

Sex and Nest Type Influence Avian Blood Parasite Prevalence in a High Elevation Bird Community

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Abstract

Background - Prevalence of avian haemosporidian parasites and the factors influencing infection in the Colorado Rocky Mountains are largely unknown. With climate change expected to promote the expansion of vector and avian blood parasite distributions, baseline knowledge and continued monitoring of the prevalence and diversity of these parasites is needed.

Methods - Using an occupancy modeling framework, we conducted a survey of haemosporidian parasite species infecting an avian community in the Colorado Rocky Mountains in order to estimate prevalence and diversity of blood parasites and to investigate species-level and individual-level characteristics that may influence infection.

Results - We estimated prevalence and diversity of avian haemosporidia across 24 bird species, detecting 39 parasite haplotypes. We found that open cup nesters have higher *Haemoproteus* prevalence than cavity or ground nesters. Additionally, we found that male Ruby-crowned Kinglets, White-crowned Sparrows, and Wilson's Warblers have higher *Haemoproteus* prevalence compared to other host species.

Conclusions - Our study presents baseline knowledge of haemosporidian parasite presence, prevalence, and diversity among avian species in the Colorado Rocky Mountains and adds to our knowledge of host-parasite relationships of blood parasites and their avian hosts.

Background

Parasitism is an important driver of ecological and evolutionary processes (Tompkins and Begon 1999, Schmid Hempel 2011) as parasites may regulate host population size (e.g., Hochachka and Dhondt), affect species interactions (e.g., Ricklefs 2010), and create selection pressures in wild populations (e.g., Laine 2009). Compounding effects of parasites with other factors such as climate change, invasive species, habitat loss, or harsh environmental conditions can also drive populations to low numbers, predisposing them to local or global extinctions (Yuill 1986, Minchella and Scott 1991, Gulland 1995, Holmes 1995).

Haemosporida (Phylum: Apicomplexa) are protozoan parasites that infect the blood cells of vertebrates and are transmitted by dipteran vectors (Valkiunas 2004). These blood parasites – haemosporidian parasites – are distributed worldwide and infect a number of vertebrates, including mammals (Witsenburg et al. 2012), reptiles (Staats and Schall 1996), and birds (Valkiunas 2004). Blood parasites go through sexual reproduction in dipteran vectors and are transmitted to vertebrate hosts during vectors' blood meals (Valkiunas 2004). Once in a competent host, the parasite makes its way into the host's bloodstream where asexual reproduction occurs and the infected host becomes a reservoir, carrying developed gametocytes within its red blood cells (Valkiunas 2004).

Among vertebrates, birds are hosts to the highest diversity of haemosporidian parasites, with records of birds being infected with over 200 morphologically distinct haemosporidian parasite species (Valkiunas

2004, Bensch et al. 2009, Valkiunas et al. 2014) and over 3000 unique haplotypes (Bensch et al. 2009). The three parasite genera that infect birds include *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* (Valkiunas 2004, Mullen and Durden 2009). Negative effects of infection can be due to changes in host behavior (Bosholn et al. 2016) or to severe physiological responses, resulting in high mortality rates during the acute phase of infection (van Riper and van Riper 1986, Atkinson et al. 2000). Clinical signs associated with acute haemosporidian parasite infections include anorexia, hemolytic anemia, lethargy, and depression (Mullen and Durden 2009). Avian hosts can suffer declines in reproductive success (Ortego et al. 2008, Knowles et al. 2010) and reduced lifespan when enduring chronic infections (Asghar et al. 2015). Within species, factors such as age, sex, immune status, and degree of exposure may also contribute to variation in host susceptibility and mortality (Mullen and Durden 2009).

Spatial and temporal dynamics of avian haemosporidian parasite occurrence are governed by environmental, ecological, and demographic characteristics (LaPointe et al. 2005, Wood et al. 2007, Lachish et al. 2011, Rooyen et al. 2013). In temperate environments, seasonality has a strong influence on survival and development of both parasites and insect vectors, as mosquitoes emerge during the spring and are active until the end of the summer (Balenghien et al. 2006). This increase in parasites and vectors coincides with the breeding season for most avian species, when resource allocation is diverted to reproduction instead of immune function (Stearns 1992). In addition, congregations of breeding birds and their vectors are beneficial to avian blood parasites as frequency of infection is dependent on host and vector abundance (LaPointe et al. 2005, Medeiros et al. 2015). Our study took place during the breeding season, allowing us to survey avian blood parasites at a time when infection frequency is expected to be highest.

The intensity and seasonality of haemosporidian parasite transmission tends to vary by elevation (LaPointe 2001). Negative correlations between elevation and abundance of mosquitos, the main vectors of avian blood parasites, have been found in many systems including the mountains in Colorado's Front Range (Eisen et al. 2001, Barker et al. 2009), where our study site is located. Many parasites have elevation limits because of the constraints of lower ambient temperatures encountered at higher elevations, though distributions are expanding with climate change as transmission of most vector-borne parasites may be enhanced by higher ambient temperatures (e.g. Samuel et al. 2015, La Pointe et al. 2010). Changes in environmental conditions for vectors, such as an increase in mean air temperature and declining precipitation, support the expansion of haemosporidian parasites into habitats where lower temperatures previously limited transmission (Atkinson et al. 2014, Paz 2015). *Culex tarsalis* and *C. pipiens* are important vectors of avian blood parasites at lower elevations in northeastern Colorado but the low abundance of these species at higher elevations may mean that avian blood parasites have not yet established in areas such as Rocky Mountain National Park (Eisen et al. 2008). However, little research has been done on the distribution of haemosporidian parasites in Colorado, especially in high elevation communities like those in the Colorado Rockies.

