

**PREVALENCE AND DIVERSITY OF AVIAN HAEMOSPORIDIAN PARASITES
IN NORTH AMERICAN RAPTORS**

by
Laura Kwasnoski

A thesis submitted to the Faculty of the University of Delaware in partial
fulfillment of the requirements for the degree of Master's of Science in Wildlife Ecology

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Laura Kwasnoski

Approved: _____
Vincenzo Ellis, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
Jacob Bowman, Ph.D.
Chair of the Department of Entomology and Wildlife Ecology (ENWC)

Approved: _____
Brian Farkas, Ph.D.
Dean of the College of Agriculture and Natural Resources (CANR)

Approved: _____
Louis F. Rossi, Ph.D.
Vice Provost for Graduate and Professional Education and
Dean of the Graduate College

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Table 1.1 All raptors tested for haemosporidian parasites (*Haemoproteus*, *Leucocytozoon*, *Plasmodium*) at five avian rehabilitation centers across the United States. General location of rehabilitation centers provided, but names withheld for privacy and instead assigned a site number. “Number of Raptors Screened” refers to individual birds tested and “Number of Raptor Species Screened” indicates the number of raptor species analyzed from the center. The number of birds with *Haemoproteus*, *Leucocytozoon* and *Plasmodium* infections are reported for each rehabilitation center. In the case of co-infections (i.e., a bird infected by > 1 parasite genus), each infection is reported in its appropriate column (e.g., a bird infected by both *Haemoproteus* and *Plasmodium* is counted in both the *Haemoproteus* column and the *Plasmodium* column).

Raptor Rehabilitation Center	Location (State)	Number of Raptors Screened	Number of Raptor Species Screened	Number of <i>Haemoproteus</i> Infections	Number of <i>Leucocytozoon</i> Infections	Number of <i>Plasmodium</i> Infections
Site 1	CA	85	5	6	33	36
Site 2	CO	74	9	10	19	36
Site 3	MO	248	14	90	31	72
Site 4	NC	138	14	24	5	33
Site 5	FL	195	17	27	1	28
TOTALS		740		157	89	205

Table 1.2 Total number of avian haemosporidian infections (“*Haem.*” = *Haemoproteus*, “*Leuc.*” = *Leucocytozoon*, “*Plas.*” = *Plasmodium*) and co-infections (*Haem./Leuc.*, *Haem./Plas.*, *Leuc./Plas.*, *Haem./Leuc./Plas.*) in raptor hosts with a sample size of 10 or more individuals from each site. Individuals with co-infections are reported both in the column for each individual genus infection and in the co-infection columns. For example, a bird with a *Haemoproteus* and *Plasmodium* co-infection would be reported in “*Haem.*”, “*Plas.*” and “*Haem./Plas.*”. Host species scientific names are found in Supplementary Table S1.2.

Raptor Rehab. Center	Host Species	Individuals Screened	<i>Haem.</i>	<i>Leuc.</i>	<i>Plas.</i>	<i>Haem./Leuc.</i>	<i>Haem./Plas.</i>	<i>Leuc./Plas.</i>	<i>Haem./Leuc./Plas.</i>
Site 1	Great-Horned Owl	32	4	23	11	2	1	6	1
	Red-Tailed Hawk	35	1	7	17	0	1	7	0
Site 2	Cooper's Hawk	10	0	2	8	0	0	2	0
	Great-Horned Owl	14	5	8	2	4	1	1	0
	Red-Tailed Hawk	30	2	5	14	0	2	2	0
	Swainson's Hawk	13	1	2	8	0	0	1	1
Site 3	Bald Eagle	10	0	0	1	0	0	0	0
	Barred Owl	48	39	10	10	8	7	0	2
	Cooper's Hawk	34	4	2	20	0	2	2	0
	Great-Horned Owl	37	25	15	6	9	1	1	3
	Red-Shouldered Hawk	31	7	0	15	0	6	0	0
	Red-Tailed Hawk	55	6	2	19	0	4	2	0
Site 4	Barred Owl	36	14	5	5	2	0	1	0
	Black Vulture	15	0	0	4	0	0	0	0
	Red-Shouldered Hawk	23	2	0	8	0	1	0	0
	Red-Tailed Hawk	31	1	0	12	0	1	0	0
Site 5	Bald Eagle	11	0	0	2	0	0	0	0
	Barred Owl	26	11	0	4	0	3	0	0
	Black Vulture	12	0	1	1	0	0	0	0
	Osprey	24	3	0	1	0	0	0	0
	Red-Shouldered Hawk	77	7	0	12	0	1	0	0
	Red-Tailed Hawk	10	0	0	3	0	0	0	0

Table 2.1 Samples analyzed from each site. The number of birds sampled and the number of birds infected with each parasite genus (*Haemoproteus*, *Leucocytozoon*, *Plasmodium*) are reported. One bird from Utah was infected with both *Haemoproteus* and *Leucocytozoon* and was therefore reported once in the “Number of *Haemoproteus* Infections” column and once in the “Number of *Leucocytozoon* Infections” column.

Sampling Location	Number of Samples	Number of <i>Haemoproteus</i> Infections	Number of <i>Leucocytozoon</i> Infections	Number of <i>Plasmodium</i> Infections
Delaware, USA	35	11	0	0
Utah, USA	21	10	1	0

Table 2.2 Number of birds infected with each parasite lineage from each site. Parasite lineage co-infections were recorded once in each column for which they had an infection. For example, a Delaware bird infected with both BNOW02 and BNOW03 was reported once in both the “BNOW02” and “BNOW03” columns. Unknown *Haemoproteus* infections were co-infections (as determined by double peaks in the sequencing chromatogram) that we could not separate into distinct lineages. The parasite genus is reported in parentheses below the lineage name (abbreviated as follows: “*Haemo.*” = *Haemoproteus*, “*Leuco.*” = *Leucocytozoon*).

Parasite Lineages						
Sampling Location	BNOW02 (<i>Haemo.</i>)	BNOW03 (<i>Haemo.</i>)	FASPA02 (<i>Leuco.</i>)	FASPA03 (<i>Haemo.</i>)	SALAU01 (<i>Haemo.</i>)	Unknown <i>Haemo.</i> Infection
Delaware, USA	2	9	0	0	1	0
Utah, USA	2	5	1	1	1	2

Table 2.3 Additional parasite lineage information from other studies that identified the haemosporidian lineages found in this study. Existing parasite lineage data were extracted from the MalAvi database. Parasite lineage genus is reported in parentheses below the lineage name (abbreviated as follows: “*Haemo.*” = *Haemoproteus*, “*Leuco.*” = *Leucocytozoon*). Regional location data are provided when possible and countries listed by 2-digit abbreviation (Italy = “IT”, Mexico = “MX”, United States of America = “US”, Uruguay = “UY”).

Parasite Lineage	Host Species	Location	Publication
BNOW02 (<i>Haemo.</i>)	American barn owl (<i>Tyto furcula</i>)	California, US	Ishak et al. 2008
	American kestrel	Delaware, US	This paper
	American kestrel	Utah, US	This paper
BNOW03 (<i>Haemo.</i>)	American barn owl	California, US	Ishak et al. 2008
	American kestrel	US	Outlaw & Ricklefs 2009
	American kestrel	San Luis Potosi, MX	Tinajero et al. 2019
	American kestrel	Delaware, US	This paper
	American kestrel	Utah, US	This paper

FASPA02 (<i>Leuco.</i>)	American kestrel	California, US	Walther et al. 2016
	Merlin (<i>Falco columbarius</i>)	Livorno, IT	Nardoni et al. 2020
	American kestrel	Utah, US	This paper
FASPA03 (<i>Haemo.</i>)	American kestrel	San Luis Potosi, MX	Tinajero et al. 2019
	American kestrel	Utah, US	This paper
SALAU01 (<i>Haemo.</i>)	Golden-billed saltator (<i>Saltator aurantiirostris</i>)	UY	Durrant et al. 2006
	American kestrel	US	Outlaw & Ricklefs 2009
	American kestrel	Delaware, US	This paper
	American kestrel	Utah, US	This paper

SUPPLEMENTARY TABLES

Table S1.1 Results from Spearman’s rank correlation test evaluating the correlation between the prevalence of the haemosporidian genera among raptor species (n = 11 with sample size greater than or equal to 10 across all sites) and among sites (n = 5, using all individuals sampled).

