and 2018. Table 5.4E contains the results of the ANCOM-BC analysis of differential abundance in bacterial phlya between 2018 and 2019. Table 5.4F contains the results of the ANCOM-BC analysis of differential abundance in bacterial phlya between 2017 and 2019.

Table 5.4A Differential abundance in bacterial genera between 2017 and 2018.

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Acidobacteriota	*Subgroup 17*	-0.02	0.18	-0.37	0.33	1
Actinobacteriota	*Conexibacter*	1.15	0.26	0.63	1.66	**<0.01**
Actinobacteriota	*Euzebyaceae* (UG)	-2.05	0.27	-2.58	-1.52	**<0.01**
Actinobacteriota	*Frankiales*	-0.9	0.22	-1.34	-0.47	**0.01**
Actinobacteriota	*Gaiellales* (UF)	-0.09	0.12	-0.33	0.15	1
Actinobacteriota	*Nocardioides*	0.71	0.19	0.33	1.09	**0.03**
Actinobacteriota	*Parafrigoribacterium*	-0.02	0.1	-0.22	0.18	1
Cyanobacteria	*Aliterella*	0.73	0.23	0.29	1.18	0.14
Desulfobacterota	*Desulfosarcinaceae* (UG)	-0.45	0.21	-0.87	-0.04	1
Firmicutes	*Clostridium sensu stricto 1*	0.39	0.12	0.15	0.62	0.15
Firmicutes	*Limnochordaceae*	-1.45	0.18	-1.79	-1.1	**0**
Firmicutes	*Lutispora*	0.32	0.21	-0.1	0.73	1
MBNT15	*MBNT15*	0.54	0.12	0.3	0.79	**<0.01**
Patescibacteria	*Saccharimonadales*	0.15	0.09	-0.02	0.33	1
Planctomycetota	*Fimbriiglobus*	-0.32	0.27	-0.86	0.22	1
Planctomycetota	*Gemmata*	-0.54	0.32	-1.16	0.09	1
Planctomycetota	*Gemmataceae* (UG)	-0.35	0.38	-1.1	0.39	1
Planctomycetota	*Pirellula*	0.64	0.18	0.29	0.99	**0.04**
Planctomycetota	*Tundrisphaera*	-0.4	0.33	-1.05	0.26	1

Table 5.4A continued

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Proteobacteria	*Alpha I cluster*	-0.68	0.19	-1.05	-0.31	**0.04**
Proteobacteria	*Amaricoccus*	-0.24	0.12	-0.47	-0.01	1
Proteobacteria	*Aquisphaera*	-0.23	0.13	-0.48	0.03	1
Proteobacteria	*Coxiella*	0.23	0.2	-0.17	0.62	1
Proteobacteria	*D05-2*	-0.71	0.25	-1.19	-0.22	0.51
Proteobacteria	*Diplorickettsiaceae* (UG)	-0.67	0.32	-1.28	-0.05	1
Proteobacteria	*Geminicoccus*	-1.38	0.37	-2.1	-0.65	**0.02**
Proteobacteria	*Legionella*	0.37	0.16	0.06	0.68	1
Verrucomicrobiota	*Neochlamydia*	-0.53	0.25	-1.02	-0.04	1

Table 5.4B Differential abundance in bacterial genera between 2018 and 2019.

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Acidobacteriota	*Subgroup 17*	-0.58	0.13	-0.84	-0.32	**<0.01**
Actinobacteriota	*Conexibacter*	-0.3	0.19	-0.68	0.08	1
Actinobacteriota	*Euzebyaceae* (UG)	-0.49	0.19	-0.87	-0.12	1
Actinobacteriota	*Frankiales*	-0.01	0.17	-0.33	0.31	1
Actinobacteriota	*Gaiellales* (UF)	3.02	0.12	2.78	3.26	**<0.01**
Actinobacteriota	*Nocardioides*	-0.08	0.18	-0.43	0.27	1
Actinobacteriota	*Parafrigoribacterium*	-0.34	0.09	-0.52	-0.16	**0.05**
Cyanobacteria	*Aliterella*	4.39	0.18	4.04	4.74	**0**
Desulfobacterota	*Desulfosarcinaceae* (UG)	-0.46	0.16	-0.79	-0.14	0.85
Firmicutes	*Clostridium sensu stricto 1*	0.04	0.12	-0.2	0.29	1
Firmicutes	*Limnochordaceae*	-0.18	0.1	-0.38	0.02	1
Firmicutes	*Lutispora*	-3.38	0.15	-3.68	-3.08	**<0.01**
MBNT15	*MBNT15*	-0.48	0.13	-0.74	-0.22	0.04
Patescibacteria	*Saccharimonadales*	0.1	0.09	-0.08	0.27	1
Planctomycetota	*Fimbriiglobus*	-0.87	0.21	-1.27	-0.47	**<0.01**
Planctomycetota	*Gemmata*	-0.71	0.22	-1.15	-0.27	0.27
Planctomycetota	*Gemmataceae* (UG)	-1.81	0.28	-2.36	-1.26	**<0.01**
Planctomycetota	*Pirellula*	-0.21	0.16	-0.53	0.11	1
Planctomycetota	*Tundrisphaera*	-0.73	0.24	-1.21	-0.26	0.45

Table 5.4B continued

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Proteobacteria	*alphaI cluster*	0.2	0.14	-0.07	0.47	1
Proteobacteria	*Amaricoccus*	0.71	0.12	0.47	0.95	**<0.01**
Proteobacteria	*Aquisphaera*	1.25	0.13	0.99	1.51	**<0.01**
Proteobacteria	*Coxiella*	-0.61	0.16	-0.92	-0.29	**0.03**
Proteobacteria	*D05-2*	-0.27	0.16	-0.58	0.04	1
Proteobacteria	*Diplorickettsiaceae* (UG)	-0.45	0.24	-0.92	0.01	1
Proteobacteria	*Geminicoccus*	-0.99	0.29	-1.55	-0.43	0.09
Proteobacteria	*Legionella*	0.42	0.14	0.13	0.7	0.68
Verrucomicrobiota	*Neochlamydia*	-0.46	0.21	-0.87	-0.05	1

Table 5.4C Differential abundance in bacterial genera between 2017 and 2019.

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Acidobacteriota	*Subgroup 17*	-0.54	0.15	-0.83	-0.25	**0.03**
Actinobacteriota	*Conexibacter*	0.91	0.24	0.44	1.39	**0.02**
Actinobacteriota	*Euzebyaceae* (UG)	-2.48	0.24	-2.96	-2	**<0.01**
Actinobacteriota	*Frankiales*	-0.85	0.22	-1.29	-0.41	**0.02**
Actinobacteriota	*Gaiellales* (UF)	2.99	0.12	2.74	3.23	**<0.01**
Actinobacteriota	*Nocardioides*	0.68	0.17	0.34	1.03	**0.01**
Actinobacteriota	*Parafrigoribacterium*	0	0	0	0	**<0.01**
Cyanobacteria	*Aliterella*	5.18	0.18	4.81	5.54	**<0.01**
Desulfobacterota	*Desulfosarcinaceae* (UG)	-0.86	0.18	-1.22	-0.5	**<0.01**
Firmicutes	*Clostridium sensu stricto 1*	0.49	0.11	0.28	0.7	**<0.01**
Firmicutes	*Limnochordaceae*	-1.56	0.17	-1.9	-1.23	**<0.01**
Firmicutes	*Lutispora*	-3.02	0.17	-3.36	-2.68	**<0.01**
MBNT15	*MBNT15*	0.12	0.09	-0.06	0.31	1
Patescibacteria	*Saccharimonadales*	0.31	0.08	0.15	0.48	**0.02**
Planctomycetota	*Fimbriiglobus*	-1.12	0.25	-1.62	-0.63	**<0.01**
Planctomycetota	*Gemmata*	-1.19	0.29	-1.76	-0.62	**0.01**
Planctomycetota	*Gemmataceae* (UG)	-2.12	0.31	-2.73	-1.52	**<0.01**
Planctomycetota	*Pirellula*	0.49	0.16	0.18	0.8	0.32
Planctomycetota	*Tundrisphaera*	-1.08	0.3	-1.66	-0.5	**0.04**

Table 5.4C continued

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Proteobacteria	*alphaI cluster*	-0.42	0.2	-0.8	-0.03	1
Proteobacteria	*Amaricoccus*	0.53	0.13	0.28	0.79	**0.01**
Proteobacteria	*Aquisphaera*	1.09	0.15	0.8	1.37	**<0.01**
Proteobacteria	*Coxiella*	-0.32	0.16	-0.63	-0.02	1
Proteobacteria	*D05-2*	-0.92	0.24	-1.38	-0.45	**0.02**
Proteobacteria	*Diplorickettsiaceae* (UG)	-1.07	0.29	-1.64	-0.5	**0.04**
Proteobacteria	*Geminicoccus*	-2.32	0.32	-2.96	-1.69	**<0.01**
Proteobacteria	*Legionella*	0.85	0.14	0.57	1.13	**<0.01**
Verrucomicrobiota	*Neochlamydia*	-0.92	0.21	-1.34	-0.5	**<0.01**

Table 5.4D Differential abundance in bacterial phlya between 2017 and 2018.

Phylum	Log fold change	Standard Error	Lower C.I.	Upper C.I.	p_{adj}
Cyanobacteria	0.66	0.23	0.22	1.1	0.06
Firmicutes	0.14	0.18	-0.21	0.49	1
MBNT15	0.44	0.16	0.12	0.75	0.11
Patescibacteria	0.4	0.14	0.12	0.68	0.08
Planctomycetota	-0.22	0.26	-0.73	0.29	1
Proteobacteria	-0.6	0.25	-1.08	-0.12	0.25
Verrucomicrobiota	-0.32	0.22	-0.76	0.11	1

Table 5.4E Differential abundance in bacterial phlya between 2018 and 2019.

Phylum	Log fold change	Standard Error	Lower C.I.	Upper C.I.	p_{adj}
Cyanobacteria	3.98	0.17	3.65	4.31	**<0.01**
Firmicutes	-1.31	0.16	-1.63	-0.99	**<0.01**
MBNT15	-0.48	0.15	-0.76	-0.19	**0.02**
Patescibacteria	0.32	0.13	0.06	0.58	0.31
Planctomycetota	-1.71	0.19	-2.08	-1.33	**<0.01**
Proteobacteria	-0.43	0.18	-0.78	-0.07	0.37
Verrucomicrobiota	-0.33	0.2	-0.72	0.05	1

Table 5.4F Differential abundance in bacterial phlya between 2017 and 2019.