Across species, factors such as nest type and migration strategy can explain variation in host susceptibility. Open-cup nesting has been linked to higher blood parasite prevalence in numerous studies

due to higher vector exposure for incubating individuals compared to cavity or ground nesting birds (Gonzalez et al. 2014, Matthews et al. 2015, Smith et al. 2018). Migration has important implications for the emergence and spread of infectious disease-causing parasites due to long-distance movements and exposure to diverse habitats of infected hosts. Establishment of parasites and expansion of their ranges may take place through migration of host species as parasites are able to survive at higher elevations as environmental conditions become more suitable for parasites and vectors (McKay and Hoyer 2016). Migratory birds can harbor high intensity infections and are host to biologically diverse haemosporidian parasite species (Ricklefs et al. 2016), allowing them to act as a source of infection to non-migratory birds who may be more susceptible to blood parasites due to lack of previous exposure at higher elevations (Bueno et al. 2010, Yoshimura et al. 2014). With the potential for migratory birds to spread avian blood parasites to new areas, and a warming climate allowing for the spread of blood parasites into new environments, parasite surveillance is needed in bird communities, even those previously thought to be in areas with low parasite prevalence.

Parasite surveys can serve as early indicators of disease outbreaks that could affect the health of avian populations. The study of blood parasites requires knowledge of baseline levels of haemosporidian parasite infection in target host populations to aid in detection of temporal shifts of parasite diversity to evaluate changes in prevalence of infection. Using an occupancy approach, we conducted multiple screenings for blood parasites per host in order to better estimate detection probability of haemosporidian parasites within host species (Mosher et al. 2019, MacKenzie et al. 2018). Occupancy modeling approaches are useful in wildlife disease ecology because they acknowledge that uncertainty, such as false negative results, exist when using imperfect diagnostic tests (McClintock et al. 2010, Lachish et al. 2012). Variation in detection among multiple screenings of the same blood sample supports the need to use an occupancy modelling framework in order to take detection probability into account in wildlife disease studies (MacKenzie et al. 2018).

The objectives of our study were to: 1) conduct a survey of haemosporidian parasite species infecting an avian community in the Colorado Front Range Rocky Mountains in order to obtain baseline prevalence and diversity estimates at high elevation where avian parasite surveys have not taken place, and 2) to test differences in prevalence and diversity for various individual and species characteristics. Our hypotheses related to the second objective regarding specific host and environmental predictor variables are presented in Table 1.

Methods

Study system

Our study area was located at the Colorado State University Mountain Campus in Larimer County, Colorado, USA (N40.5611, W105.5978), within a mountain valley at an elevation of 2,750 meters. The valley is a breeding site for numerous bird species and no prior research on avian blood parasites has been conducted there to our knowledge.

Data collection

We collected data during the summers of 2017 and 2018. The field portion of our study began in early June when birds begin breeding and continued through the end of the breeding season, around mid-August. We captured birds using mist nets set in sites with high passerine activity. Netting sites were in riparian, forested, and edge habitats. Song playbacks were used to attract birds to nets, providing larger sample sizes to facilitate comparisons of parasite prevalence across host species.

Captured birds were identified at the species level and banded. Sex and age were determined when possible based on guidelines from Pyle (1997), morphological measurements were taken (tarsus length (mm), wing chord (mm), mass (g)), and 10-20 μ L of blood were collected by brachial venipuncture and stored on Nobuto Blood Filter Strips for later DNA extraction. All birds were handled and sampled under a Federal Bird Banding permit from the USGS Bird Banding Laboratory and in accordance with approved guidelines of the Institutional Animal Care and Use Committee of Colorado State University (Protocol 17-7309A).

DNA extraction, PCR amplification, and sequencing

We assessed Haemosporidian parasite infection prevalence and parasite diversity using molecular techniques. We took a 2 mm hole punch of blood-soaked Nobuto Blood Filter Strip (with \sim 15 μ L of blood) for each bird and extracted DNA using the Qiaquick DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA), following the manufacturer's dried blood spot protocol. We stored extracted DNA at -20 °C prior to screening. We screened an aliquot of DNA for parasite presence using a nested polymerase chain reaction (PCR) protocol to amplify a segment of mitochondrial DNA (mtDNA) from the cytochrome *b* gene as outlined in Hellgren et al. (2004). Primers HaemNF1 and HaemNR3 were used to amplify an initial 617-bp segment of mtDNA from species of haemosporidian parasites. The conditions for this PCR were as follows: 30 seconds at 94°C, 30 seconds at 50°C, and 45 seconds at 72°C for 20 cycles. The samples were incubated before the cyclic reaction at 94°C for 3 minutes and after the cyclic reaction at 72°C for 10 minutes. An aliquot of the product (1 μ L) from the first PCR reaction was used in a second reaction amplifying a 479-bp segment of *Haemoproteus* and *Plasmodium* lineages using primers HaemF and HaemR2 (Hellgren et al. 2004). The conditions of the second round of PCRs are as follows: 30 seconds at 50°C, and 45 seconds at 72°C for 35 cycles. PCR screening was repeated three times for each sample, and each plate (96 samples) included two positive controls (one for *Haemoproteus* and one for *Plasmodium*) and one negative control. All PCR reactions were performed at a final volume of 25 μ L using illustra PureTaq Ready-To-Go™ beads (GE Healthcare) with freeze-dried, pre-formulated reagents. We ran 5 μ L of the final product on a 2% agarose gel to screen for parasite presence. For host individuals infected with haemosporidian parasites, the final PCR product was cleaned with ExoSAP (ThermoFisher Scientific) prior to sequencing.