Among Species (n=11)			Among Sites (n=5)		
Parasite Genera Comparison	Correlation Coefficient (ρ)	<i>P</i> value	Parasite Genera Comparison	Correlation Coefficient (ρ)	<i>P</i> value
<i>Haemoproteus</i> vs. <i>Plasmodium</i>	-0.16	0.630	<i>Haemoproteus</i> vs. <i>Plasmodium</i>	-0.50	0.450
<i>Plasmodium</i> vs. <i>Leucocytozoon</i>	0.54	0.085	<i>Plasmodium</i> vs. <i>Leucocytozoon</i>	0.90	0.083
<i>Leucocytozoon</i> vs. <i>Haemoproteus</i>	0.08	0.819	<i>Leucocytozoon</i> vs. <i>Haemoproteus</i>	-0.60	0.350

Table S1.2 List of all raptor species sampled at rehabilitation centers. Additional information including common name, scientific name, four-digit alphanumeric (banding) code and bird family classification listed.

Common Name	Scientific Name	4-Digit Alphanumeric Code	Raptor Family
American Kestrel	<i>Falco sparverius</i>	AMKE	<i>Falconidae</i>
Bald Eagle	<i>Haliaeetus leucocephalus</i>	BAEA	<i>Accipitridae</i>
Barn Owl (American Barn Owl)	<i>Tyto alba</i> (<i>Tyto furcata</i>)	ABOW	<i>Tytonidae</i>
Barred Owl	<i>Strix varia</i>	BDOW	<i>Strigidae</i>
Black Vulture	<i>Coragyps atratus</i>	BLVU	<i>Cathartidae</i>
Broad-Winged Hawk	<i>Buteo platypterus</i>	BWHA	<i>Accipitridae</i>
Burrowing Owl	<i>Athene cunicularia</i>	BUOW	<i>Strigidae</i>
Cooper's Hawk	<i>Astur cooperii</i>	COHA	<i>Accipitridae</i>
Eastern Screech Owl	<i>Megascops asio</i>	EASO	<i>Strigidae</i>
European Eagle Owl	<i>Bubo bubo</i>	EUEO	<i>Strigidae</i>
Golden Eagle	<i>Aquila chrysaetos</i>	GOEA	<i>Accipitridae</i>
Great-Horned Owl	<i>Bubo virginianus</i>	GHOW	<i>Strigidae</i>
Harris's Hawk	<i>Parabuteo unicinctus</i>	HAHA	<i>Accipitridae</i>
Merlin	<i>Falco columbarius</i>	MERL	<i>Falconidae</i>
Mississippi Kite	<i>Ictinia mississippiensis</i>	MIKI	<i>Accipitridae</i>
Northern Goshawk (American Goshawk)	<i>Accipiter gentilis</i> (<i>Astur atricapillus</i>)	NOGO/ AGOS	<i>Accipitridae</i>
Osprey	<i>Pandion haliaetus</i>	OSPR	<i>Pandionidae</i>
Peregrine Falcon	<i>Falco peregrinus</i>	PEFA	<i>Falconidae</i>
Red-Shouldered Hawk	<i>Buteo lineatus</i>	RSHA	<i>Accipitridae</i>
Red-Tailed Hawk	<i>Buteo jamaicensis</i>	RTHA	<i>Accipitridae</i>
Sharp-Shinned Hawk	<i>Accipiter striatus</i>	SSHA	<i>Accipitridae</i>

Short-Eared Owl	<i>Asio flammeus</i>	SEOW	<i>Strigidae</i>
Short-Tailed Hawk	<i>Buteo brachyurus</i>	STHA	<i>Accipitridae</i>
Swainson's Hawk	<i>Buteo swainsoni</i>	SWHA	<i>Accipitridae</i>
Swallow-Tailed Kite	<i>Elanoides forficatus</i>	STKI	<i>Accipitridae</i>
Tawny Eagle	<i>Aquila rapax</i>	TAEA	<i>Accipitridae</i>
Turkey Vulture	<i>Cathartes aura</i>	TUVU	<i>Cathartidae</i>
Western Screech Owl	<i>Megascops kennicottii</i>	WESO	<i>Strigidae</i>

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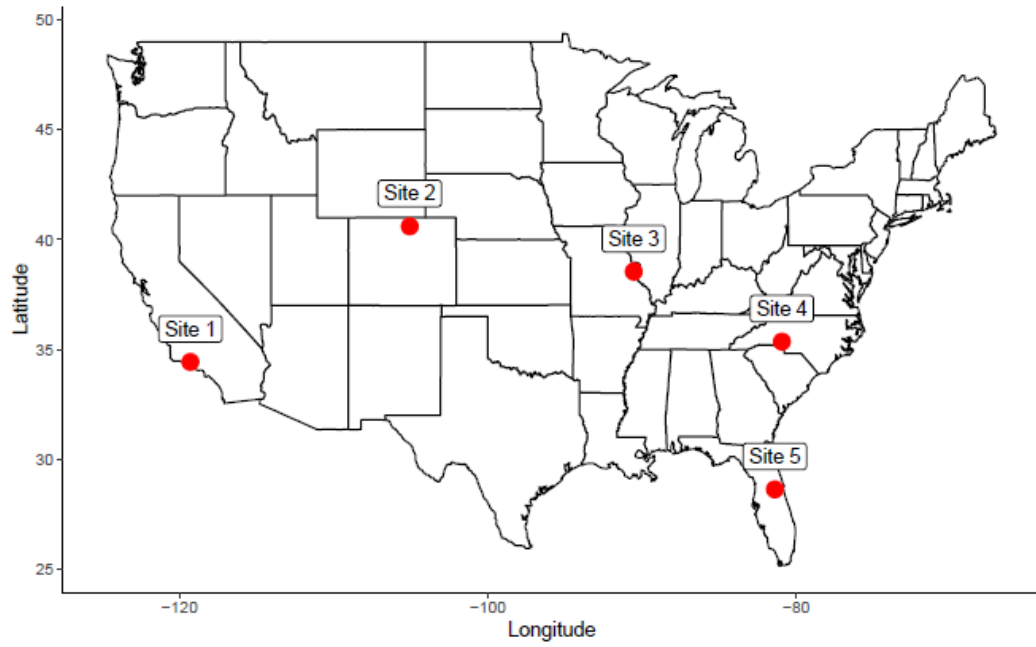


Figure 1.1 Locations of raptor rehabilitation centers from which samples were collected in 2021 and 2022.