Phylum	Log fold change	Standard Error	Lower C.I.	Upper C.I.	p_{adj}
Cyanobacteria	4.65	0.2	4.26	5.04	**<0.01**
Firmicutes	-1.16	0.18	-1.51	-0.81	**<0.01**
MBNT15	-0.03	0.13	-0.28	0.22	1
Patescibacteria	0.73	0.12	0.48	0.97	**<0.01**
Planctomycetota	-1.92	0.22	-2.35	-1.48	**<0.01**
Proteobacteria	-1.02	0.22	-1.45	-0.58	**<0.01**
Verrucomicrobiota	-0.65	0.19	-1.03	-0.27	**0.01**

Table 5.5 Results of ANCOM-BC analysis identifying significant differences in pair-wise comparisons between seasons, including natural log fold change, standard errors, confidence limits and adjusted p-values, with a p_{adj} <0.05 denoting significance. Positive log fold change values indicates that the first season was significantly enriched for the bacterial phylum or genus while a negative log fold change value indicated that the second season was significantly enriched. For example, an uncultured genus in family *Vicinamibacterales* was significantly enriched in spring when compared against summer. Table **5.5A** contains the results of the ANCOM-BC analysis of differential abundance in bacterial genera between spring and summer. Table **5.5B** contains the results of the ANCOM-BC analysis of differential abundance in bacterial genera between summer and fall. Table **5.5C** contains the results of the ANCOM-BC analysis of differential abundance in bacterial genera between spring and fall. Table **5.5D** contains the results of the ANCOM-BC analysis of differential abundance in bacterial phlya between spring and summer. Table **5.5E** contains the results of the ANCOM-BC analysis of differential abundance in bacterial phlya between summer and fall. Table **5.5F** contains the results of the ANCOM-BC analysis of differential abundance in bacterial phlya between spring and fall.

Table 5.5A Differential abundance in bacterial genera between spring and summer.

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Acidobacteriota	*Vicinamibacterales* (UF)	1.14	0.31	0.54	1.74	**0.03**
Actinobacteriota	*67-14*	2.01	0.32	1.37	2.64	**<0.01**
Actinobacteriota	*Actinomycetospora*	-0.16	0.09	-0.34	0.03	1
Actinobacteriota	*Arthrobacter*	0.75	0.19	0.38	1.11	**0.01**
Actinobacteriota	*Euzebyaceae* (UG)	0.09	0.14	-0.19	0.37	1
Actinobacteriota	*Frankiales*	0.95	0.23	0.49	1.41	**0.01**
Bacteroidota	*Flavobacterium*	-0.15	0.13	-0.39	0.1	1
Bacteroidota	*Sporocytophaga*	0.04	0.09	-0.14	0.23	**<0.01**
Cyanobacteria	*Aliterella*	-0.68	0.53	-1.71	0.35	1
Dependentiae	*Babeliales*	1.78	0.31	1.17	2.4	**<0.01**
Firmicutes	*Bacillus*	1.19	0.33	0.55	1.84	**0.05**
Firmicutes	*Cellulosimicrobium*	-0.06	0.09	-0.24	0.12	**<0.01**
Firmicutes	*Clostridium sensu stricto 1*	-0.73	0.21	-1.15	-0.31	1
Firmicutes	*Erysipelatoclostridia*	-0.32	0.14	-0.59	-0.05	1
Firmicutes	*Paenibacillus*	-1.76	0.27	-2.3	-1.23	**<0.01**
Myxococcota	*Nannocystaceae* (UG)	1.65	0.33	1	2.29	**<0.01**
Patescibacteria	*LWQ8*	0.02	0.29	-0.56	0.59	1
Patescibacteria	*Parcubacteria*	-0.01	0.09	-0.19	0.16	**<0.01**
Planctomycetota	*Gemmataceae* (UG)	-2.46	0.39	-3.21	-1.7	**<0.01**
Planctomycetota	*Planctomicrobium*	0.01	0.08	-0.15	0.18	**<0.01**

Table 5.5A continued

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Proteobacteria	*A21b*	-0.12	0.09	-0.3	0.05	1
Proteobacteria	*Alkanindiges*	-0.26	0.08	-0.42	-0.1	**<0.01**
Proteobacteria	*Amaricoccus*	0.88	0.29	0.31	1.44	1
Proteobacteria	*Anaeromyxobacter*	0.9	0.24	0.43	1.37	**0.03**
Proteobacteria	*Aureimonas*	1.03	0.27	0.5	1.56	**0.02**
Proteobacteria	*Burkholderia-Caballeronia-Paraburkholderia*	-0.17	0.23	-0.63	0.28	1
Proteobacteria	*Caulobacter*	-0.51	0.15	-0.81	-0.22	1
Proteobacteria	*D05-2*	0.3	0.16	-0.01	0.6	1
Proteobacteria	*Diplorickettsiaceae* (UG)	-0.45	0.31	-1.05	0.16	1
Proteobacteria	*Ensifer*	-0.13	0.13	-0.4	0.13	1
Proteobacteria	*Escherichia-Shigella*	-0.31	0.21	-0.73	0.11	1
Proteobacteria	*Geminicoccus*	-0.64	0.34	-1.32	0.03	1
Proteobacteria	*Lysobacter*	1.99	0.29	1.41	2.56	**<0.01**
Proteobacteria	*Massilia*	1.33	0.3	0.74	1.92	**<0.01**
Proteobacteria	*Ralstonia*	1.52	0.34	0.85	2.18	**<0.01**
Proteobacteria	*Reyranella*	0.94	0.27	0.4	1.48	1
Proteobacteria	*Thiomicrospiraceae* (UG)	0	0.2	-0.38	0.39	1
Proteobacteria	*Xanthobacteraceae* (UG)	1.36	0.26	0.85	1.87	**<0.01**
Verrucomicrobiota	*Christensenellaceae R-7 group*	-0.45	0.23	-0.9	0	1
Verrucomicrobiota	*Chthoniobacter*	0.5	0.33	-0.15	1.15	1
Verrucomicrobiota	*Luteolibacter*	1.17	0.27	0.65	1.69	**<0.01**
Verrucomicrobiota	*Neochlamydia*	-0.9	0.43	-1.74	-0.06	1

Table 5.5B Differential abundance in bacterial genera between summer and fall.

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Acidobacteriota	*Vicinamibacterales (UF)*	-1.24	0.29	-1.81	-0.66	**<0.01**
Actinobacteriota	*67-14*	-2.66	0.31	-3.27	-2.06	**<0.01**
Actinobacteriota	*Actinomycetospora*	-0.03	0.07	-0.16	0.11	1
Actinobacteriota	*Arthrobacter*	0	0	0	0	NA
Actinobacteriota	*Euzebyaceae (UG)*	1.02	0.15	0.72	1.32	**<0.01**
Actinobacteriota	*Frankiales*	-0.81	0.23	-1.25	-0.36	1
Bacteroidota	*Flavobacterium*	-0.14	0.11	-0.34	0.07	1
Bacteroidota	*Sporocytophaga*	0	0	0	0	NA
Cyanobacteria	*Aliterella*	-0.17	0.49	-1.14	0.8	1
Dependentiae	*Babeliales*	-1.54	0.3	-2.14	-0.95	**<0.01**
Firmicutes	*Bacillus*	-1.49	0.32	-2.11	-0.87	**<0.01**
Firmicutes	*Cellulosimicrobium*	0	0	0	0	NA
Firmicutes	*Clostridium sensu stricto 1*	0.05	0.18	-0.31	0.41	1
Firmicutes	*Erysipelatoclostridia*	-0.16	0.12	-0.39	0.08	1
Firmicutes	*Paenibacillus*	0.57	0.21	0.17	0.97	1
Myxococcota	*Nannocystaceae (UG)*	-1.55	0.31	-2.16	-0.93	**<0.01**
Patescibacteria	*LWQ8*	-0.67	0.26	-1.18	-0.15	1
Patescibacteria	*Parcubacteria*	0	0	0	0	NA
Planctomycetota	*Gemmataceae (UG)*	1.55	0.32	0.92	2.18	**<0.01**
Planctomycetota	*Planctomicrobium*	0	0	0	0	NA
Proteobacteria	*A21b*	0	0	0	0	NA
Proteobacteria	*Alkanindiges*	0.2	0.06	0.08	0.33	**<0.01**
Proteobacteria	*Amaricoccus*	-1.05	0.26	-1.57	-0.53	**0.01**
Proteobacteria	*Anaeromyxobacter*	-0.71	0.23	-1.15	-0.26	1
Proteobacteria	*Aureimonas*	-1.04	0.26	-1.54	-0.53	**0.01**
Proteobacteria	*Burkholderia-Caballeronia-Paraburkholderia*	-0.29	0.21	-0.71	0.13	1
Proteobacteria	*Caulobacter*	0.11	0.13	-0.15	0.37	1
Proteobacteria	*D05-2*	0.56	0.17	0.24	0.89	1
Proteobacteria	*Diplorickettsiaceae (UG)*	1.51	0.31	0.9	2.12	**<0.01**
Proteobacteria	*Ensifer*	0	0	0	0	NA
Proteobacteria	*Escherichia-Shigella*	-0.22	0.2	-0.6	0.17	1

Table 5.5B continued

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Proteobacteria	*Geminicoccus*	1.75	0.33	1.11	2.39	**<0.01**
Proteobacteria	*Lysobacter*	0	0	0	0	NA
Proteobacteria	*Massilia*	-1.24	0.29	-1.8	-0.67	**<0.01**
Proteobacteria	*Ralstonia*	-1.55	0.32	-2.19	-0.92	**<0.01**
Proteobacteria	*Reyranella*	-1.14	0.26	-1.65	-0.63	**<0.01**
Proteobacteria	*Thiomicrospiraceae* (UG)	-0.44	0.18	-0.8	-0.08	1
Proteobacteria	*Xanthobacteraceae* (UG)	-0.93	0.25	-1.43	-0.44	**0.03**
Verrucomicrobiota	*Christensenellaceae R-7 group*	0.01	0.21	-0.39	0.41	1
Verrucomicrobiota	*Chthoniobacter*	-1.2	0.31	-1.8	-0.6	**0.01**
Verrucomicrobiota	*Luteolibacter*	-1.15	0.25	-1.64	-0.66	**<0.01**
Verrucomicrobiota	*Neochlamydia*	0.13	0.39	-0.63	0.89	1

Table 5.5C Differential abundance in bacterial genera between spring and fall.