The sequencing reaction of 10 μ L contained 0.25 μ L BigDye™ (Thermo Fisher Scientific), 2.275 μ L BigDye™, 1 μ L of each nested secondary PCR primer (HaemF and HaemR2), 1 μ L of PCR product, and 5.475 μ L molecular grade ddH₂O. Cycle sequencing was conducted at 94 °C for 2 min; 40 cycles of amplification

at 85 °C for 10 s; 53 °C for 10 s and 60 °C for 2.5 min. The sequencing reactions were cleaned-up using 600 µl of Sephadex® G-50 solution per sample prior to analysis on an automated ABI 3500 Genetic Analyzer. Forward and reverse reads were assembled and edited using Geneious Prime 2019.0.4 (<https://www.geneious.com>). Mixed sequences, as indicated by double peaks in a chromatogram, were considered co-infections (Lutz et al. 2015). We identified all sequences at the genus level using the Basic Local Alignment Search Tool (BLAST) feature in the MalAvi database (Bensch et al., 2009; <http://mbio-serv2.mbioekol.lu.se/Malavi/>), a database for avian blood parasites. Mitochondrial haplotypes, that is, sequences differing by one or more bases (<100% identity) from known parasite lineages, were considered unique lineages (Hellgren et al. 2004).

Statistical Analyses

We repeated each PCR assay three times for each DNA sample in order to obtain a parasite detection history composed of 1s and 0s for each individual with 1 signifying at least one detected parasite and 0 indicating no parasite detected. With this detection history, we estimated the probability of parasite detection along with the proportion of individuals infected with blood parasites, corrected for detection probability. We analyzed detection histories for avian haemosporidian parasites using the single-season occupancy model in Program MARK (White and Burnham 1999) to estimate prevalence (i.e., occupancy) of each genus of parasite for each bird species as well as across species (Eads et al. 2015, MacKenzie et al. 2017). In a typical occupancy framework, randomly selected “sites” are surveyed on multiple occasions within a period where occupancy state is assumed not to change. Repeated survey occasions at each site allow estimation of two parameters: occupancy (ψ), the probability that a site is occupied by the species of interest, and detection probability (p), the probability that the species is detected during a given occasion if the site is occupied (MacKenzie et al. 2006). In our study, each blood sample from an individual bird is analogous to a site, the species of interest are *Haemoproteus* and *Plasmodium* parasites, and the repeated survey occasions are multiple replicates of PCR assays for each DNA sample. Reinterpreting the model parameters for parasite detection gives ψ_i as the prevalence of a parasite infection, and p_i as the probability of detecting a parasite(s) in site i , given the presence of the parasite(s) in the host.

We carried out analyses for *Haemoproteus* and *Plasmodium* parasite prevalence separately. We constructed a candidate model set for an all-species analysis that included all birds captured and sampled, as well as a species-specific candidate model set for each host species with at least 20 DNA samples (ten species). In order to address any individual heterogeneity that may exist, we used the random effects model in Program MARK to incorporate any heterogeneity beyond our predictions in each model. Our model set consisted of all possible combinations of predictor variables (Table 1) and we used an information-theoretic approach for model ranking and selection (Burnham and Anderson 2002). We calculated Akaike weights (w_i ; the weight of evidence in favor of each model being the best model compared to the rest of the models in the set) and considered the variables with a cumulative weight greater than 0.5 to be the most important (Barbieri and Berger 2004).

Results

In 2017, we captured 232 birds, and in 2018, we captured 206 birds. Of the 438 birds captured, 180 were males, 206 were females, and for the remainder sex could not be determined. We captured 24 hatch-year birds, 135 second-year birds, 241 after second-year birds, and we could not determine age in the remaining 38 birds. Body condition indices (mass:tarsus) ranged from 0.20 to 25.71 g/mm. We collected molecular data from a total of 437 birds belonging to 24 species over the two years of the study (Table 2).

Haemosporidian Parasite Diversity

In total, we detected 10 *Plasmodium* and 29 *Haemoproteus* cytochrome b haplotypes. Thirty-three haplotypes had a 100% match to current sequences deposited in the Malawi database (Table 2; Bensch et al. 2009), and the other 6 sequences were considered novel haplotypes. The most common *Haemoproteus* lineages were TURDUS2 and SISKIN1, which were detected in 15 and 13 individuals, respectively. The most common *Plasmodium* lineage was PADOM11, which was detected in 6 individuals. Only 3 individuals were found to be infected with both *Plasmodium* and *Haemoproteus*, 1 Warbling Vireo (*Vireo gilvus*) and 2 Lincoln's Sparrows (*Melospiza lincolni*). The greatest diversity was obtained from the Wilson's Warbler (*Cardellina pusilla*; 13), the Warbling Vireo (12), and the White-crowned Sparrow (*Zonotrichia leucophrys*; 11), which also had some of the largest sample sizes.

Haemoproteus

We detected *Haemoproteus* parasites in at least one PCR (out of 3) in 109/437 birds, a naïve (without taking detection probability or covariates into account) *Haemoproteus* prevalence of nearly 25% (109/437). Nest type and year were considered important variables associated with *Haemoproteus* prevalence in the all-species analysis, with variable weights of 0.54 and 0.85 (Table 3). Tree nesters in 2018 had the highest *Haemoproteus* overall prevalence, estimated at 0.38 (± 0.06 ; Figure 3.1). Prevalence was similar for cavity and ground nesters both years and was higher in 2018. In the all-species analysis, we found no evidence of unmodeled heterogeneity using a random effects model (Appendix 3.1, Table A3.1.1). PCR replicate was an important variable when considering detection probability, with a variable weight of 0.99. Detection probability was estimated at 0.62 (± 0.07 SE) for the first PCR run, 0.38 (± 0.07 SE) for the second PCR run, and 0.50 (± 0.07 SE) for the third PCR run (Figure 3.2).