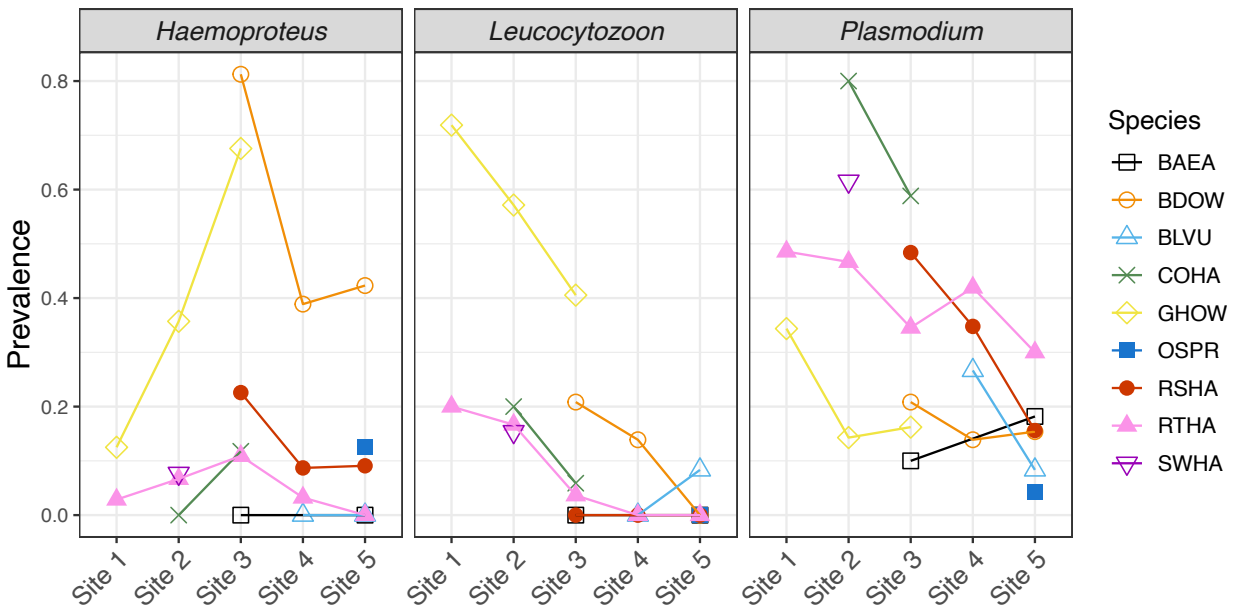


Figure 1.2 Haemosporidian parasite prevalence among raptor species across sites.

Prevalence of each parasite genus is plotted separately. Prevalence values of the same raptor species at different sites are connected by lines to make patterns easier to follow.

Raptor species (titled “Species” in the legend) are represented by four-digit alphanumeric codes that are linked to common and scientific names in Supplementary Table S1.2. Only species with 10 or more individuals sampled are reported for each site (raw data in Table 1.2).

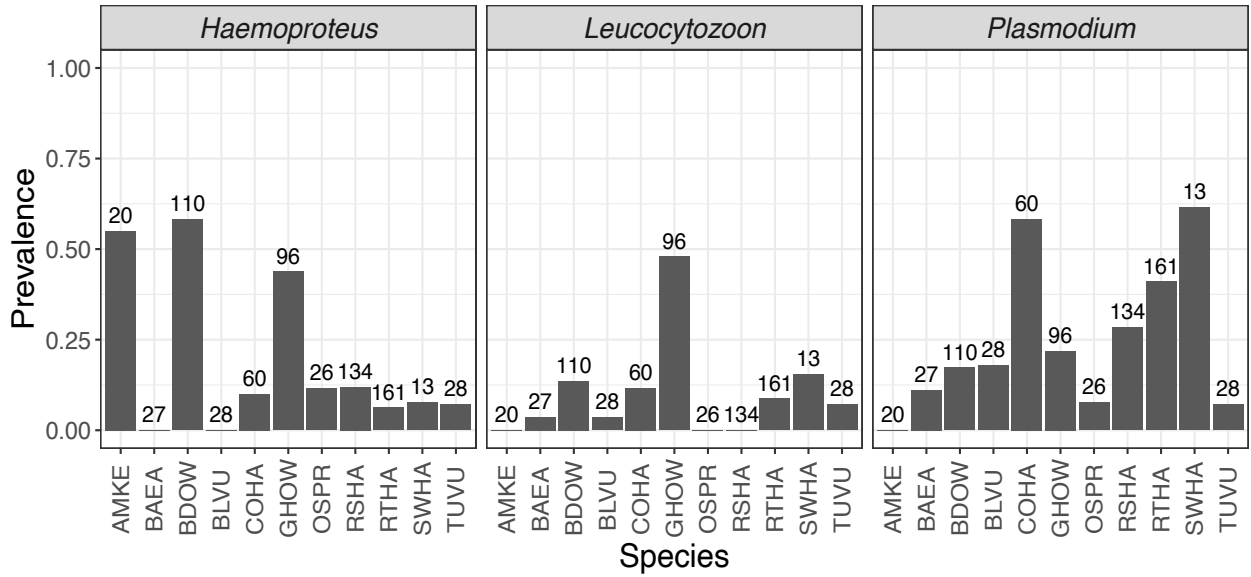


Figure 1.3 Haemosporidian prevalence (separated by parasite genus) for raptor host species after combining site data. Raptor species are represented by four-digit alphanumeric codes that are linked to common and scientific names in Supplementary Table S1.2. Only species with 10 or more individuals sampled across all sites are reported (raw data in Table 1.2); this includes more species than in the GLM analysis, which we restricted to species with sample size of 10 or more individuals within sites (Fig. 1.2). The number of raptors from each species that were screened for *Haemoproteus*, *Leucocytozoon* and *Plasmodium* are reported on top of the bars in the graph.

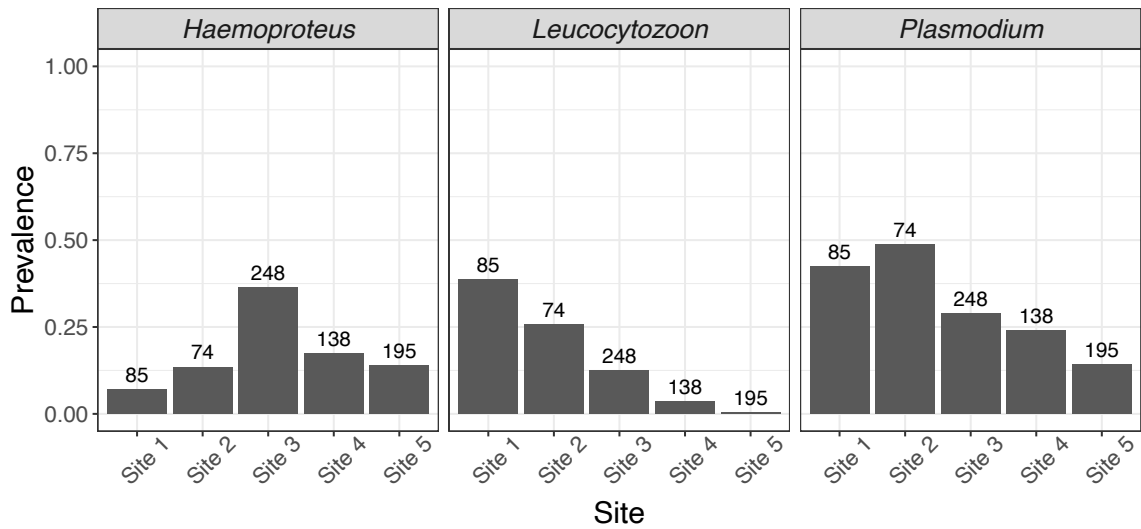


Figure 1.4 Haemosporidian prevalence (parasite genera displayed separately) at each site after combining data from all raptor species within sites. Prevalence calculated from all raptors sampled at each site and is not restricted to species with a sample size of 10 or more individuals within a site (Fig. 1.2). The number of raptors at each site that were screened for *Haemoproteus*, *Leucocytozoon* and *Plasmodium* are reported on top of the bars in the graph.