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Acidobacteriota	*Vicinamibacterales* (UF)	0.21	0.1	0.01	0.41	1
Actinobacteriota	*67-14*	0.77	0.16	0.46	1.08	**<0.01**
Actinobacteriota	*Actinomycetospora*	0.3	0.08	0.14	0.46	**0.05**
Actinobacteriota	*Arthrobacter*	0	0	0	0	NA
Actinobacteriota	*Euzebyaceae* (UG)	-1	0.17	-1.34	-0.66	**<0.01**
Actinobacteriota	*Frankiales*	-0.03	0.08	-0.18	0.13	1
Bacteroidota	*Flavobacterium*	0.4	0.09	0.23	0.57	**<0.01**
Bacteroidota	*Sporocytophaga*	0.19	0.09	0.03	0.36	1
Cyanobacteria	*Aliterella*	0.96	0.26	0.45	1.48	**0.04**
Dependentiae	*Babeliales*	-0.13	0.12	-0.36	0.11	1
Firmicutes	*Bacillus*	0.41	0.12	0.18	0.64	1
Firmicutes	*Cellulosimicrobium*	0.09	0.09	-0.08	0.26	1
Firmicutes	*Clostridium sensu stricto 1*	0.79	0.14	0.53	1.06	**<0.01**
Firmicutes	*Erysipelatoclostridium*	0.59	0.09	0.42	0.77	**<0.01**
Firmicutes	*Paenibacillus*	1.31	0.22	0.87	1.74	**<0.01**

Table 5.5C continued

Phylum	Genus	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Myxococcota	*Nannocystaceae* (UG)	0.02	0.13	-0.24	0.27	1
Patescibacteria	*LWQ8*	0.76	0.16	0.45	1.08	**<0.01**
Patescibacteria	*Parcubacteria*	0	0	0	0	NA
Planctomycetota	*Gemmataceae* (UG)	1.02	0.29	0.45	1.59	1
Planctomycetota	*Planctomicrobium*	0	0	0	0	NA
Proteobacteria	*A21b*	0.35	0.08	0.19	0.5	**<0.01**
Proteobacteria	*Alkanindiges*	0.17	0.08	0.02	0.32	1
Proteobacteria	*Amaricoccus*	0.29	0.14	0.02	0.56	1
Proteobacteria	*Anaeromyxobacter*	0	0	0	0	NA
Proteobacteria	*Aureimonas*	0.12	0.11	-0.09	0.33	1
Proteobacteria	*Burkholderia-Caballeronia-Paraburkholderia*	0.58	0.12	0.35	0.81	**<0.01**
Proteobacteria	*Caulobacter*	0.52	0.1	0.31	0.72	**<0.01**
Proteobacteria	*D05-2*	-0.74	0.14	-1.03	-0.46	**<0.01**
Proteobacteria	*Diplorickettsiaceae* (UG)	-0.95	0.2	-1.35	-0.56	**<0.01**
Proteobacteria	*Ensifer*	0.44	0.09	0.27	0.61	**<0.01**
Proteobacteria	*Escherichia-Shigella*	0.64	0.11	0.43	0.86	**<0.01**
Proteobacteria	*Geminicoccus*	-1	0.26	-1.5	-0.5	**0.02**
Proteobacteria	*Lysobacter*	0	0	0	0	NA
Proteobacteria	*Massilia*	0.02	0.12	-0.21	0.25	1
Proteobacteria	*Ralstonia*	0.15	0.14	-0.12	0.42	1
Proteobacteria	*Reyranella*	0.31	0.1	0.12	0.5	1
Proteobacteria	*Thiomicrospiraceae* (UG)	0.55	0.09	0.36	0.73	**<0.01**
Proteobacteria	*Xanthobacteraceae* (UG)	-0.32	0.09	-0.49	-0.14	1
Verrucomicrobiota	*Christensenellaceae R-7 group*	0.55	0.14	0.29	0.82	**0.01**
Verrucomicrobiota	*Chthoniobacter*	0.81	0.15	0.51	1.11	**<0.01**
Verrucomicrobiota	*Luteolibacter*	0.09	0.1	-0.1	0.29	1
Verrucomicrobiota	*Neochlamydia*	0.88	0.22	0.44	1.32	**0.01**

Table 5.5D Differential abundance in bacterial phlya between spring and summer.

Phylum	Log fold change	Std. Error	Lower C.I.	Upper C.I.	p_{adj}
Actinobacteriota	0.95	0.24	0.47	1.42	**<0.01**
Campilobacterota	-0.58	0.22	-1.02	-0.14	0.19
Chloroflexi	0.99	0.33	0.34	1.63	**0.05**
Dependentiae	1.04	0.3	0.46	1.62	**0.01**
Desulfobacterota	-0.6	0.22	-1.04	-0.17	0.13
Fibrobacterota	-0.73	0.16	-1.05	-0.41	**<0.01**
Gemmatimonadota	-0.29	0.22	-0.72	0.15	1
MBNT15	-0.53	0.2	-0.92	-0.14	0.15
Myxococcota	1.55	0.32	0.93	2.17	**<0.01**
Patescibacteria	-0.81	0.26	-1.32	-0.3	**0.04**
Planctomycetota	-1.12	0.32	-1.74	-0.5	**0.01**
Proteobacteria	0.78	0.2	0.4	1.17	**<0.01**

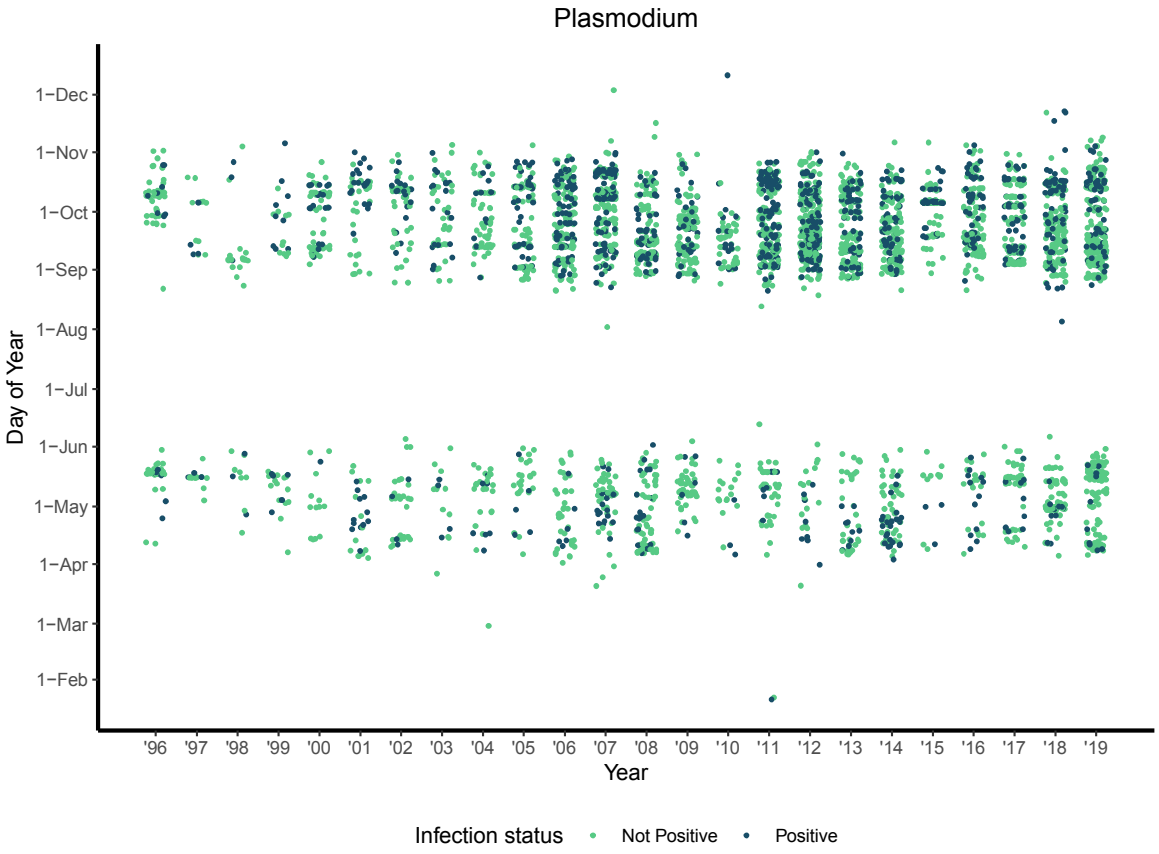
Table 5.5E Differential abundance in bacterial phlya between summer and fall.

Phylum	Log fold change	Std. Error	Lower CI	Upper CI	p_{adj}
Actinobacteriota	0.41	0.23	-0.05	0.87	1
Campilobacterota	-1.95	0.23	-2.41	-1.5	**<0.01**
Chloroflexi	1.09	0.31	0.49	1.69	**0.01**
Dependentiae	0.85	0.28	0.3	1.39	**0.04**
Desulfobacterota	-1.23	0.21	-1.64	-0.82	**<0.01**
Fibrobacterota	-1	0.15	-1.29	-0.7	**<0.01**
Gemmatimonadota	-0.68	0.21	-1.09	-0.28	**0.02**
MBNT15	-1.26	0.18	-1.6	-0.91	**<0.01**
Myxococcota	1.44	0.3	0.84	2.04	**<0.01**
Patescibacteria	-0.38	0.24	-0.85	0.1	1
Planctomycetota	-1.11	0.28	-1.66	-0.56	**<0.01**
Proteobacteria	-0.77	0.19	-1.15	-0.4	**<0.01**

Table 5.5F Differential abundance in bacterial phlya between spring and fall.