Of all species, the Warbling Vireo, American Robin (*Turdus migratorius*), and Wilson's Warbler had the highest naïve *Haemoproteus* prevalences, at 59%, 35%, and 32%, respectively. In the species-specific analyses (Appendix 3.1), all species had an estimated individual heterogeneity of nearly zero. In terms of prevalence (ψ), sex was considered an important variable for the Ruby-crowned Kinglet (*Regulus calendula*), the White-crowned Sparrow, and the Wilson's Warbler (Figure 3.3), with variable weights of 0.57, 0.54, and 0.51, respectively (Table 4). BCI was also considered an important covariate of prevalence for the Red-breasted Nuthatch (*Sitta canadensis*) and the Ruby-crowned Kinglet (Figure 3.5), with variable weights of 0.69 and 0.84, respectively (Table 4). No species had variable weights above 0.5 for age or

year. For detection probability (p), PCR run was an important variable for the Lincoln's Sparrow and the White-crowned Sparrow, with variable weights of 0.96 and 0.50, respectively (Figure 3.5).

Plasmodium

We detected *Plasmodium* parasites in at least one PCR replicate in 23 out of 437 birds, which is a total naïve *Plasmodium* prevalence of 5.3%. When analyzing all species together, we detected no heterogeneity using a random effects model. We found no predictor variable with a cumulative variable AICc weight of at least 0.5, and therefore none of our hypothesized variables were considered important in predicting *Plasmodium* infection in the all-species analysis.

The Wilson's Warbler and Lincoln's Sparrow had the most infected individuals per species, with three birds positive for *Plasmodium* in each. Because of the low number of positives per species, species-specific analyses could not be carried out for *Plasmodium* prevalence.

Discussion

Avian haemosporidian parasites in the Colorado Rocky Mountains are little studied. Within the MalAvi database, only one study (Marzal et al. 2011) reported sampling birds in Colorado, and this study was focused solely on House Sparrows (*Passer domesticus*). Because of the limited amount of data, the factors influencing avian haemosporidian parasite infection in the Colorado Rocky Mountains are largely unknown. A warming climate is expected to aid in the expansion of parasite distributions, and baseline knowledge and continued monitoring of the prevalence and diversity of these parasites is needed. This is especially true of high elevation resident host species that are more susceptible to infection and may be more heavily impacted by the spread of haemosporidian parasites.

In this study, we present baseline knowledge of haemosporidian parasite presence, prevalence, and diversity across a suite of avian species in the Colorado Rocky Mountains. Among the 437 birds of 24 species sampled, thirty-nine unique haemosporidian parasite haplotypes were detected, 21 species had at least 1 infected individual, and *Haemoproteus* parasites had a larger host-breadth and had much higher prevalence compared to *Plasmodium*. In addition, 6 novel haplotypes were detected among 3 different species. Using an occupancy-modelling framework to account for imperfect detection of avian blood parasites, we found that nest type is an important species-level factor influencing *Haemoproteus* parasitism at our study site, with open cup nesters having a higher prevalence compared to cavity and ground nesters. We also found that sex and BCI are important individual-level factors associated with *Haemoproteus* parasitism in some species, with males and birds with higher BCI having a higher blood parasite prevalence.

Haemosporidian lineage diversity

Diversity of haemosporidian parasites in wild birds was high, with a total of 39 lineages of *Haemoproteus* and *Plasmodium* species from 21 of the 24 avian species that were sampled (Table 2). *Plasmodium*

lineage parasites are considered generalists in terms of host breadth, while *Haemoproteus* lineages are generally considered to be host-specific (Hellgren et al. 2009). However, we identified *Haemoproteus* in a wider range of bird species than *Plasmodium* and found that *Haemoproteus* parasites were more prevalent overall.

The small sample sizes obtained for many host species limit us from determining host specificity of the obtained lineages; however, some patterns were still apparent and most of these centered on the family Turdidae. One lineage, TURDUS2, infects many families of birds throughout Europe (Hellgren et al. 2007a), Asia (Hellgren et al. 2007b), and the United States (Oakgrove et al. 2014), yet most detections have occurred in the Turdidae (Thrush family). Accordingly, American Robins, a member of the Turdidae, at our study site had the highest proportion of TURDUS2 detections compared to other species. As this lineage was found in numerous species at our study site, American Robins may be acting as a reservoir for this parasite lineage. According to the MalAvi database, the lineage TUMIG07, has only been detected in the American Robin and Hermit Thrush (*Catharus guttatus*) in Alaska (Oakgrove et al. 2014). In our study, individuals from both of these species were found to be positive for the TUMIG07 lineage, along with six other species suggesting that this lineage is relatively common at our study site. The VIGIL07 lineage has only been detected in the Vireonidae family in California (Walther et al. 2016), New Mexico (Marroquin-Flores unpublished data), and Michigan (Smith et al. 2018), and, in our study, the highest proportion of individuals positive for this lineage was in the Warbling Vireo. Of the 7 detections of the TUMIG08 lineage in the MalAvi database, 4 have been from the American Robin (Oakgrove et al. 2014). Accordingly, 2 of the 4 detections of this lineage at our study site were from American Robins, with one detection in a Lincoln Sparrow, and the other in a Warbling Vireo. The POETR01 lineage has been mainly detected in the Thrush family as well (Oakgrove et al. 2014), and our one detection of this lineage was in an American Robin and therefore agrees with previous detections.