CHAPTER 1

PREVALENCE OF HAEMOSPORIDIAN PARASITES IN CAPTIVE RAPTORS ACROSS THE UNITED STATES

ABSTRACT

Avian haemosporidians are common vector-transmitted parasites that infect birds. Although avian haemosporidians have been studied extensively in songbirds, less is known about haemosporidian prevalence in raptors, particularly in the United States. We used molecular methods to test blood samples collected from raptors at five wildlife rehabilitation centers across the United States for avian haemosporidians. We identified parasites from the genera *Haemoproteus*, *Leucocytozoon*, and *Plasmodium* using a multiplex PCR and determined prevalence by raptor host species and site. Almost half of the raptors tested (47%) were infected with at least one parasite, with *Plasmodium* detected at the highest frequency (28%) followed by *Haemoproteus* (21%), and *Leucocytozoon* (12%). Prevalence of each parasite genus varied among host species. For example, *Haemoproteus* prevalence was higher in American Kestrels (*Falco sparverius*), Barred Owls (*Strix varia*), Great-Horned Owls (*Bubo virginianus*) than in Bald Eagles (*Haliaeetus leucocephalus*) or Black Vultures (*Coragyps atratus*). Prevalence also varied among sites for all three parasite genera. However, we did not detect a species by site

interaction effect for prevalence, suggesting that variation in prevalence among raptor species is relatively constant across the region we investigated. This work adds to our understanding of raptor disease ecology at a regional geographic scale. Future analyses will focus on the diversity and distribution of parasite genetic lineages among these host species and sites

INTRODUCTION

The prevalence of parasite infections among host individuals has often been related to aspects of the host species' biology (Arneberg 2001; Ricklefs 1992). While it is known that parasite prevalence can vary over space (Byers et al. 2008), comparative analyses typically assume that relative parasite prevalence among host species is constant (e.g., host species A generally has higher prevalence than host species B across their distributions). However, this is an important assumption to test empirically. Under a scenario of localized coevolution driven by limited host or parasite gene flow among populations (Thompson 2005), relative parasite prevalence among host species may be location dependent. Such a result would call for a re-evaluation of studies linking host traits to parasite prevalence and this is particularly important to assess in understudied host species.

Birds and their avian haemosporidian parasites (phylum: Apicomplexa, order Haemosporida) are ideal for investigating variation in prevalence over space and among host species. Avian haemosporidians are globally distributed, dipteran-vectorated parasites of birds (Garnham 1966; Santiago-Alarcon et al. 2012; Valkiūnas 2004). These parasites are primarily grouped into three genera: (i) *Haemoproteus*, which are vectorated by biting midges (*Culicoides* spp.) and louse flies (Hippoboscidae); (ii) *Leucocytozoon*, which are vectorated by black flies (Simuliidae); and (iii) *Plasmodium*, which are vectorated by mosquitoes (Culicidae) (Valkiūnas 2004). Genus-level haemosporidian prevalence has been related to life history traits of birds (Hamilton and Zuk 1982; Ricklefs 1992; Ricklefs et al. 2018; Scheuerlein and Ricklefs 2004), however many relationships are not

consistent among studies (Ellis et al. 2020). Avian haemosporidian prevalence is known to vary over both time (Bensch et al. 2007; Bensch and Åkesson 2003) and space (Fallon et al. 2005), but the possibility of a host species-by-location statistical interaction effect has not often been tested. Furthermore, much of the avian haemosporidian research concerns songbird hosts and other small birds that are readily captured in mist-nets; larger birds are less frequently sampled as they require more specialized capture techniques and thus have received less attention.

One such understudied group of avian hosts, particularly in the United States, is raptors, here defined as birds of prey including eagles, hawks, harriers, kites, accipiters (*Accipitridae*), osprey (*Pandionidae*), vultures (*Cathartidae*), owls (*Tytonidae/Strigidae*) and falcons (*Falconidae*). Sampling of avian haemosporidians in raptors has been mostly limited to rehabilitation centers outside of the United States (Krone et al. 2008; Ciloglu et al. 2016; Pornpanom et al. 2021; Harl et al. 2022; Nourani et al. 2022; Araujo et al. 2023; Martín-Maldonado et al. 2023). There are exceptions, including a study by Outlaw and Ricklefs (2009) that sampled *ca.* 200 raptors from rehabilitation centers in the United States and Spain. Wild raptors have also been sampled to determine haemosporidian prevalence and how prevalence varies in relation to other variables (e.g., environmental conditions, contaminants, urbanization) (Chakarov et al. 2008; Chakarov et al. 2015; Frixione and Rodríguez-Estrella 2023; Ishak et al. 2008; Kwasnoski et al. 2019; Leppert et al. 2008; Walther et al. 2016; Tinajero et al. 2019; Wiegmann et al. 2021).

Avian haemosporidians are detected and identified through microscopy (Garnham 1966; Valkiūnas 2004), through PCR and DNA sequencing (Hellgren et al. 2004), or

both. Genetic sequences of the parasite mitochondrial cytochrome *b* gene allow for genetic lineages to be identified (Bensch et al. 2009). A recent multiplex PCR assay was developed to allow for simultaneous identification of haemosporidian parasites from all three genera with comparable sensitivity to previously established PCR assays (Ciloglu et al. 2019).

Here we use that multiplex PCR protocol (Ciloglu et al. 2019) to test raptor blood samples for avian haemosporidian parasites. We screened over 700 samples from five wildlife rehabilitation centers across the United States to determine prevalence. We tested whether prevalence varied among raptor species or sites (i.e., rehabilitation centers). Importantly, we also tested for a statistical interaction between raptor species and site – this allowed us to determine if relative prevalence among host species was constant or varied over space. We also evaluated whether prevalence of one haemosporidian genus was correlated with prevalence of another genus.

METHODS

Sample Collection

Raptor blood samples were collected in 2021 and 2022 from five rehabilitation centers by center staff (Fig. 1.1, Table 1.1). Blood was added to a lysis buffer and stored at room temperature until DNA extraction using an isopropanol and ammonium acetate extraction protocol (Svensson and Ricklefs 2009; Matthews et al. 2016). Extracted raptor DNA samples were tested for avian haemosporidian infection using the molecular methods described below.

Molecular Screening for Avian Haemosporidians

We quantified DNA using a Nanodrop One spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and diluted samples to obtain DNA concentrations of *ca.* 25 ng/ μ l for testing. Samples with low DNA concentrations were air-dried, re-hydrated with ultrapure water, re-quantified and diluted to the appropriate concentrations. Samples with high DNA concentrations were diluted with ultrapure water and re-quantified to confirm target concentration.

We tested the samples for infection using a single-reaction multiplex PCR that distinguishes among parasites from the genera *Haemoproteus*, *Leucocytozoon*, and *Plasmodium*. To do this, we used primers that bind to known sequences of mitochondrial DNA and generate fragments with lengths unique to each parasite genus (Ciloglu et al. 2019): HMF 5' - ATGGATGTCAATTACCACAATC – 3' and HMR 5' -

GGGAAGTTTATCCAGGAAGTT – 3' for *Haemoproteus*, LMF 5' -
TGGAACAATAATTGSATTATTTACAYT – 3' and LMR 5' -
AACATATCATATTCCATCCATTTAGATTA – 3' for *Leucocytozoon*, and PMF 5'-
CCTCACGAGTCGATCAGG – 3' and PMR 5' – GGAAACCGGCGCTAC – 3' for
Plasmodium.

For each sample, we combined 5.0 µl of Qiagen 2X Master Mix (Qiagen, Germantown, MD, USA), 0.2 µl of each primer (at 10 µM concentration), 1.8 µl of ultrapure water, and 2.0 µl of extracted DNA. We used the following thermal profile: 15 minute initial denaturation (95 °C); 35 cycles of denaturation (94 °C) for 30 seconds, annealing (59 °C) for 90 seconds, extension (72 °C) for 30 seconds; 10 minutes of final extension (72 °C). The resulting products have unique base pair lengths for *Haemoproteus* (525-533 bp), *Leucocytozoon* (218 bp) and *Plasmodium* (377-379 bp) (Ciloglu et al. 2019).

We identified infections by genus and checked for successful amplification using gel electrophoresis with 2% agarose gels stained with GelRed (Biotium, Fremont, CA, USA) and visualized with a GelDoc Go (Bio-Rad, Hercules, CA, USA). All PCRs included a positive control (DNA of all three parasite genera in a single sample) and a negative control (ultrapure water).

Statistical Analyses

Overall Parasite Prevalence

We counted the number of individual birds infected with *Haemoproteus*, *Leucocytozoon* and *Plasmodium* (Table 1.1) and divided this number by the total number of birds sampled to calculate the prevalence of each parasite genus. We calculated prevalence among host species within and among sites. We formatted and plotted the data in R v.4.4.2 (R Core Team 2024) with functions from the R packages found in the package tidyverse v.2.0.0 (Wickham et al. 2019).