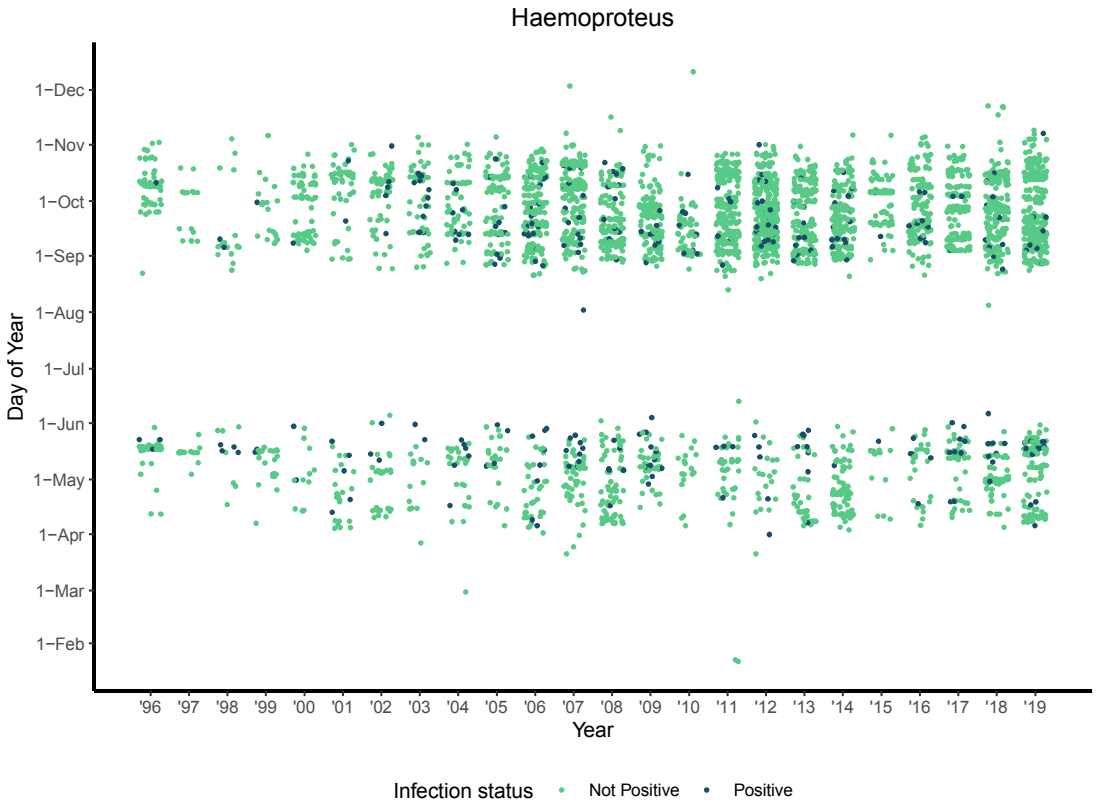
Phylum	Log fold change	Std. Error	Lower CI	Upper CI	p_{adj}
Actinobacteriota	-0.47	0.13	-0.72	-0.22	**0.01**
Campilobacterota	-1.31	0.16	-1.63	-0.99	**<0.01**
Chloroflexi	0.17	0.16	-0.15	0.49	1
Dependentiae	-0.13	0.13	-0.39	0.12	1
Desulfobacterota	-0.56	0.13	-0.82	-0.3	**<0.01**
Fibrobacterota	-0.2	0.1	-0.4	0	0.93
Gemmatimonadota	-0.33	0.11	-0.54	-0.12	**0.04**
MBNT15	-0.66	0.14	-0.94	-0.38	**<0.01**
Myxococcota	-0.05	0.13	-0.31	0.21	1
Patescibacteria	0.5	0.13	0.24	0.75	**<0.01**
Planctomycetota	0.07	0.21	-0.34	0.48	1
Proteobacteria	-1.49	0.16	-1.81	-1.18	**<0.01**

6 Supplemental Figures and Tables, Chapter 3

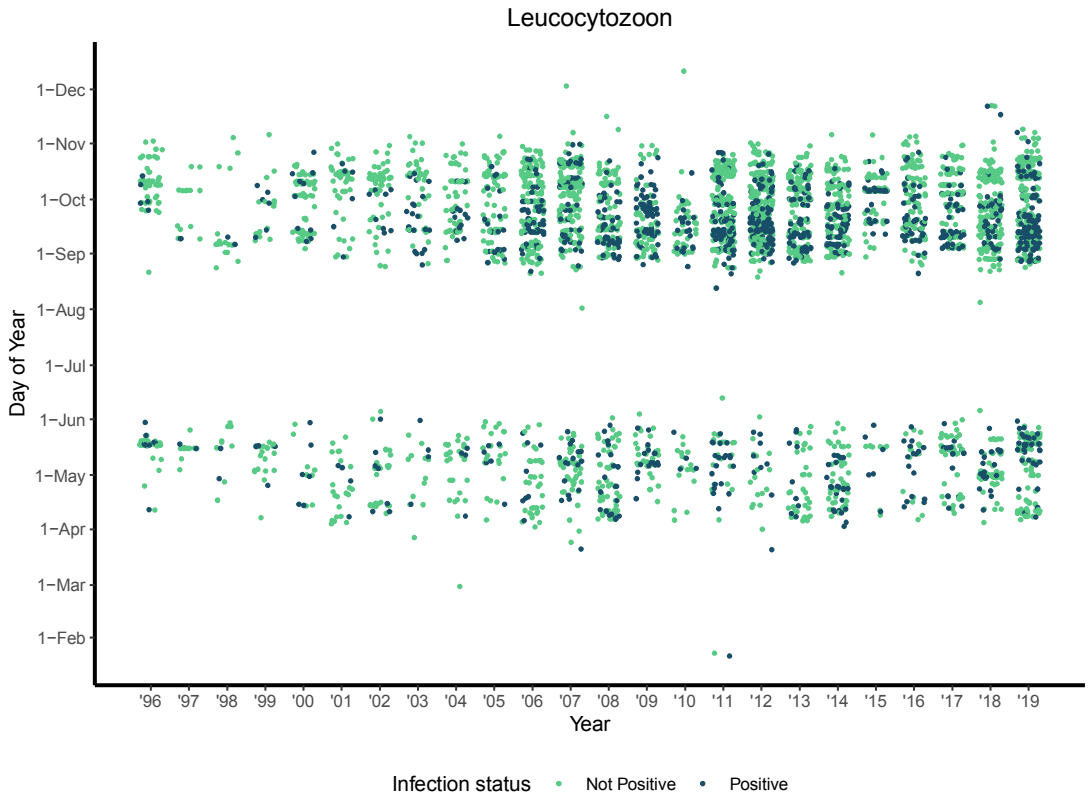


6.1A Incidences of *Plasmodium* infection.

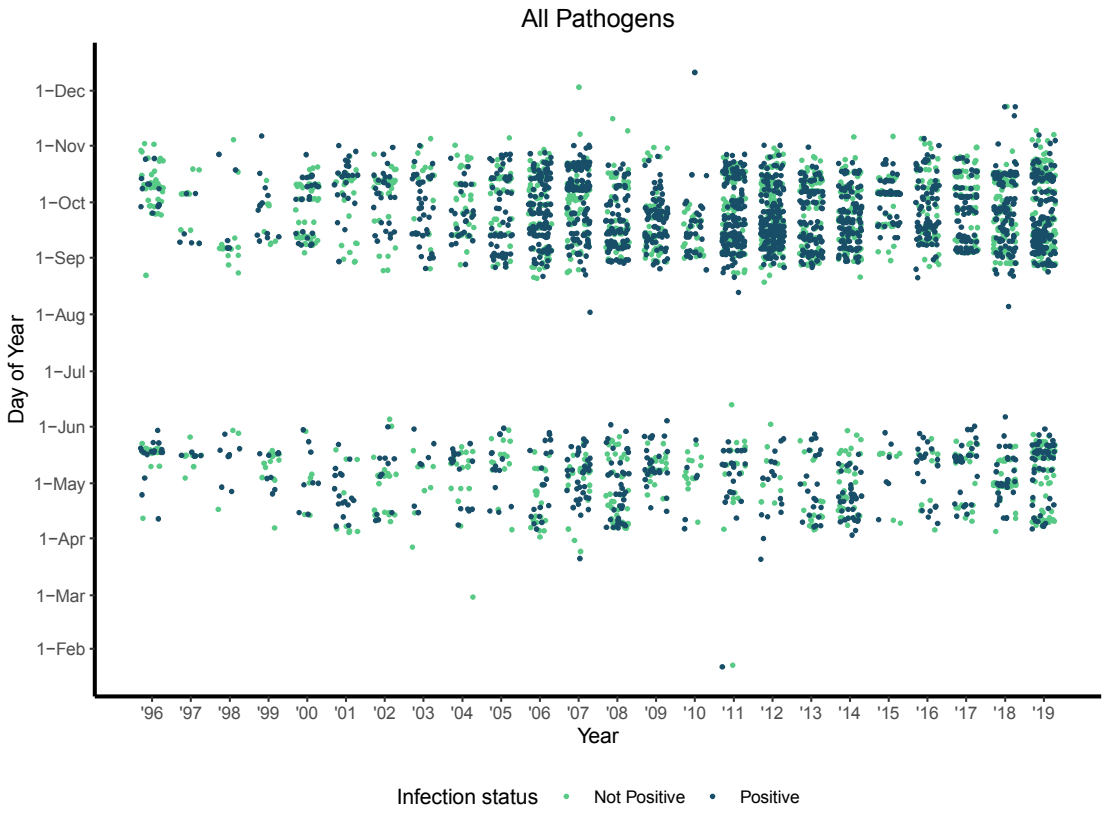
Figure 6.1 Distribution of samples over time colored according to positive or not positive status for Plasmodium (**3.2A**), Haemoproteus (**3.2B**) or Leucocytozoon (**3.2C**). Figure **3.2D** displayed the distribution of samples that screened positive for at least one of the pathogens, indicating the overall positivity of all samples.



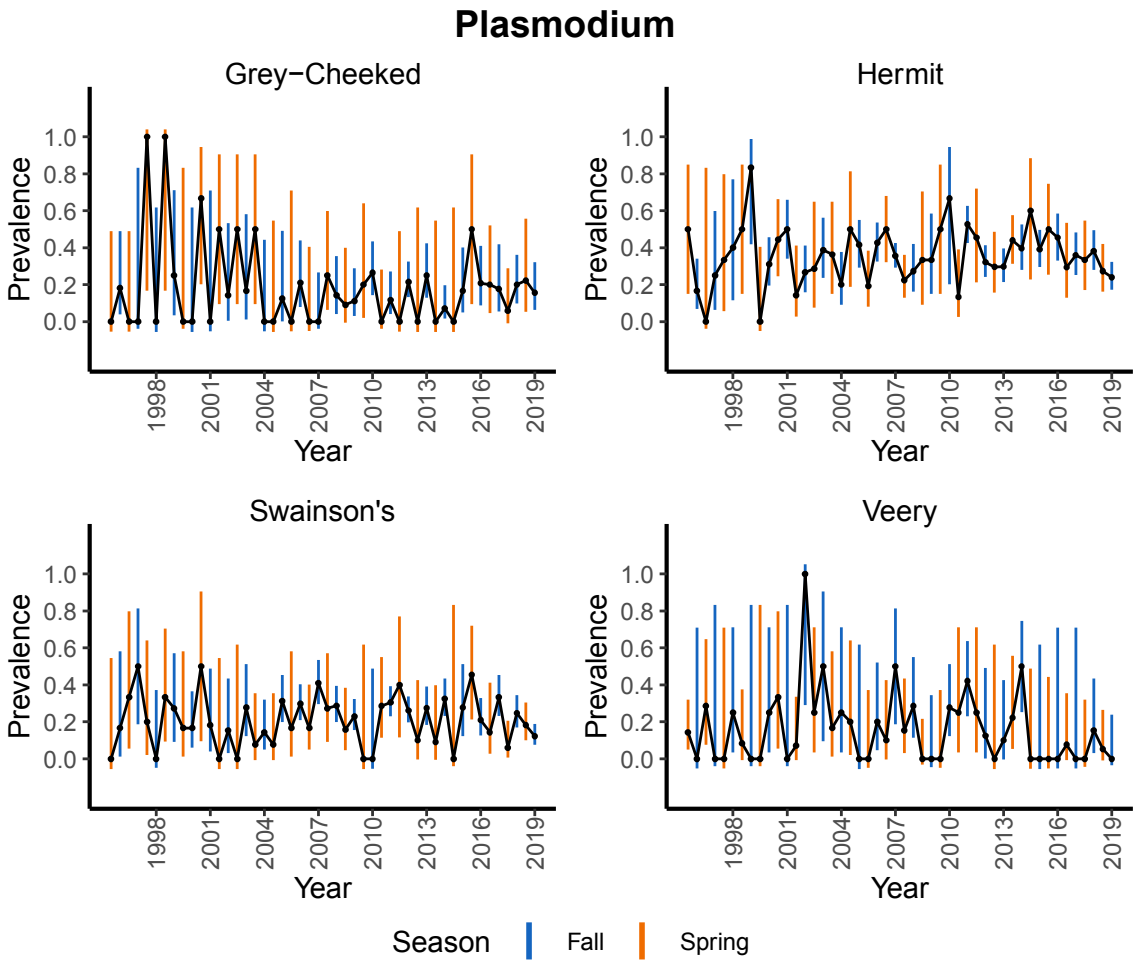
6.1B Incidences of *Haemoproteus* infection.