Patterns across host species

When analyzing *Haemoproteus* prevalence across host species, nest type and year were important variables associated with infection, with open-cup nesters in 2018 having the highest estimated prevalence of 0.38 (± 0.06 ; Figure 3.1). Open-cup nesting has been linked to higher blood parasite prevalence in other studies (Gonzalez et al. 2014, Matthews et al. 2015, Smith et al. 2018), indicating that *Haemoproteus* vectors – biting midges – are more likely to come in contact with species that have open-cup nests than with ground or cavity nesters, perhaps because open-cup nesters are more vulnerable to exposure to blood feeding by the vectors.

Overall *Haemoproteus* prevalence also displayed marked variation between years in our study, with 2018 having a higher overall prevalence compared to 2017 (Figure 3.1). Interannual variation in avian blood parasite prevalence is common and has been found in many studies (e.g., Bensch et al. 2007, Wood et al. 2007, Lachish et al. 2011, Podmokła et al. 2014). One potential explanation for this annual variation is that the vectors responsible for *Haemoproteus* transmission fluctuate in abundance in response to weather variation (e.g., temperature and rainfall), which alter the habitat and microclimate they require for

breeding. Higher prevalence may therefore occur in years when conditions are more favorable for vectors. Alternatively, annual variation in host demography and population dynamics could also play a role in driving this annual variation (Anderson and May 1986, Atkinson and Samuel 2010).

Age, sex, BCI, and migration were not considered important variables across species. These variables have been linked to higher haemosporidian parasite prevalence in other studies (e.g., Hatchwell et al. 2001, Deviche et al. 2005, Garvin et al. 2006, Calero-Riestra and Garcia 2016), thus site and broader geographic variation are important factors to consider when describing relationships between patterns of prevalence and host life history traits, and these relationships (or lack thereof) should be interpreted with caution. Further studies are needed to address the influence of host traits on patterns of avian haemosporidian parasite infection and to determine whether such patterns exist and persist at large spatial scales and across a wider host-parasite community.

When analyzing *Plasmodium* prevalence among host species, no associations were found between prevalence and species-level traits, likely due to the low number of individuals that were positive for the parasite. Low abundance of *Plasmodium* parasites and vectors in the host community can limit the transmission of blood parasites and may explain low prevalence. Elevation governs the distribution of parasites belonging to different genera, with *Plasmodium* parasites being more prevalent at lower altitudes and *Haemoproteus* parasite prevalence increasing with elevation (Rooyen et al. 2013). Accordingly, Eisen et al. (2008) found that *Culex* spp. mosquitoes, the main vectors of *Plasmodium* parasites, had not yet established in areas in and around Rocky Mountain National Park. Associations between exposure to mosquitoes and *Plasmodium* prevalence across host species has been demonstrated (Medeiros et al. 2015), supporting the idea that *Plasmodium* vectors may be absent, or in low numbers at our study site.

Patterns of Haemoproteus infection within individual species

Sex was associated with *Haemoproteus* infection in the Ruby-crowned Kinglet, White-crowned Sparrow, and Wilson's Warbler (Figure 3.4). Sex-related differences in haemosporidian parasite prevalence are often observed in nature, however, sex-bias in parasitism is not universal and consistent, and often varies between and within host-parasite systems (McCurdy et al. 1998). Contrary to our prediction, our study demonstrated a strong male-biased parasite prevalence in the three species mentioned above, with Ruby-crowned Kinglet having the largest difference between sexes (0.53 in males vs. 0.01 in females). Although the greater stress of reproduction in females might translate to weakened immune responses (Møller et al. 1999), there is overwhelming evidence that sex-associated hormones can directly influence the differential susceptibility of each sex to infections (Loye and Zuk 1991). For example, testosterone has immunosuppressive effects in many species, leading to higher susceptibility of males to parasite infections (Zuk 1996, Zuk and McKean 1996, Hughes and Randolph 2001). This is not the case for every host-parasite relationship, as was illustrated by our failure to find an association between parasite prevalence and sex in the other seven species that we analyzed.

BCI was positively associated with *Haemoproteus* infection in the Red-breasted Nuthatch and the Ruby-crowned Kinglet when species were analyzed individually (Figure 3.5). Similar results have been found in other species such as the American Kestrel (*Falco sparverius*), the Yellow-rumped Warbler (*Setophaga coronata*), and the Great Tit (*Parus major*; Dawson and Bortolotti 2000, Cozzarolo et al. 2018, Ots and Horak 1998). This positive correlation may be due to infected individuals with lower body condition having lower capture probability. If infected individuals with low body condition are less active and are less likely to fly into mist nets, that leaves only infected individuals with greater body condition to be caught. Similarly, if individuals with low body condition are unable to survive the acute stage of *Haemoproteus* infection, then this may leave more infected individuals with higher body condition. The eight other species analyzed in our study did not show an apparent relationship between prevalence and BCI, which is a common result in wildlife studies given that host condition and its responsiveness to infection could change in response to foraging resources that fluctuate in space and time (Schultz et al. 2010, Sanchez et al. 2018). Some parasites cause minimal or no effects on condition in certain host taxa (Sanchez et al. 2018), and some infections might only exert negative fitness effects during stressful periods or under resource limitation (Khan and Fallis 1970, Applegate 1971).