Variation in Parasite Prevalence Among Raptor Species, Sites and their Interaction

We restricted the dataset to raptor host species with at least 10 individuals sampled at each site to minimize error in our estimates of prevalence (Table 1.2). For all the following statistical analyses, we used that restricted dataset comprising 614 individuals of 9 host species. For each parasite genus, we ran a generalized linear model (GLM) in R with a binomial error distribution (function `glm()` with argument `family = "binomial"`) with infection status (infected coded as 1, uninfected coded as 0) as the response variable and raptor species, site, and their interaction as explanatory variables. We calculated "Type II" Analysis of Deviance tables for each GLM using the `Anova()` function in the R package `car` v.3.1-1 (Fox and Weisberg 2019); those results include a likelihood ratio χ^2 test statistic, degrees of freedom, and *P* value for each explanatory variable to evaluate statistical significance.

Correlating Parasite Prevalence Between Parasite Genera

Finally, we tested for correlations in prevalence between parasite genera among host species and sites. We used non-parametric Spearman's rank correlation tests with the `cor.test()` function (`method = "spearman"`) in R (Table S1.1).

RESULTS

Overall Prevalence

Overall, 47% of raptors were infected with at least one haemosporidian parasite (349/740) and 13% (95/740) of raptors were infected with more than one parasite genera. *Plasmodium* was the most prevalent parasite genus (28% prevalence; 205/740), followed by *Haemoproteus* (21%; 157/740), and *Leucocytozoon* (12%; 89/740) (Table 1.1).

Testing the Effects of Site, Species, and a Site by Species Interaction on Prevalence

Haemoproteus prevalence varied by site ($\chi^2 = 49.5$, $df = 4$, $P < 0.001$) and by raptor host species ($\chi^2 = 150.4$, $df = 8$, $P < 0.001$). However, the site by species interaction effect was not significant ($\chi^2 = 3.7$, $df = 9$, $P = 0.931$; Fig. 1.2). Therefore, there was significant variation in prevalence among sites and raptor species. For example, American Kestrels (*Falco sparverius*; 55%, 11/20), Barred Owls (*Strix varia*; 58%, 64/110) and Great-Horned Owls (*Bubo virginianus*; 43%, 42/96) had the highest prevalence of *Haemoproteus* while birds from other species like Black Vultures (*Coragyps atratus*) and Bald Eagles (*Haliaeetus leucocephalus*) were not infected with *Haemoproteus* (Fig. 1.3). Site 3 had the highest *Haemoproteus* prevalence (36%, 90/248) and Site 1 had the lowest (7%, 6/85; Fig. 1.4).

Leucocytozoon prevalence also varied by site ($\chi^2 = 27.6$, $df = 4$, $P < 0.001$) and by raptor species ($\chi^2 = 74.7$, $df = 8$, $P < 0.001$), but the site by species interaction was not significant ($\chi^2 = 8.0$, $df = 9$, $P = 0.631$; Fig. 1.2). Great-Horned Owls had the highest prevalence of *Leucocytozoon* (48%, 46/96), while American Kestrels, Osprey (*Pandion*

haliaetus), and Red-shouldered Hawks (*Buteo lineatus*) had no *Leucocytozoon* infections (Fig. 1.3). *Leucocytozoon* prevalence was highest at Site 1 (39%, 33/85) while Site 5 had the lowest (<1%, 1/195; Fig. 1.4).

Almost all raptor species included in the analysis were infected with *Plasmodium* (Fig. 1.3). As with the other parasite genera, *Plasmodium* prevalence varied by site ($\chi^2 = 14.2$, $df = 4$, $P = 0.007$) and raptor species ($\chi^2 = 41.2$, $df = 8$, $P < 0.001$), but the site by species interaction was not significant ($\chi^2 = 9.3$, $df = 9$, $P = 0.407$; Fig. 1.2). Swainson's Hawks (*Buteo swainsonii*) had the highest prevalence of *Plasmodium* (62%, 8/13) followed by Cooper's Hawks (*Astur cooperi*; 58%, 35/60); American Kestrels were not infected with *Plasmodium* (Fig. 1.3). Sites 1 (42%, 36/85) and 2 (49%, 36/74) had the highest *Plasmodium* prevalence while Site 5 (14%, 28/195) had the lowest (Fig. 1.4).

Testing for Correlations in Prevalence Between Parasite Genera

Prevalence was not significantly correlated between any of the parasite genera; neither among host species, nor among sites (Table S1.1).

DISCUSSION

Nearly half of the raptors we tested (47%) were infected with at least one parasite genus. *Plasmodium* infections (28%) were the most common, followed by *Haemoproteus* (21%), and *Leucocytozoon* (12%). Prevalence of all three parasite genera varied significantly by host species (Fig. 1.3) and site (Fig. 1.4) but we did not detect a significant site by host species interaction (Fig. 1.2). This finding suggests that the relative values of prevalence among raptor species generally remained constant across the region we investigated. Prevalence was not correlated between parasite genera (Table S1.1). Haemosporidian infection is common in raptors across the United States, and infection prevalence is plausibly related to both host species traits and environmental traits, the latter of which will require further testing across the landscape to precisely describe. Importantly, prevalence at the parasite genus-level measures prevalence across all congeneric lineages and does not examine the prevalences of specific lineages. Therefore, the mechanisms underlying these patterns will require description of the parasite genetic lineages causing the infections.

Under a scenario of localized coevolution, one might expect variation in prevalence among host species to depend on site (Ricklefs 2010). However, here we show that variation in prevalence among host species is independent of site (Fig. 1.2). This finding probably should not be interpreted as an absence of localized coevolution, as coevolution in haemosporidian parasites might operate at the level of parasite species or genetic lineage instead at the level of genus (Ricklefs et al. 2016). The genus-level

prevalence we calculated may more closely reflect overall tolerance or resistance of bird species to infection.

Numerous studies have investigated avian traits as explanatory factors for prevalence with mixed results (Dunn et al. 2011; Fecchio et al. 2017; Filion et al. 2020; Granthon and Williams 2017; Grieves et al. 2023; Isaksson et al. 2013). Perhaps one of the best supported relationships is one between avian incubation period and genus-level haemosporidian prevalence (Ricklefs 1992; Ricklefs et al. 2018). While we do not have adequate data to test this, variation in prevalence among host species in our dataset may also be due to vector preference for certain species (Hamer et al. 2009). It is also known that prevalence shows phylogenetic signal among avian hosts (Ellis et al. 2020; Gupta et al. 2020), plausibly a result of related host species have more similar immune systems than more distantly related species (O'Connor et al. 2016); we did not have sufficient raptor species diversity to effectively evaluate this hypothesis with our data.

We also found variation in prevalence among sites. This finding might occur because sites are spread across the United States (Fig. 1.1) and encompass a wide range of environmental and climatic characteristics. Temperature, elevation, precipitation, geography, and effects of deforestation, climate change, and urbanization have been hypothesized to affect avian haemosporidian transmission, with mixed results (Sehgal 2015). Here, we found that all sites had relatively high *Plasmodium* prevalence. However, the western-most sites had higher proportions of *Leucocytozoon* infections, which have been found at higher prevalence in forested areas of higher elevation (Reis et al. 2020). In contrast, the central site had the highest *Haemoproteus* prevalence.

However, we acknowledge that we do not know where birds became infected (i.e., infections could have been acquired before birds were brought into rehabilitation centers).

Outlaw and Ricklefs (2009) sampled captive raptors from the United States and Spain but found a much lower overall prevalence than we found (15% vs. 47%). Prevalence in our study was also higher than those found in several other studies of captive raptors (Giorgiadis et al. 2020, 13% from Germany and France; Martín-Maldonado et al. 2023, 35% from Spain; Muñoz et al. 1999, 31% in nocturnal raptors and 43% in diurnal raptors from Spain; Krone et al. 2001, Falconiformes 11% and Strigiformes 13% from Germany; Pornpanom et al. 2021, 7% from Thailand). However, Barino et al. (2021) found over 72% of captive nocturnal raptors from Brazil infected with haemosporidians. It is unclear what mechanisms account for such large differences in prevalence among studies (and presumably among host species), although the studies differ in sampling year, host species, and geographic location.