6.1C Incidences of *Leucocytozoon* infection.

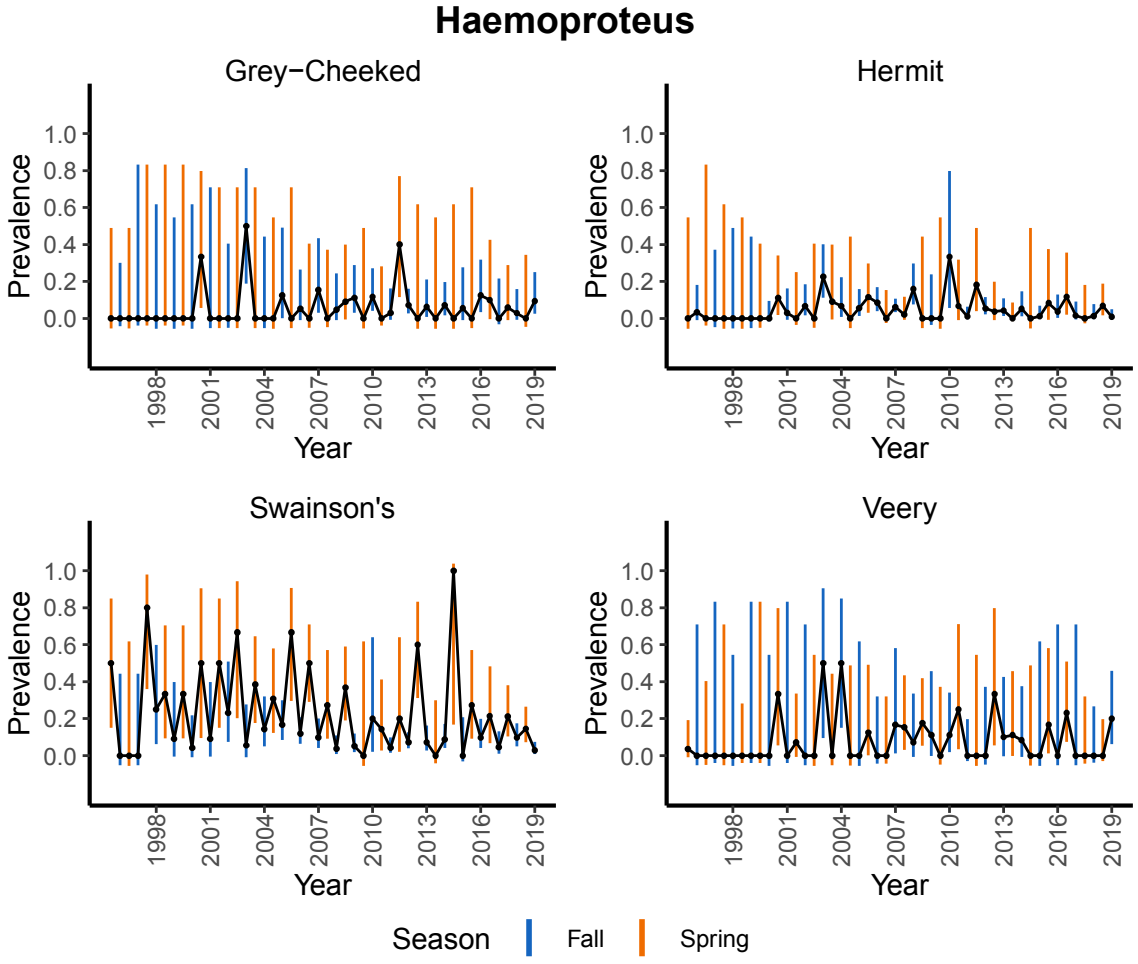


6.1D Incidences of overall infection.

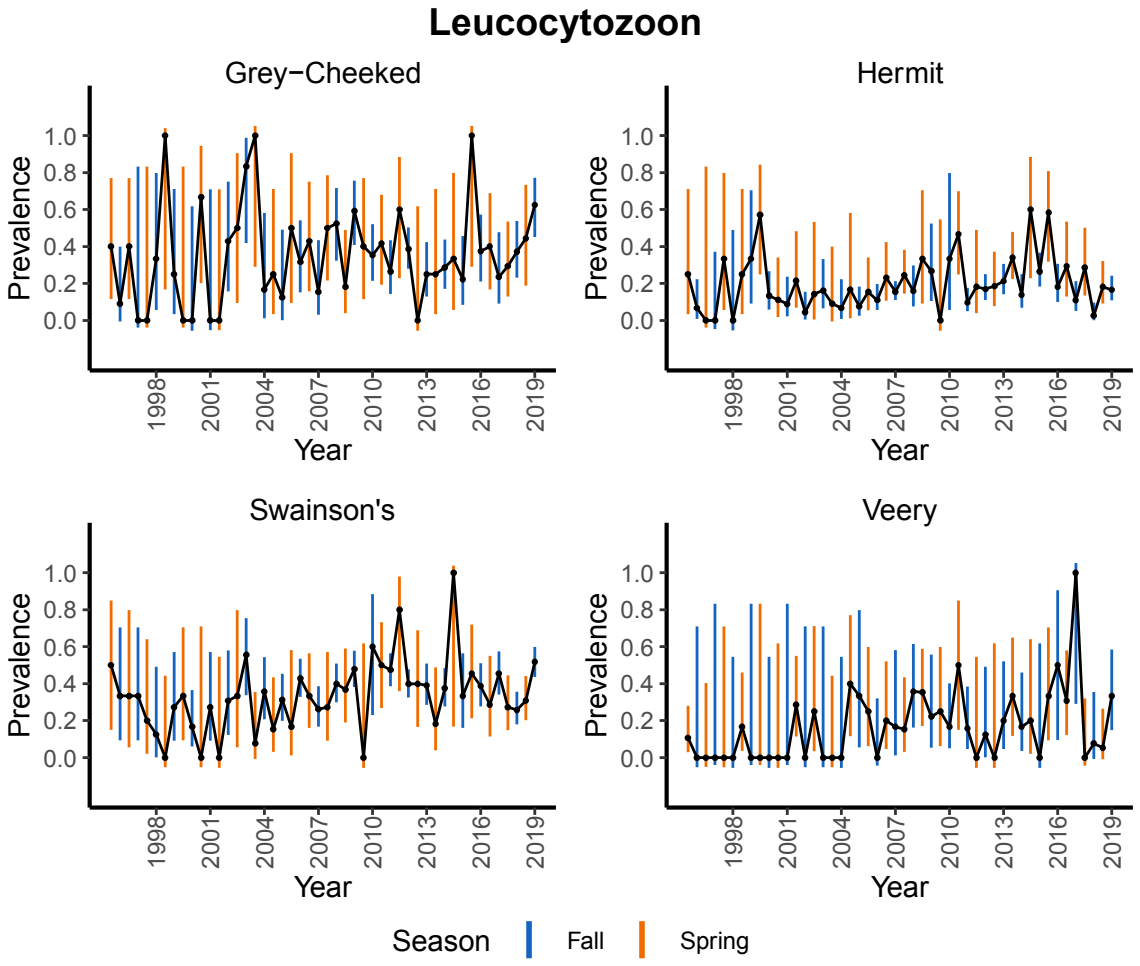


6.2A Observed prevalence and confidence intervals of *Plasmodium*.

Figure 6.2 Observed prevalence of *Plasmodium* (**6.2A**), *Haemeoproteus* (**6.2B**), and *Leucocytozoon* (**6.2C**) with 95% binomial confidence intervals. Confidence intervals are colored according to which season birds were collected in, with orange representing spring migratory periods and blue representing fall migratory periods. Data derived prevalence per time period is denoted by a black dot.



6.2B Observed prevalence and confidence intervals of *Haemeoproteus.*



6.2C Observed prevalence and confidence intervals of *Leucocytozoon.*

Table 6.1 Distribution of birds per time period, including the number of birds screened per time period, the number of individuals positive for each of the three haemosporidian pathogens and the prevalence of each pathogen. These data are show for all birds (**6.1A**) and for each host species separately (**6.1B**: Grey-Cheeked thrush, **6.1C**: Hermit thrush, **6.1D**: Swainson's thrush, **6.1E**: Veery).

Table 6.1A Prevalence of pathogens per time period in all birds.