We found no relationship between *Haemoproteus* prevalence and individual-level traits (sex, age, BCI) in the American Robin, Mountain Chickadee (*Poecile gambeli*), Pine Siskin (*Carduelis pinus*), or Dark-eyed Junco (*Junco hyemalis*), contrary to our hypotheses. Our results suggest that the individual-level traits examined in this study may not be important predictors of *Haemoproteus* infection for all species. However, we did identify a pattern in nest type indicating that aspects of avian life history and ecology shape, to a limited extent, their parasite community and the proportion of individuals infected by blood parasites.

Detection probability

PCR replicate was an important variable associated with detection probability for *Haemoproteus* infection for three individual species (Lincoln Sparrow, Warbling Vireo, and White-Crowned Sparrow) as well as for the all-species analysis, with PCR results varying among the three PCR runs (Figures 3.2 and 3.3). Nested PCR assays for haemosporidian parasites are known to be vulnerable to false negative results for parasite intensities at very low samples (Ishtiaq et al. 2017) and is most likely responsible for the variation we found between PCR replicates.

Detection probability for *Plasmodium* parasites was estimated at 0.30, which could be due to a decreased detection for *Plasmodium* based on blood samples (Svensson-Coelho et al. 2016) because *Plasmodium* enters latent, exoerythrocytic phases during chronic infection and may even be absent in the blood stream (Valkiunas 2004). Thus, sampling peripheral blood may not allow for detection of all true infections with *Plasmodium*, leading to underestimates of prevalence.

Conclusion

Our results suggest that open cup nesting birds in the Colorado Rocky Mountains are commonly infected with avian blood parasites and that male Ruby-crowned Kinglets, White-crowned Sparrows, and Wilson's Warblers have higher prevalence compared to females of these species. Prevalence of avian haemosporidian parasites and the factors influencing infection in the Colorado Rocky Mountains are largely unknown, though our study presents baseline knowledge of blood parasite presence, prevalence, and diversity across avian species in the region. With climate change expected to support the expansion of avian blood parasite distributions, monitoring of avian haemosporidian parasites should continue in order to detect changes in prevalence and diversity over time. Our study is the only avian blood parasite survey conducted in the Colorado Rocky Mountains, to date; additional research in this area examining host-parasite relationships would help to determine whether anthropogenic changes – such as climate change – leading to potential changes in vector communities or parasite distributions may pose a threat to resident avian populations.

Declarations

Ethics approval and consent to participate – Permission for capturing and taking blood samples from birds was approved by the Colorado State University Institution for Animal Care and Use Committee (Protocol 17-7309A).

Consent for publication – Not applicable.

Availability of data and materials - The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests - The authors declare that they have no competing interests.

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Authors' contributions – MR and KH conceived the idea. MR, KH, and PD designed the field study. MR carried out the laboratory work with the instruction and laboratory equipment from KH and AP. MR conducted the analysis with guidance from PD. MR wrote the paper and KH, PD, and AP reviewed the manuscript.

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Tables

Due to technical limitations, table 1,2,3,4 is only available as a download in the Supplemental Files section.