The data we present here provide an initial quantification of variation in avian haemosporidian genus-level prevalence among several North American raptor species at several sites across the United States. We found that variation in prevalence among host species is relatively constant across a broad region. It is therefore interesting to speculate what host traits account for the variation in prevalence we identified. Targeted sampling of raptor species will allow us to build a dataset to address this question. Raptors have been less well studied than songbirds and other small landbirds, so our work adds to what is known about this group. Furthermore, climate change is likely affecting raptor

distributions (Martínez-Ruiz et al. 2023), and having a baseline in parasite prevalence will allow us to determine how climate change affects prevalence. Therefore, this work also contributes information that may be important for raptor conservation.

ETHICS STATEMENT

Permits and IACUC

Permit # (Site 1): Federal: MB030997-0; State (CA): WR-10038

Permit # (Site 2): Federal: MB725655-0; State (CO): Reh-277

Permit # (Site 3): Federal: MB682643-0; State (MO): 44999

Permit # (Site 4): Federal: MB685213-0; State (NC): WR25000468

Permit # (Site 5): Federal: MB672801; State (FL): 906-000027

IACUC (Rhode's College): 129-21

CHAPTER 2

AVIAN HAEMOSPORIDIAN PARASITE PREVALENCE AND DIVERSITY IN TWO POPULATIONS OF THE AMERICAN KESTREL (*FALCO SPARVERIUS*)

ABSTRACT

Parasite communities vary among host species and across space. However, little is known about differences in parasite communities between geographically and genetically distinct populations of the same host species. American kestrels (*Falco sparverius*) are small falcons with regionally distinct genetic populations across North America. We sampled kestrels from Delaware and Utah for avian haemosporidian parasites (genera: *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) and used molecular barcoding of the parasite cytochrome *b* gene (*cyt b*) to quantify parasite genetic lineage diversity. We identified four lineages of *Haemoproteus* parasites and one *Leucocytozoon* lineage infecting kestrels. A comparison with previous studies suggests that most of these lineages are largely restricted to kestrels. We found similar infection prevalence and lineage composition between the sites. All kestrels sampled in Utah were adults (i.e., sampled after hatch year), but in Delaware we found adult birds had a higher infection

prevalence than juveniles (i.e., hatch-year birds). Despite harboring largely the same parasite lineages, kestrels are unlikely to disperse between Utah and Delaware. The similarity in parasite lineages in the two kestrel populations could be due to a number of factors including broadly distributed vector species (of which little is known), movement of alternative and undetected host species, or transmission during migration or on overwintering grounds. Alternatively, the *cyt b* gene might not capture recent genetic differentiation among the parasites. Future studies should explore these various possibilities to understand the mechanisms underpinning parasite distributions across genetically structured host populations.

INTRODUCTION

Parasite communities can change across a landscape due to dispersal limitations of the parasites and/or turnover in the presence or abundance of host species. For example, the species and prevalence of ectoparasites (fleas and mites) of small mammals have been found to change across space and correlate with changes in host species distributions and climate variation (Krasnov et al. 2020). Landscape topography may be another impediment to parasite dispersal (Poulin and Krasnov 2010), unless parasites are highly mobile (Eriksson et al. 2019). Similarly, vector-transmitted parasites may disperse more readily than their hosts (Eriksson et al. 2019), particularly if their vectors are highly mobile. Such parasite dispersal could lead to hosts with distinct population genetic structure being infected by a single genetic population of parasites across their range. More parasite surveys across the ranges of dispersal limited host species are needed to begin to create generalizations about parasite dispersal and its consequences for hosts.

Avian haemosporidian parasites are ideal for investigating parasite distributions across host populations. Avian haemosporidians are well-studied, vector-transmitted, and globally distributed with the exception of Antarctica (Garnham 1966; Valkiūnas 2004). These parasites are generally classified into the taxonomic genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*. Parasites from each genus are transmitted by different dipteran vectors: *Haemoproteus* is vectored by biting midges (family: Ceratopogonidae) or louse flies (family: Hippoboscidae), *Plasmodium* is vectored by mosquitoes (family: Culicidae), and *Leucocytozoon* is vectored by black flies (family: Simuliidae) (Atkinson et al. 2008; Santiago-Alarcon et al. 2012; Valkiūnas 2004). Infections in birds can be

determined by microscopic examination of blood smears (Valkiūnas 2004) and/or molecular screening. Molecular barcoding of the parasite's cytochrome *b* gene (*cyt b*) is commonly used to identify the genetic parasite lineage (Hellgren et al. 2004); a single nucleotide difference in the sequenced region of *cyt b* is the criterion typically used to define a unique lineage (Bensch et al. 2009). Avian haemosporidians range from specialists to generalists (Ellis and Bensch 2018) and some can be found throughout the ranges of their host species (Ellis et al. 2015; Huang et al. 2025). When statistically controlling for host species turnover, avian haemosporidian lineage turnover appears unrelated to geographic distance (Ellis et al. 2015) and infections of the same haemosporidian lineages have been found across a species' range (Fallon et al. 2006; Szöllösi et al. 2011), even spanning continents (Ellis et al. 2019). Therefore, one can hypothesize that the composition of haemosporidian lineages is likely to be similar among genetically distinct host populations, however more evidence is needed, particularly from under-sampled host populations like raptors.

American kestrels (*Falco sparverius*) are a small falcon species widely distributed across the Americas with 17 recognized sub-species, two of which are found in the United States (Ferguson-Lees and Christie 2001; Gill et al. 2024). Among kestrels in the United States and Canada, there five genetically distinct populations (“eastern”, “western”, “Texas”, “Florida” and “Alaska”; Ruegg et al. 2021). Kestrels are partial migrants that display differential intraspecific migration, with birds at more temperate breeding-season latitudes migrating shorter distances or not migrating at all compared to kestrels from higher latitudes (Goodrich and Smith 2008; Goodrich et al. 2012; Heath et

al. 2012). Efforts to track migrating kestrels are ongoing (Crandall and Craighead 2019; Hunt et al. 2023). Avian haemosporidian parasites have been surveyed in kestrels from several parts of their range. In California, wild kestrels were sampled during the breeding season and 37% were infected with *Leucocytozoon* (Walther et al. 2016). In Texas, a *Haemoproteus* lineage (FASPA04) was identified infecting kestrels (Keith et al. 2022). *Haemoproteus* parasites have also been found in kestrels sampled from the southern Chihuahuah Desert of central Mexico (Tinajero et al. 2019) and the Baja California peninsula (Frixione and Rodríguez-Estrella 2023). Given that the range-wide genetic population structure of North American kestrels has been established (Ruegg et al. 2021), kestrels are an ideal host species to investigate parasite turnover across their range.

Here we sampled avian haemosporidians in wild kestrels from Utah and Delaware. These two locations likely represent the genetically distinct “western” and “eastern” kestrel populations of North America, respectively (Ruegg et al. 2021). We used a multiplex PCR to identify parasite infections (Ciloglu et al. 2019) and sequenced *cyt b* to identify parasite lineages in infected birds (Bensch et al. 2009). We then investigated whether the two host populations were infected by the same or different parasite lineages to better understand how parasites are distributed across host populations. We also investigated the host specificity of the lineages we identified by comparing our results with the distributions of those lineages identified in previous studies.

METHODS

Study Sites and Sample Collection

American kestrels were sampled at two sites across North America in collaboration with one non-profit and one state agency. Locations were chosen from two geographically and likely genetically distinct populations of American kestrels – the “eastern” and “western” groups represented by Delaware and Utah, respectively (Ruegg et al. 2021).