			Plasmodium		*Haemoproteus*		*Leucocytozoon*		Total	
Year	Seas.	Num. Scr.	Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.
1996	Spring	41	6	14.6%	3	7.3%	8	19.5%	16	39.0%
1996	Fall	50	8	16.0%	1	2.0%	5	10.0%	12	24.0%
1997	Spring	15	3	20.0%	0	0.0%	3	20.0%	5	33.3%
1997	Fall	15	5	33.3%	0	0.0%	2	13.3%	6	40.0%
1998	Spring	11	3	27.3%	4	36.4%	2	18.2%	8	72.7%
1998	Fall	21	4	19.0%	2	9.5%	2	9.5%	7	33.3%
1999	Spring	24	6	25.0%	2	8.3%	5	20.8%	10	41.7%
1999	Fall	22	9	40.9%	1	4.5%	7	31.8%	14	63.6%
2000	Spring	17	2	11.8%	2	11.8%	7	41.2%	10	58.8%
2000	Fall	76	19	25.0%	1	1.3%	10	13.2%	26	34.2%
2001	Spring	27	12	44.4%	5	18.5%	4	14.8%	17	63.0%
2001	Fall	49	20	40.8%	3	6.1%	6	12.2%	24	49.0%
2002	Spring	36	4	11.1%	4	11.1%	8	22.2%	13	36.1%
2002	Fall	68	17	25.0%	7	10.3%	9	13.2%	30	44.1%
2003	Spring	16	4	25.0%	2	12.5%	4	25.0%	8	50.0%
2003	Fall	57	19	33.3%	12	21.1%	20	35.1%	38	66.7%
2004	Spring	34	7	20.6%	6	17.6%	4	11.8%	15	44.1%
2004	Fall	71	12	16.9%	8	11.3%	13	18.3%	31	43.7%
2005	Spring	28	5	17.9%	4	14.3%	6	21.4%	14	50.0%
2005	Fall	134	42	31.3%	13	9.7%	30	22.4%	74	55.2%
2006	Spring	42	6	14.3%	8	19.0%	8	19.0%	19	45.2%
2006	Fall	196	67	34.2%	18	9.2%	51	26.0%	117	59.7%
2007	Spring	61	17	27.9%	9	14.8%	17	27.9%	35	57.4%

Table 6.1A continued

Year	Seas.	Num. Scr.	*Plasmodium*		*Haemoproteus*		*Leucocytozoon*		Total	
			Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.
2007	Fall	274	97	35.4%	21	7.7%	49	17.9%	147	53.6%
2008	Spring	81	18	22.2%	6	7.4%	21	25.9%	39	48.1%
2008	Fall	159	42	26.4%	12	7.5%	55	34.6%	93	58.5%
2009	Spring	53	6	11.3%	11	20.8%	17	32.1%	29	54.7%
2009	Fall	148	30	20.3%	9	6.1%	68	45.9%	90	60.8%
2010	Spring	20	3	15.0%	0	0.0%	4	20.0%	6	30.0%
2010	Fall	60	16	26.7%	8	13.3%	19	31.7%	34	56.7%
2011	Spring	45	7	15.6%	4	8.9%	21	46.7%	24	53.3%
2011	Fall	264	97	36.7%	7	2.7%	77	29.2%	159	60.2%
2012	Spring	25	8	32.0%	5	20.0%	9	36.0%	16	64.0%
2012	Fall	346	92	26.6%	22	6.4%	109	31.5%	184	53.2%
2013	Spring	43	9	20.9%	8	18.6%	9	20.9%	21	48.8%
2013	Fall	205	56	27.3%	12	5.9%	57	27.8%	105	51.2%
2014	Spring	74	25	33.8%	1	1.4%	23	31.1%	37	50.0%
2014	Fall	192	58	30.2%	14	7.3%	52	27.1%	107	55.7%
2015	Spring	14	3	21.4%	1	7.1%	6	42.9%	6	42.9%
2015	Fall	126	42	33.3%	2	1.6%	33	26.2%	63	50.0%
2016	Spring	31	12	38.7%	5	16.1%	16	51.6%	21	67.7%
2016	Fall	143	43	30.1%	11	7.7%	44	30.8%	82	57.3%
2017	Spring	54	10	18.5%	9	16.7%	17	31.5%	31	57.4%
2017	Fall	149	48	32.2%	4	2.7%	43	28.9%	80	53.7%
2018	Spring	81	10	12.3%	8	9.9%	20	24.7%	32	39.5%
2018	Fall	217	61	28.1%	11	5.1%	40	18.4%	102	47.0%
2019	Spring	127	25	19.7%	11	8.7%	30	23.6%	57	44.9%
2019	Fall	307	51	16.6%	11	3.6%	117	38.1%	148	48.2%

Table 6.1B Prevalence of pathogens per time period in all Grey-Cheeked thrush.

Year	Seas.	Num. Scr.	*Plasmodium*		*Haemoproteus*		*Leucocytozoon*		Total	
			Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.
1996	Spring	5	0	0.0%	0	0.0%	2	40.0%	2	40.0%
1996	Fall	11	2	18.2%	0	0.0%	1	9.1%	2	18.2%
1997	Spring	5	0	0.0%	0	0.0%	2	40.0%	2	40.0%
1997	Fall	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%
1998	Spring	1	1	100.0%	0	0.0%	0	0.0%	1	100.0%
1998	Fall	4	1	25.0%	0	0.0%	1	25.0%	2	50.0%
1999	Spring	1	1	100.0%	0	0.0%	1	100.0%	1	100.0%
1999	Fall	4	1	25.0%	0	0.0%	1	25.0%	2	50.0%
2000	Spring	2	0	0.0%	0	0.0%	1	50.0%	1	50.0%
2000	Fall	3	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2001	Spring	3	2	66.7%	1	33.3%	2	66.7%	3	100.0%
2001	Fall	2	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2002	Spring	2	1	50.0%	0	0.0%	0	0.0%	1	50.0%
2002	Fall	7	1	14.3%	0	0.0%	3	42.9%	4	57.1%
2003	Spring	2	1	50.0%	0	0.0%	1	50.0%	2	100.0%
2003	Fall	6	1	16.7%	3	50.0%	5	83.3%	6	100.0%
2004	Spring	2	1	50.0%	0	0.0%	2	100.0%	2	100.0%
2004	Fall	7	0	0.0%	0	0.0%	1	14.3%	1	14.3%
2005	Spring	4	0	0.0%	0	0.0%	1	25.0%	1	25.0%
2005	Fall	10	1	10.0%	1	10.0%	2	20.0%	3	30.0%
2006	Spring	2	0	0.0%	0	0.0%	1	50.0%	1	50.0%
2006	Fall	19	4	21.1%	1	5.3%	6	31.6%	9	47.4%
2007	Spring	7	0	0.0%	0	0.0%	3	42.9%	3	42.9%
2007	Fall	13	0	0.0%	2	15.4%	2	15.4%	4	30.8%
2008	Spring	8	2	25.0%	0	0.0%	4	50.0%	4	50.0%
2008	Fall	21	3	14.3%	1	4.8%	11	52.4%	11	52.4%
2009	Spring	11	1	9.1%	1	9.1%	2	18.2%	3	27.3%

Table 6.1B continued

Year	Seas.	Num. Scr.	*Plasmodium*		*Haemoproteus*		*Leucocytozoon*		Total	
			Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.
2009	Fall	28	3	10.7%	3	10.7%	16	57.1%	20	71.4%
2010	Spring	5	1	20.0%	0	0.0%	2	40.0%	2	40.0%
2010	Fall	34	9	26.5%	4	11.8%	12	35.3%	21	61.8%
2011	Spring	12	0	0.0%	0	0.0%	5	41.7%	5	41.7%
2011	Fall	34	4	11.8%	1	2.9%	9	26.5%	14	41.2%
2012	Spring	5	0	0.0%	2	40.0%	3	60.0%	4	80.0%
2012	Fall	71	15	21.1%	5	7.0%	28	39.4%	40	56.3%
2013	Spring	3	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2013	Fall	32	8	25.0%	2	6.3%	8	25.0%	14	43.8%
2014	Spring	4	0	0.0%	0	0.0%	1	25.0%	1	25.0%
2014	Fall	42	3	7.1%	3	7.1%	12	28.6%	17	40.5%
2015	Spring	3	0	0.0%	0	0.0%	1	33.3%	1	33.3%
2015	Fall	18	3	16.7%	1	5.6%	4	22.2%	8	44.4%
2016	Spring	2	1	50.0%	0	0.0%	2	100.0%	2	100.0%
2016	Fall	24	5	20.8%	3	12.5%	9	37.5%	15	62.5%
2017	Spring	10	2	20.0%	1	10.0%	4	40.0%	6	60.0%
2017	Fall	17	3	17.6%	0	0.0%	4	23.5%	7	41.2%
2018	Spring	17	1	5.9%	1	5.9%	5	29.4%	7	41.2%
2018	Fall	35	7	20.0%	1	2.9%	13	37.1%	18	51.4%
2019	Spring	9	2	22.2%	0	0.0%	4	44.4%	5	55.6%
2019	Fall	32	5	15.6%	3	9.4%	20	62.5%	21	65.6%

Table 6.1C Prevalence of pathogens per time period in all Hermit thrush.

Year	Seas.	Num. Scr.	*Plasmodium*		*Haemoproteus*		*Leucocytozoon*		Total	
			Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.
1996	Spring	4	2	50.0%	0	0.0%	1	25.0%	3	75.0%
1996	Fall	30	5	16.7%	1	3.3%	2	6.7%	7	23.3%
1997	Spring	0	0	0.0%	0	0.0%	0	0.0%	0	0.0%
1997	Fall	8	2	25.0%	0	0.0%	0	0.0%	2	25.0%
1998	Spring	3	1	33.3%	0	0.0%	1	33.3%	2	66.7%
1998	Fall	5	2	40.0%	0	0.0%	0	0.0%	2	40.0%
1999	Spring	4	2	50.0%	0	0.0%	1	25.0%	3	75.0%
1999	Fall	7	5	71.4%	0	0.0%	3	42.9%	6	85.7%
2000	Spring	7	0	0.0%	0	0.0%	4	57.1%	4	57.1%
2000	Fall	45	14	31.1%	0	0.0%	6	13.3%	18	40.0%
2001	Spring	18	8	44.4%	2	11.1%	2	11.1%	11	61.1%
2001	Fall	35	18	51.4%	2	5.7%	3	8.6%	19	54.3%
2002	Spring	14	2	14.3%	0	0.0%	3	21.4%	4	28.6%
2002	Fall	46	12	26.1%	4	8.7%	2	4.3%	17	37.0%
2003	Spring	7	2	28.6%	0	0.0%	1	14.3%	3	42.9%
2003	Fall	31	12	38.7%	7	22.6%	5	16.1%	19	61.3%
2004	Spring	11	4	36.4%	1	9.1%	1	9.1%	5	45.5%
2004	Fall	31	7	22.6%	2	6.5%	2	6.5%	11	35.5%
2005	Spring	6	3	50.0%	0	0.0%	1	16.7%	4	66.7%
2005	Fall	54	22	40.7%	3	5.6%	4	7.4%	26	48.1%
2006	Spring	26	5	19.2%	3	11.5%	4	15.4%	10	38.5%
2006	Fall	82	35	42.7%	7	8.5%	9	11.0%	45	54.9%
2007	Spring	26	13	50.0%	0	0.0%	6	23.1%	17	65.4%
2007	Fall	194	69	35.6%	12	6.2%	30	15.5%	102	52.6%
2008	Spring	49	11	22.4%	1	2.0%	12	24.5%	23	46.9%
2008	Fall	44	12	27.3%	7	15.9%	7	15.9%	21	47.7%
2009	Spring	6	2	33.3%	0	0.0%	2	33.3%	4	66.7%