Figures

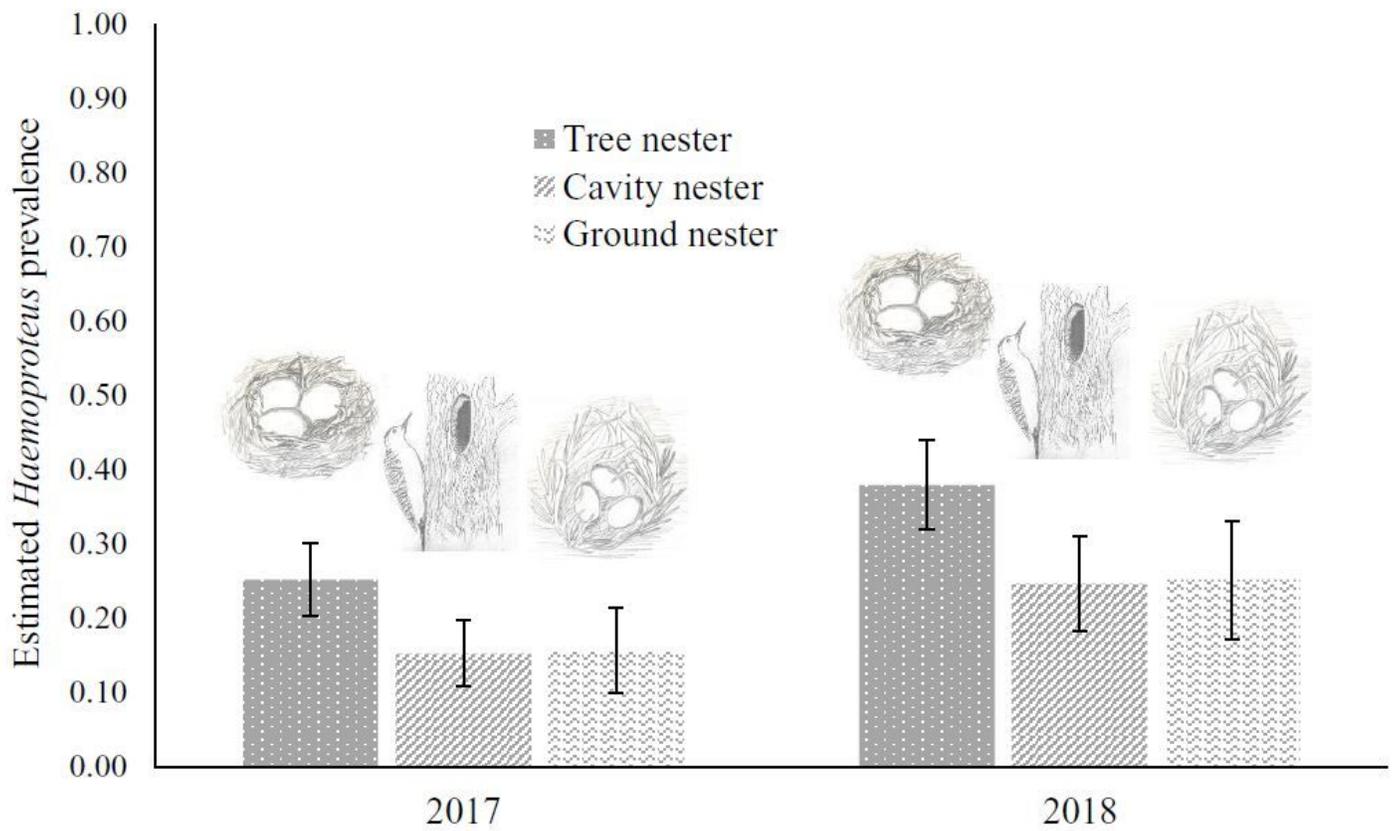


Figure 1

Estimated *Haemoproteus* prevalence (± SE) for each nest type (tree, cavity, and ground) between each study year in a bird community in the Colorado Rocky Mountains.

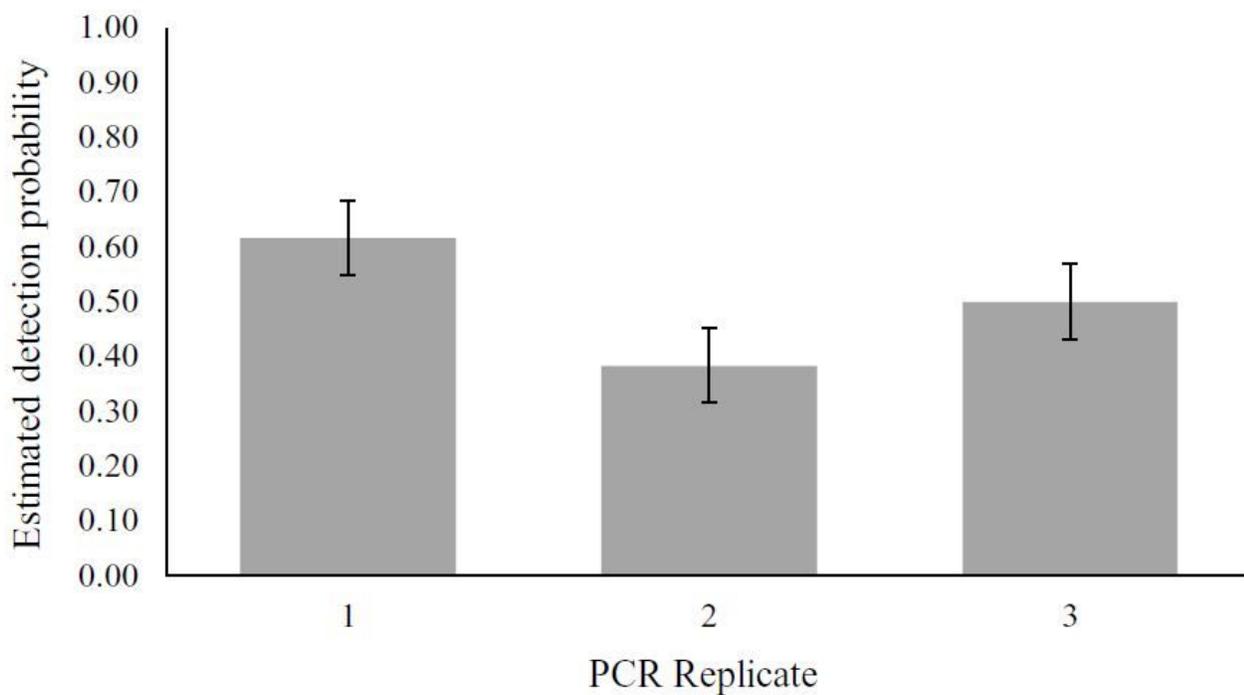


Figure 2

Estimated detection probability ($p \pm SE$) of avian Haemoproteus for each PCR replicate in the all-species analysis for birds sampled in a high elevation bird community in the Colorado Rocky Mountains.