From May to June 2024, 21 wild kestrels were captured and had small blood samples taken by HawkWatch International (HWI) biologists in the greater metropolitan area of Salt Lake City, Utah (centroid coordinates of sample locations: 40.76111935, -111.9850279). HWI spearheads a long-term kestrel demography study monitoring over 500 nest boxes and approximately 130 kestrel pairs during the kestrel breeding season each year. All the kestrels sampled by HWI were adults (i.e., after hatch-year birds).

From March 2023 to June 2024, 49 wild kestrels were sampled across the state of Delaware (centroid coordinates of sample locations: 39.6152001, -75.60631524) by state biologists and trained professionals at Delaware’s Department of Natural Resources and Environmental Control (DNREC) Division of Fish and Wildlife. DNREC maintains and regularly monitors approximately 90 nest boxes throughout Delaware during the kestrel breeding season (March to July) as well as opportunistically sampling non-breeding birds throughout the year.

Following permits and protocols established within each organization, wild kestrels were safely captured using methods including bal-chatri traps, hand-grabbing

from nest boxes, and mist net trapping (with and without animatronic owl decoy lures). Each bird was sexed, aged (juveniles or hatch year “HY” birds including nestlings; adult or and after hatch year “AHY” birds), fitted with a federally issued metal leg band, and measured, before *ca.* 50-70 μ l of whole blood was collected from their brachial, femoral or jugular veins. Kestrels were then released or returned to nest boxes. Blood samples were immediately added to 1.5 μ l microcentrifuge tubes pre-loaded with RNAlater Stabilization Solution (ThermoFisher Scientific, Waltham, MA, USA) and frozen at -20 °C within 24 hours of sampling.

Molecular Screening and DNA Sequencing

All kestrel blood samples were sent to the University of Delaware (Newark, DE) for molecular analysis. We extracted DNA from the blood samples using a DNeasy Blood and Tissue Kit (Qiagen, San Francisco, CA, USA) and quantified DNA concentrations using a Nanodrop One Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). DNA samples were diluted with ultrapure water to a concentration of *ca.* 25 ng/ μ l.

We tested each sample for *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* infection using a multiplex polymerase chain reaction (PCR; Ciloglu et al. 2019). The multiplex PCR uses the following primers: HMF 5' - ATTGGATGTCAATTACCACAATC – 3' and HMR 5' - GGGAAGTTTATCCAGGAAGTT – 3' for *Haemoproteus*, PMF 5' - CCTCACGAGTCGATCAGG – 3' and PMR 5' – GGAAACCGGCGCTAC – 3' for *Plasmodium*, and LMF 5' - TGGAACAATAATTGSATTATTTACAYT – 3' and LMR 5' -

AACATATCATATTCCATCCATTTAGATTA – 3' for *Leucocytozoon*. In addition to 0.2 µl of each primer (at 10 µM concentration) and 2 µl of extracted DNA (25 ng/µl), we combined 5.0 µl of Qiagen 2X Master Mix (Qiagen, San Francisco, CA, USA) and 1.8 µl ultrapure water into reaction wells. Reagents were mixed, centrifuged, and run on a MiniAmp Plus Thermal Cycler (ThermoFisher Scientific, Waltham, MA, USA) with the following thermal profile: 15 minute initial denaturation (95 °C); 35 cycles of 30 second denaturation (94 °C), 90 second annealing (59 °C) and 30 second extension (72 °C); 10 minute final extension (72 °C). We ran 5 µl of multiplex PCR product from each sample mixed with 1 µl of 6X TriTrack loading dye (ThermoFisher Scientific, Waltham, MA, USA) through 2% agarose gels stained with GelRed (Biotium Inc., Fremont, CA, USA) and visualized the results with a GelDoc Go (Bio-Rad, Hercules, CA, USA). Bands from each sample were compared to bands from a triple-infection positive control (i.e., a mix of DNA from birds infected with all three parasite genera) to confirm infection. We also included a 100 bp DNA ladder to verify amplified fragment lengths in each gel and an ultrapure water negative control in all PCRs and gels.

We amplified the molecular barcoding region of *cyt b* from samples infected with haemosporidians as demonstrated by the multiplex PCR. To amplify *cyt b*, we used a standard nested PCR (Hellgren et al. 2004). This two-step process included an initial PCR, where we combined 2 µl of extracted DNA (25 ng/µl) with 12.5 µl DreamTaq Master Mix 2X (ThermoFisher Scientific, Waltham, MA, USA), 9.5 µl ultrapure water, and 1 µl of each 10 µM primer (forward: HaemNFI 5'-CATATATTAAGAGAAITATGGAG-3'; reverse: HaemNR3 5'-

ATAGAAAGATAAGAAATACCATTC-3') and amplified DNA with the following thermal profile: 2 minute initial denaturation (95 °C); 20 cycles of 30 second denaturation (95 °C), 30 second annealing (50 °C) and 60 second extension (72 °C); 10 minute final extension (72 °C). Then, we then ran a second PCR reaction for *Haemoproteus/Plasmodium* or *Leucocytozoon* as described in Hellgren et al. (2004). This included combining 1 µl of PCR product from the first reaction with 12.5 µl of DreamTaq Master Mix 2X, 8.5 µl ultrapure water and 1 µl of each primer (10 µM concentration; *Haemoproteus/Plasmodium* primers: forward HaemF 5'-ATGGTGCTTTCGATATATGCATG-3', reverse HaemR2 5'-GCATTATCTGGATGTGATAATGGT-3'; *Leucocytozoon* primers: forward HaemFL 5'-ATGGTGTTTTAGATACTTACATT-3', reverse HaemR2L 5'-CATTATCTGGATGAGATAATGGIGC-3'). The sample was then run through the following PCR thermal profile: 2 minute initial denaturation (95 °C); 35 cycles of 30 second denaturation (95 °C), 30 second annealing (50 °C) and 60 second extension (72 °C); 10 minute final extension (72 °C). To confirm successful amplification, we followed the same gel and visualization protocol as described previously. We used a negative control (ultrapure water) and positive control (DNA from a bird with a previously sequenced infection) in all reactions; the second reaction used a new negative control (ultrapure water) and the negative control from the first reaction (a "nested negative").

We sent PCR products from the second ("nested") reaction to Azenta Life Sciences (South Plainfield, NJ, USA) for exo-sap purification and Sanger sequencing in both the forward and reverse directions. We analyzed the Sanger sequences in Geneious

R.11.1.5 (<https://www.geneious.com>) and used BLAST to compare the sequences to the MalAvi database (Bensch et al. 2009). All parasite lineage data from this study have been submitted to MalAvi.

Statistical Analyses

To test for differences in parasite lineage composition and prevalence, we used Fisher's Exact Tests with the function *fisher.test()* in R v.4.4.2 (R Core Team 2024) We tested whether parasite lineage composition and prevalence differed among the two sites and whether prevalence varied with bird sex and age. We summarized and plotted our data using packages hosted in the R package tidyverse v.2.0.0 (Wickham et al. 2019).

RESULTS

Similar Prevalence in Utah and Delaware

We found 31.4% (11/35) of kestrels in Delaware infected with a parasite (all were *Haemoproteus*) and 47.6% (10/21) of birds from Utah infected (10 *Haemoproteus* infections, 1 *Leucocytozoon*) (Table 2.1). The prevalence of *Haemoproteus* did not differ significantly between the Delaware and Utah populations (odds ratio = 0.511, $P = 0.264$). Since there was only one *Leucocytozoon* infection, we did not test for a difference in the prevalence of that genus.

Prevalence in Relation to Age and Sex

Infection by *Haemoproteus* varied significantly by kestrel age in Delaware (odds ratio = 26.784, $P < 0.001$) with more adult birds (75%; 9/12) infected than juveniles (8.7%; 2/23). Within Delaware, the prevalence of *Haemoproteus* was lower in male (13.3%, 2/15) than female kestrels (45%, 9/20) but this difference was not statistically significant (odds ratio = 0.197, $P = 0.069$). It is important to note that the relatively low P value in the test comparing Delaware males and females (0.069) was influenced by the ages of the birds sampled. There were many more adult females sampled (8/9 infected) than adult males (1/3 infected; odds ratio = 11.094, $P = 0.127$) and there was no difference between juvenile females (1/11 infected) and males (1/12 infected), which were more evenly sampled (odds ratio = 1.095, $P = 1$). At the Utah site, *Haemoproteus* prevalence did not vary significantly by sex (log odds = 0.531, $P = 0.659$; 3/8 males and 7/13 females infected).