Table 6.1C continued

Year	Seas.	Num. Scr.	*Plasmodium*		*Haemoproteus*		*Leucocytozoon*		Total	
			Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.
2009	Fall	15	5	33.3%	0	0.0%	4	26.7%	7	46.7%
2010	Spring	4	2	50.0%	0	0.0%	0	0.0%	2	50.0%
2010	Fall	3	2	66.7%	1	33.3%	1	33.3%	3	100.0%
2011	Spring	15	2	13.3%	1	6.7%	7	46.7%	7	46.7%
2011	Fall	93	49	52.7%	1	1.1%	9	9.7%	53	57.0%
2012	Spring	11	5	45.5%	2	18.2%	2	18.2%	7	63.6%
2012	Fall	113	36	31.9%	6	5.3%%	19	16.8%%	50	44.2%%
2013	Spring	27	8	29.6%	1	3.7%	5	18.5%	12	44.4%
2013	Fall	94	28	29.8%	4	4.3%	20	21.3%	45	47.9%
2014	Spring	50	22	44.0%	0	0.0%	17	34.0%	29	58.0%
2014	Fall	58	23	39.7%	3	5.2%	8	13.8%	32	55.2%
2015	Spring	5	3	60.0%	0	0.0%	3	60.0%	3	60.0%
2015	Fall	87	34	39.1%	1	1.1%	23	26.4%	45	51.7%
2016	Spring	12	6	50.0%	1	8.3%	7	58.3%	8	66.7%
2016	Fall	55	25	45.5%	2	3.6%	10	18.2%	30	54.5%
2017	Spring	17	5	29.4%	2	11.8%	5	29.4%	9	52.9%
2017	Fall	64	23	35.9%	1	1.6%	7	10.9%	28	43.8%
2018	Spring	21	7	33.3%	0	0.0%	6	28.6%	12	57.1%
2018	Fall	76	29	38.2%	1	1.3%	2	2.6%	32	42.1%
2019	Spring	44	12	27.3%	3	6.8%	8	18.2%	21	47.7%
2019	Fall	121	29	24.0%	1	0.8%	20	16.5%	43	35.5%

Table 6.1D Prevalence of pathogens per time period in all Swainson's thrush.

Year	Seas.	Num. Scr.	*Plasmodium* Num. Pos.	*Plasmodium* Prev.	*Haemoproteus* Num. Pos.	*Haemoproteus* Prev.	*Leucocytozoon* Num. Pos.	*Leucocytozoon* Prev.	Total Num. Pos.	Total Prev.
1996	Spring	4	0	0.0%	2	50.0%	2	50.0%	3	75.0%
1996	Fall	6	1	16.7%	0	0.0%	2	33.3%	3	50.0%
1997	Spring	3	1	33.3%	0	0.0%	1	33.3%	1	33.3%
1997	Fall	6	3	50.0%	0	0.0%	2	33.3%	4	66.7%
1998	Spring	5	1	20.0%	4	80.0%	1	20.0%	5	100.0%
1998	Fall	8	0	0.0%	2	25.0%	1	12.5%	2	25.0%
1999	Spring	7	2	28.6%	2	28.6%	1	14.3%	4	57.1%
1999	Fall	11	3	27.3%	1	9.1%	3	27.3%	6	54.5%
2000	Spring	7	2	28.6%	2	28.6%	2	28.6%	5	71.4%
2000	Fall	24	4	16.7%	1	4.2%	4	16.7%	7	29.2%
2001	Spring	2	1	50.0%	1	50.0%	0	0.0%	1	50.0%
2001	Fall	11	2	18.2%	1	9.1%	3	27.3%	5	45.5%
2002	Spring	4	0	0.0%	2	50.0%	0	0.0%	2	50.0%
2002	Fall	13	2	15.4%	3	23.1%	4	30.8%	7	53.8%
2003	Spring	3	0	0.0%	2	66.7%	1	33.3%	2	66.7%
2003	Fall	18	5	27.8%	1	5.6%	10	55.6%	11	61.1%
2004	Spring	13	1	7.7%	5	38.5%	1	7.7%	7	53.8%
2004	Fall	29	4	13.8%	4	13.8%	10	34.5%	16	55.2%
2005	Spring	13	1	7.7%	4	30.8%	2	15.4%	7	53.8%
2005	Fall	67	19	28.4%	9	13.4%	23	34.3%	44	65.7%
2006	Spring	6	1	16.7%	4	66.7%	1	16.7%	5	83.3%
2006	Fall	85	26	30.6%	10	11.8%	36	42.4%	61	71.8%
2007	Spring	18	3	16.7%	9	50.0%	6	33.3%	13	72.2%
2007	Fall	61	25	41.0%	6	9.8%	16	26.2%	37	60.7%
2008	Spring	11	3	27.3%	3	27.3%	3	27.3%	7	63.6%
2008	Fall	80	23	28.8%	3	3.8%	32	40.0%	51	63.8%
2009	Spring	19	3	15.8%	7	36.8%	7	36.8%	14	73.7%

Table 6.1D continued

Year	Seas.	Num. Scr.	*Plasmodium* Num. Pos.	*Plasmodium* Prev.	*Haemoproteus* Num. Pos.	*Haemoproteus* Prev.	*Leucocytozoon* Num. Pos.	*Leucocytozoon* Prev.	Total Num. Pos.	Total Prev.
2009	Fall	96	22	22.9%	5	5.2%	46	47.9%	60	62.5%
2010	Spring	3	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2010	Fall	5	0	0.0%	1	20.0%	3	60.0%	3	60.0%
2011	Spring	14	4	28.6%	2	14.3%	7	50.0%	10	71.4%
2011	Fall	118	36	30.5%	5	4.2%	56	47.5%	82	69.5%
2012	Spring	5	2	40.0%	1	20.0%	4	80.0%	4	80.0%
2012	Fall	154	40	26.0%	11	7.1%	61	39.6%	92	59.7%
2013	Spring	10	1	10.0%	6	60.0%	4	40.0%	8	80.0%
2013	Fall	69	19	27.5%	5	7.2%	27	39.1%	43	62.3%
2014	Spring	11	1	9.1%	0	0.0%	2	18.2%	3	27.3%
2014	Fall	80	26	32.5%	7	8.8%	30	37.5%	50	62.5%
2015	Spring	1	0	0.0%	1	100.0%	1	100.0%	1	100.0%
2015	Fall	18	5	27.8%	0	0.0%	6	33.3%	10	55.6%
2016	Spring	11	5	45.5%	3	27.3%	5	45.5%	9	81.8%
2016	Fall	62	13	21.0%	6	9.7%	24	38.7%	36	58.1%
2017	Spring	14	2	14.3%	3	21.4%	4	28.6%	9	64.3%
2017	Fall	66	22	33.3%	3	4.5%	30	45.5%	43	65.2%
2018	Spring	33	2	6.1%	7	21.2%	9	27.3%	13	39.4%
2018	Fall	91	22	24.2%	9	9.9%	24	26.4%	48	52.7%
2019	Spring	55	10	18.2%	8	14.5%	17	30.9%	29	52.7%
2019	Fall	140	18	12.9%	4	2.9%	72	51.4%	78	55.7%

Table 6.1E Prevalence of pathogens per time period in all Veery.

Year	Seas.	Num. Scr.	*Plasmodium*		*Haemoproteus*		*Leucocytozoon*		Total	
			Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.	Num. Pos.	Prev.
1996	Spring	28	4	14.3%	1	3.6%	3	10.7%	8	28.6%
1996	Fall	3	0	0.0%	0	0.0%	0	0.0%	0	0.0%
1997	Spring	7	2	28.6%	0	0.0%	0	0.0%	2	28.6%
1997	Fall	0	0	0.0%	0	0.0%	0	0.0%	0	0.0%
1998	Spring	2	0	0.0%	0	0.0%	0	0.0%	0	0.0%
1998	Fall	4	1	25.0%	0	0.0%	0	0.0%	1	25.0%
1999	Spring	12	1	8.3%	0	0.0%	2	16.7%	2	16.7%
1999	Fall	0	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2000	Spring	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2000	Fall	4	1	25.0%	0	0.0%	0	0.0%	1	25.0%
2001	Spring	4	1	25.0%	1	25.0%	0	0.0%	2	50.0%
2001	Fall	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2002	Spring	16	1	6.3%	2	12.5%	5	31.3%	6	37.5%
2002	Fall	2	2	100.0%	0	0.0%	0	0.0%	2	100.0%
2003	Spring	4	1	25.0%	0	0.0%	1	25.0%	1	25.0%
2003	Fall	2	1	50.0%	1	50.0%	0	0.0%	2	100.0%
2004	Spring	8	1	12.5%	0	0.0%	0	0.0%	1	12.5%
2004	Fall	4	1	25.0%	2	50.0%	0	0.0%	3	75.0%
2005	Spring	5	1	20.0%	0	0.0%	2	40.0%	2	40.0%
2005	Fall	3	0	0.0%	0	0.0%	1	33.3%	1	33.3%
2006	Spring	8	0	0.0%	1	12.5%	2	25.0%	3	37.5%
2006	Fall	10	2	20.0%	0	0.0%	0	0.0%	2	20.0%
2007	Spring	10	1	10.0%	0	0.0%	2	20.0%	2	20.0%
2007	Fall	6	3	50.0%	1	16.7%	1	16.7%	4	66.7%
2008	Spring	13	2	15.4%	2	15.4%	2	15.4%	5	38.5%
2008	Fall	14	4	28.6%	1	7.1%	5	35.7%	10	71.4%
2009	Spring	17	0	0.0%	3	17.6%	6	35.3%	8	47.1%

Table 6.1E continued

Year	Seas.	Num. Scr.	*Plasmodium* Num. Pos.	*Plasmodium* Prev.	*Haemoproteus* Num. Pos.	*Haemoproteus* Prev.	*Leucocytozoon* Num. Pos.	*Leucocytozoon* Prev.	Total Num. Pos.	Total Prev.
2009	Fall	9	0	0.0%	1	11.1%	2	22.2%	3	33.3%
2010	Spring	8	0	0.0%	0	0.0%	2	25.0%	2	25.0%
2010	Fall	18	5	27.8%	2	11.1%	3	16.7%	7	38.9%
2011	Spring	4	1	25.0%	1	25.0%	2	50.0%	2	50.0%
2011	Fall	19	8	42.1%	0	0.0%	3	15.8%	10	52.6%
2012	Spring	4	1	25.0%	0	0.0%	0	0.0%	1	25.0%
2012	Fall	8	1	12.5%	0	0.0%	1	12.5%	2	25.0%
2013	Spring	3	0	0.0%	1	33.3%	0	0.0%	1	33.3%
2013	Fall	10	1	10.0%	1	10.0%	2	20.0%	3	30.0%
2014	Spring	9	2	22.2%	1	11.1%	3	33.3%	4	44.4%
2014	Fall	12	6	50.0%	1	8.3%	2	16.7%	8	66.7%
2015	Spring	5	0	0.0%	0	0.0%	1	20.0%	1	20.0%
2015	Fall	3	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2016	Spring	6	0	0.0%	1	16.7%	2	33.3%	2	33.3%
2016	Fall	2	0	0.0%	0	0.0%	1	50.0%	1	50.0%
2017	Spring	13	1	7.7%	3	23.1%	4	30.8%	7	53.8%
2017	Fall	2	0	0.0%	0	0.0%	2	100.0%	2	100.0%
2018	Spring	10	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2018	Fall	13	2	15.4%	0	0.0%	1	7.7%	3	23.1%
2019	Spring	19	1	5.3%	0	0.0%	1	5.3%	2	10.5%
2019	Fall	15	0	0.0%	3	20.0%	5	33.3%	7	46.7%

Table 6.2 Summary of posterior distributions for parameter values of the hierarchical statistical model including mean, 80% and 95% credible intervals (CIs), and standard deviation. Parameter values include θ_{bg} (Table **6.2A**), β (Table **6.2B**), ϕ (Table **6.2C**), β_{ann} (Table **6.2D**), ϕ_{ann} (Table **6.2E**), and λ (Table **6.2F**).