Limited Parasite Lineage Turnover between Utah and Delaware

We found four *Haemoproteus* lineages, with three of the four lineages found at both sites (Table 2.2). The lineage BNOW03 was found in the greatest number of kestrels (nine infections in Delaware, five infections in Utah) followed by BNOW02 (two in Delaware, two in Utah) and SALUR01 (one in Delaware, one in Utah). The fourth lineage, FASPA03 was only found in a single kestrel from Utah. Additionally, two birds from Utah had co-infections (likely multiple *Haemoproteus* lineages) that we were unable to identify to lineage (for a discussion of phasing co-infections with Sanger sequence data, see Drovetski et al. 2014 and Matthews et al. 2016). We also found one *Leucocytozoon* lineage, FASPA02, in a Utah kestrel. We found no statistical differences in the frequencies of parasite lineages between the two sites (BNOW02, odds ratio = 0.582, $P = 0.626$; BNOW03, odds ratio = 1.106, $P = 1$; FASPA02, odds ratio = 0, $P = 0.375$; FASPA03, odds ratio = 0, $P = 0.375$; SALAUR01, odds ratio = 0.594, $P = 1$; Table 2). Among the juvenile birds from Delaware, we found the lineage BNOW03 (2/23 infected), indicating that the lineage is transmitted in Delaware.

Lineage Distributions and Host Specificities

Of the four *Haemoproteus* and one *Leucocytozoon* lineages we identified, all except one had previously been found infecting kestrels within their range (Table 2.3). The lineage BNOW02, which we found in both Delaware and Utah kestrels, had previously only been found in an American barn owl (*Tyto furcata*; Table 2.3).

DISCUSSION

Little is known about parasite prevalence and diversity among distinct populations of American kestrels. We screened American kestrels from two geographic regions (Delaware and Utah) that presumably represent genetically distinct populations (Ruegg et al. 2021) and found that both groups harbor largely similar avian haemosporidian parasite prevalence and lineage diversity (Table 2.2). In fact, the lineages from Delaware and Utah have also been found in other parts of the kestrels' range from other genetically distinct populations (Table 2.3). The most common parasites of kestrels in our study were *Haemoproteus* parasites, in keeping with previous studies (Dawson and Bortolotti 1999; Frixione and Rodríguez-Estrella 2023; Tinajero et al. 2019; Wiehn et al. 1997). The similarity of parasite lineages among these two host populations could be caused by several factors: 1) broadly distributed vectors that readily disperse among kestrel populations (or historically dynamic ranges of either vectors or kestrels), 2) alternative (and currently undetected) host species dispersing among kestrel populations, 3) transmission of the parasites during migration or on overwintering sites, or 4) *cyt b* lineages may not reflect true population genetic structure in the parasite (i.e., the parasite might exhibit distinct genetic populations at other genetic loci; but see Hellgren et al. 2021). Considering the first possibility, we know little about the range, distribution and prevalence of vector species (particularly biting midges and black flies) that transmit haemosporidian parasites to North American birds, so considerable natural history research is needed to investigate this possibility. The second option seems unlikely because the lineages infecting kestrels in this study are largely specialists of kestrels

(Table 2.3) and it is not clear what alternative host would regularly move longitudinally across the United States to provide opportunity for parasite transmission between the populations. The third possibility is difficult to assess because we do not know the overwintering locations of kestrels from the Delaware and Utah populations (although this could be studied in the future by tracking kestrels). However, one of the *Haemoproteus* lineages (BNOW03) was found in juvenile birds in Delaware, indicating that transmission does occur on the breeding grounds. The fourth option is something to be investigated in the future. Nuclear genes evolve faster than mitochondrial genes in haemosporidians (Galen et al. 2018) and so might show evidence of population genetic structure not apparent in *cyt b* sequences (Hellgren et al. 2015; Huang et al. 2019). Genetic sequencing techniques like hybrid capture (Huang et al. 2018) and transcriptome sequencing (Galen et al. 2019) have recently facilitated sequencing avian haemosporidian nuclear DNA.

In the Delaware kestrel population, we found two juvenile birds and seven adult birds infected with the *Haemoproteus* parasite BNOW03, confirming that lineage is transmitted within the population during the breeding season. However, we do not know whether the haemosporidian lineages in this study are transmitted on the wintering grounds of kestrels. While we know kestrels can migrate as far south as Mexico (Goodrich and Smith 2008) and Nicaragua (Hunt et al. 2023), we do not know whether kestrels from Delaware and Utah migrate or overwinter in the same locations, although it has been suggested in Ruegg et al. (2021) that they do not overlap. Interestingly, the *Leucocytozoon* lineage FASPA02 found in Utah has been found in a close relative of the

American kestrel, a merlin (*Falco columbarius*), in Italy (Nardoni et al. 2020) making it one of a few parasite lineages found on multiple continents that are not connected by north-south avian migratory flyways (Ellis et al. 2019). Nuclear DNA sequencing of this lineage on both continents could be used to determine the direction of parasite dispersal (Huang et al. 2019).

In Delaware, adult kestrels were more frequently infected with haemosporidian parasites than juveniles. These results are consistent with previous studies and likely reflect the fact that these infections can be long lasting (Latta and Ricklefs 2010). There was no significant difference in infection prevalence between male and female kestrels in both Delaware and Utah. This is an expected outcome given the results of previous studies (Ellis et al. 2020; Granthon and Williams 2017; Huang et al. 2020; Jing et al. 2023).

According to the MalAvi database (updated 2025-02-25 and including the data from this study), American kestrels have been found infected with six *Haemoproteus* lineages (BNOW02, BNOW03, FASPA01, FASPA03, FASPA04, SALAUR01), one *Leucocytozoon* lineage (FASPA02), and one *Plasmodium* lineage (MYCAME02). Among the *Haemoproteus* lineages, BNOW02 and BNOW03 have been found infecting American kestrels and barn owls (*Tyto alba*), FASPA01, FASPA03, and FASPA04 have only been found infecting American kestrels, and SALAUR01 was found infecting American kestrels and a passerine, the golden-billed saltator (*Saltator aurantirostris*) in Uruguay. The *Leucocytozoon* lineage, FASPA02, has been found infecting American kestrels and a captive merlin (*Falco columbarius*) in Italy. Finally, the *Plasmodium*

lineage, MYCAME02, is a generalist that has been found infecting 20 host species from seven taxonomic orders (Passeriformes, Ciconiiformes, Anseriformes, Phoenicopteriformes, Falconiformes, Strigiformes, and Cuculiformes) across North and South America. Therefore, American kestrels appear to be typically infected by specialized parasite lineages (i.e., parasites with one or a few host species), but are infected by at least one generalist (MYCAME02).

Parasite species and lineages can turnover across space as a result of host turnover or geographic barriers to dispersal. One way to study parasite lineage turnover while controlling for the effect of host turnover is to examine parasite distributions across populations of host species. With kestrels, there is likely limited gene flow across the continental United States (Ruegg et al. 2021); however, populations in Delaware and Utah harbor similar parasite lineages (Table 2.2) and are infected at similar rates. In fact, kestrels in general seem to harbor the same suite of haemosporidian parasites across their distribution. Further analysis of parasite genetics, vector ecology, and kestrel migration/overwintering are needed to clarify underlying mechanisms. Investigations across the regional distributions of host species are important to clarify the causes of parasite distributions and infection risk.

ETHICS STATEMENT

Permits

DE Department of Natural Resources and Environmental Control – Division of Fish and
Wildlife

Permit #: 23332

Permit Holder: Jordan Brown, State of Delaware (non-game)

HawkWatch International

Permit #: 21384

Permit Holder: Jesse Watson

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