Table 6.2A θ_{bg}

Pathogen	Host	Mean	80% CI	95% CI	SE of the mean
Haemoproteus	All Hosts	-2.35	(-2.481, -2.217)	(-2.557, -2.131)	0.015
	Grey-Cheeked	-2.92	(-3.255, -2.607)	(-3.485, -2.464)	0.005
	Hermit	-3.29	(-3.562, -3.037)	(-3.743, -2.921)	0.003
	Swainson's	-1.66	(-1.802, -1.516)	(-1.882, -1.44)	0.002
	Veery	-2.66	(-3.009, -2.326)	(-3.283, -2.153)	0.008
Leucocytozoon	All Hosts	-1.12	(-1.204, -1.027)	(-1.253, -0.979)	0.001
	Grey-Cheeked	-0.61	(-0.759, -0.473)	(-0.856, -0.385)	0.004
	Hermit	-1.52	(-1.644, -1.393)	(-1.717, -1.324)	0.002
	Swainson's	-0.71	(-0.835, -0.588)	(-0.912, -0.527)	0.002
	Veery	-1.60	(-1.83, -1.386)	(-1.929, -1.272)	0.024
Plasmodium	All Hosts	-1.12	(-1.209, -1.044)	(-1.26, -0.998)	0.004
	Grey-Cheeked	-1.81	(-2.026, -1.613)	(-2.123, -1.518)	0.008
	Hermit	-0.66	(-0.764, -0.557)	(-0.826, -0.499)	0.003
	Swainson's	-1.36	(-1.493, -1.225)	(-1.568, -1.155)	0.002
	Veery	-1.82	(-2.072, -1.59)	(-2.239, -1.474)	0.006

Table 6.2B β

Pathogen	Host	Mean	80% CI	95% CI	SE of the mean
Haemoproteus	All Hosts	0.33	(0.146, 0.5)	(0.044, 0.591)	0.002
	Grey-Cheeked	0.27	(0.042, 0.567)	(0.01, 0.762)	0.005
	Hermit	0.48	(0.119, 0.834)	(0.03, 1.023)	0.004
	Swainson's	0.37	(0.113, 0.608)	(0.032, 0.731)	0.004
	Veery	0.39	(0.062, 0.782)	(0.015, 1.116)	0.013
Leucocytozoon	All Hosts	0.40	(0.256, 0.533)	(0.167, 0.606)	0.002
	Grey-Cheeked	0.50	(0.054, 1.406)	(0.015, 1.49)	0.318
	Hermit	0.34	(0.14, 0.535)	(0.045, 0.637)	0.003
	Swainson's	0.24	(0.054, 0.447)	(0.013, 0.565)	0.003
	Veery	0.40	(0.067, 0.818)	(0.017, 1.017)	0.059
Plasmodium	All Hosts	0.14	(0.027, 0.27)	(0.006, 0.327)	0.007
	Grey-Cheeked	0.20	(0.032, 0.414)	(0.008, 0.559)	0.010
	Hermit	0.13	(0.023, 0.253)	(0.006, 0.339)	0.005
	Swainson's	0.21	(0.048, 0.367)	(0.012, 0.462)	0.002
	Veery	0.51	(0.106, 0.93)	(0.027, 1.156)	0.061

Table 6.2C ϕ

Pathogen	Host	Mean	80% CI	95% CI	SE of the mean
Haemoproteus	All Hosts	0.49	(0.375, 0.634)	(0.256, 0.799)	0.004
	Grey-Cheeked	0.54	(0.148, 0.881)	(0.036, 0.966)	0.004
	Hermit	0.56	(0.372, 0.783)	(0.108, 0.928)	0.003
	Swainson's	0.42	(0.233, 0.674)	(0.037, 0.91)	0.014
	Veery	0.54	(0.133, 0.881)	(0.031, 0.966)	0.008
Leucocytozoon	All Hosts	0.75	(0.69, 0.809)	(0.655, 0.859)	0.002
	Grey-Cheeked	0.53	(0.214, 0.845)	(0.05, 0.951)	0.018
	Hermit	0.69	(0.098, 0.942)	(0.018, 0.983)	0.012
	Swainson's	0.69	(0.211, 0.906)	(0.034, 0.967)	0.009
	Veery	0.57	(0.227, 0.849)	(0.065, 0.945)	0.021
Plasmodium	All Hosts	0.52	(0.164, 0.88)	(0.014, 0.95)	0.022
	Grey-Cheeked	0.49	(0.118, 0.92)	(0.028, 0.981)	0.016
	Hermit	0.54	(0.126, 0.897)	(0.029, 0.975)	0.009
	Swainson's	0.59	(0.152, 0.895)	(0.03, 0.969)	0.007
	Veery	0.50	(0.138, 0.871)	(0.043, 0.962)	0.013

Table 6.2D β_{ann}

Pathogen	Host	Mean	80% CI	95% CI	SE of the mean
Haemoproteus	All Hosts	0.38	(0.236, 0.542)	(0.162, 0.763)	0.026
	Grey-Cheeked	0.33	(0.071, 0.615)	(0.021, 0.807)	0.003
	Hermit	0.17	(0.027, 0.35)	(0.007, 0.484)	0.001
	Swainson's	0.89	(0.705, 1.013)	(0.622, 1.232)	0.032
	Veery	0.20	(0.033, 0.412)	(0.007, 0.572)	0.003
Leucocytozoon	All Hosts	0.05	(0.006, 0.098)	(0.001, 0.14)	0
	Grey-Cheeked	0.29	(0.016, 0.994)	(0.004, 1.028)	0.248
	Hermit	0.42	(0.291, 0.541)	(0.228, 0.643)	0.002
	Swainson's	0.20	(0.085, 0.317)	(0.036, 0.379)	0.002
	Veery	0.11	(0.019, 0.248)	(0.005, 0.35)	0.006
Plasmodium	All Hosts	0.20	(0.12, 0.278)	(0.074, 0.326)	0.003
	Grey-Cheeked	0.17	(0.033, 0.343)	(0.008, 0.445)	0.004
	Hermit	0.09	(0.021, 0.178)	(0.004, 0.235)	0.002
	Swainson's	0.27	(0.142, 0.397)	(0.077, 0.473)	0.001
	Veery	0.51	(0.28, 0.731)	(0.162, 0.882)	0.008

Table 6.2E ϕ_{ann}

Pathogen	Host	Mean	80%CI	95%CI	SE of the mean
Haemoproteus	All Hosts	0.95	(0.765, 1)	(0.617, 1)	0.025
	Grey-Cheeked	0.01	(0, 0)	(0, 0)	0.001
	Hermit	0.03	(0, 0)	(0, 1)	0.002
	Swainson's	0.98	(0.98, 1)	(0.761, 1)	0.007
	Veery	0.05	(0, 0)	(0, 1)	0.003
Leucocytozoon	All Hosts	0.12	(0, 0.994)	(0, 1)	0.007
	Grey-Cheeked	0.14	(0, 1)	(0, 1)	0.025
	Hermit	0.98	(0.974, 1)	(0.753, 1)	0.002
	Swainson's	0.00	(0, 0)	(0, 0)	0.000
	Veery	0.13	(0, 1)	(0, 1)	0.050
Plasmodium	All Hosts	0.00	(0, 0)	(0, 0)	0
	Grey-Cheeked	0.02	(0, 0)	(0, 0.001)	0.001
	Hermit	0.02	(0, 0)	(0, 0.01)	0.002
	Swainson's	0.00	(0, 0)	(0, 0)	0.001
	Veery	0.00	(0, 0)	(0, 0)	0.003

Table 6.2F λ

Pathogen	Host	Mean	Length of cycle in years	80%CI	95%CI	SE of the mean
Haemoproteus	All Hosts	39.01	19.51	(29.997, 46.525)	(10.707, 47.625)	0.64
	Grey-Cheeked	23.90	11.95	(4.364, 43.887)	(2.165, 46.984)	0.28
	Hermit	35.07	17.53	(13.445, 46.331)	(4.17, 47.588)	0.39
	Swainson's	37.39	18.69	(17.601, 47.28)	(7.283, 47.821)	0.88
	Veery	22.16	11.08	(4.28, 42.845)	(1.8, 46.817)	0.48
Leucocytozoon	All Hosts	45.08	22.54	(42.07, 47.669)	(38.295, 47.92)	0.21
	Grey-Cheeked	17.82	8.91	(1.991, 38.419)	(1.985, 45.316)	5.87
	Hermit	37.60	18.80	(24.17, 46.413)	(9.369, 47.6)	0.41
	Swainson's	36.10	18.05	(15.678, 46.935)	(5.797, 47.733)	0.49
	Veery	20.38	10.19	(5.738, 44.172)	(3.548, 46.978)	1.33
Plasmodium	All Hosts	20.47	10.23	(6.515, 37.824)	(3.805, 45.658)	0.95
	Grey-Cheeked	23.18	11.59	(4.464, 42.853)	(1.898, 46.666)	0.70
	Hermit	22.14	11.07	(4.342, 42.547)	(2.218, 46.679)	0.70
	Swainson's	29.59	14.80	(9.7, 45.296)	(3.265, 47.32)	0.53
	Veery	16.32	8.16	(6.447, 36.805)	(3.675, 45.405)	1.38

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