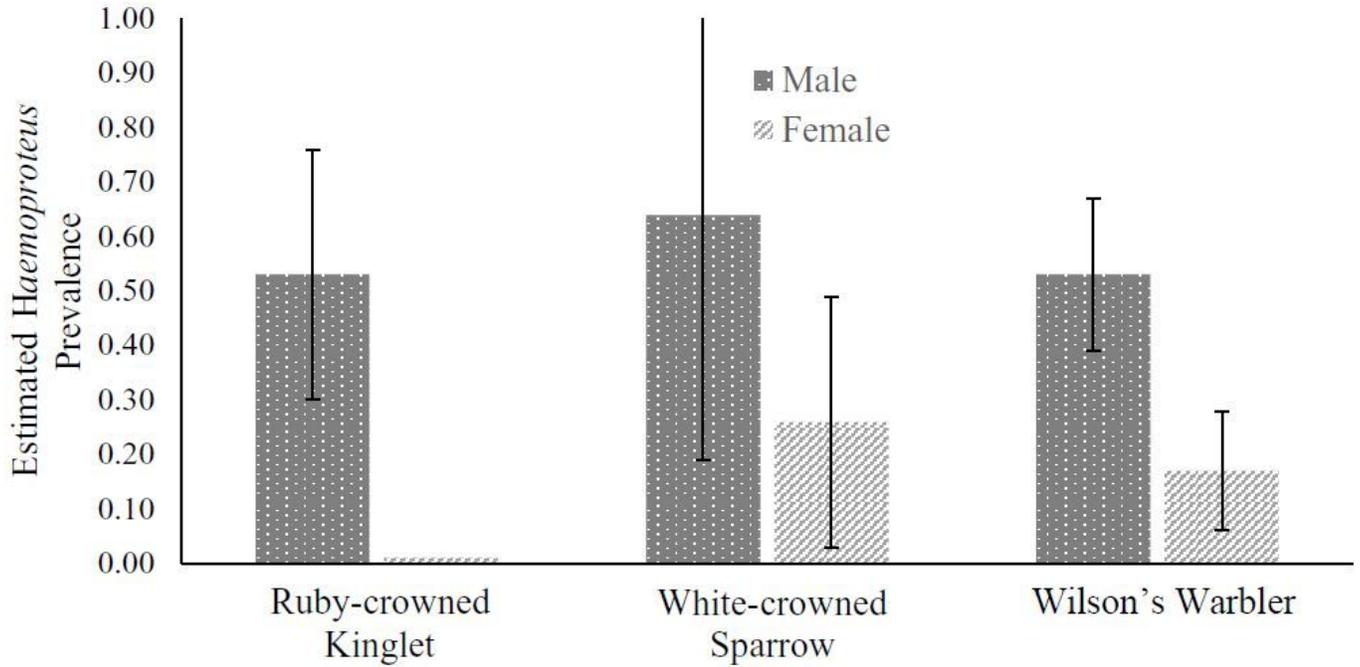


Figure 3

Estimated Haemoproteus prevalence ($\pm SE$) for male and female Ruby-crowned Kinglet, White-crowned Sparrow, and Wilson's Warbler. Note that no female Ruby-crowned Kinglets were positive in this study.

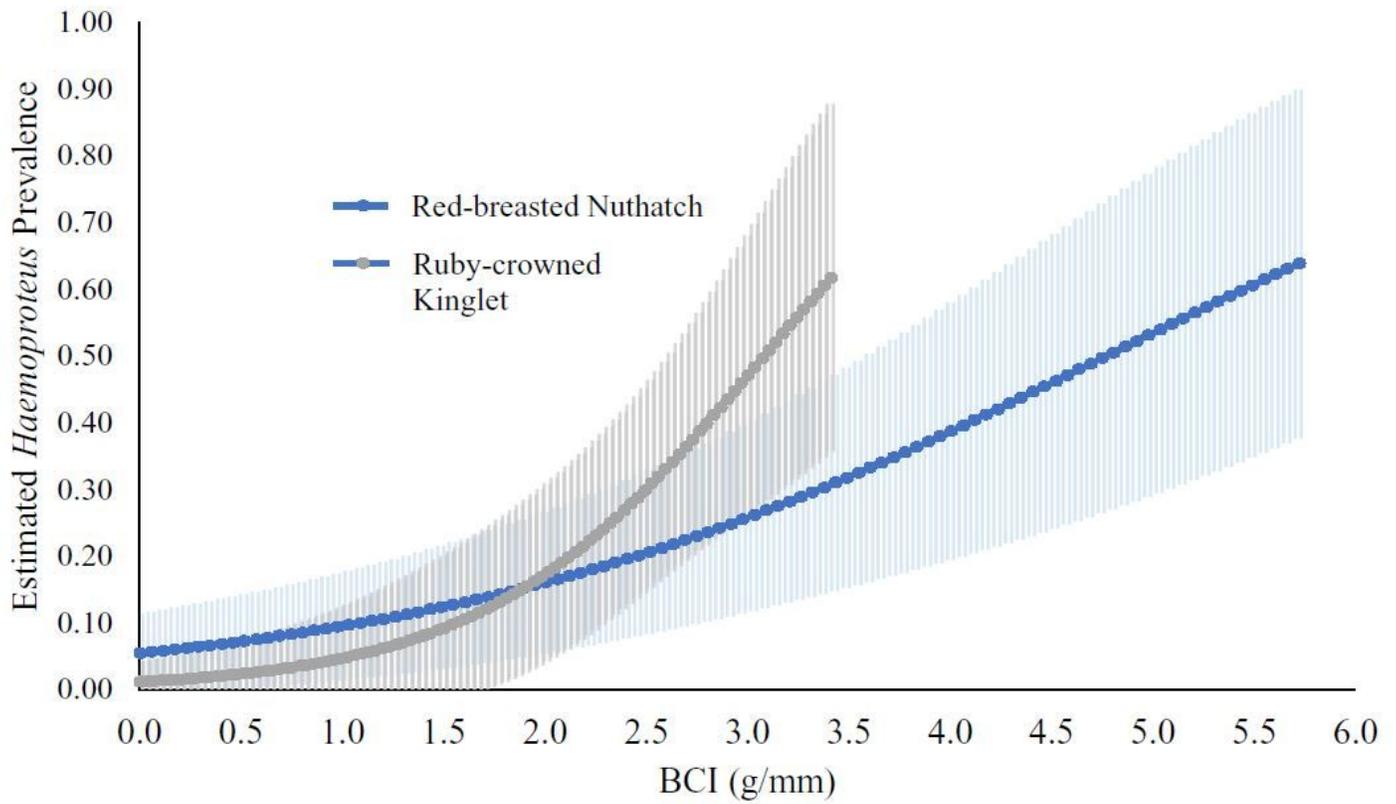


Figure 4

Estimated *Haemoproteus* prevalence (\pm SE) and body condition index for the Red-breasted Nuthatch and the Ruby-crowned Kinglet. BCI is a ratio of body mass (g) to tarsus length (mm) for each individual.

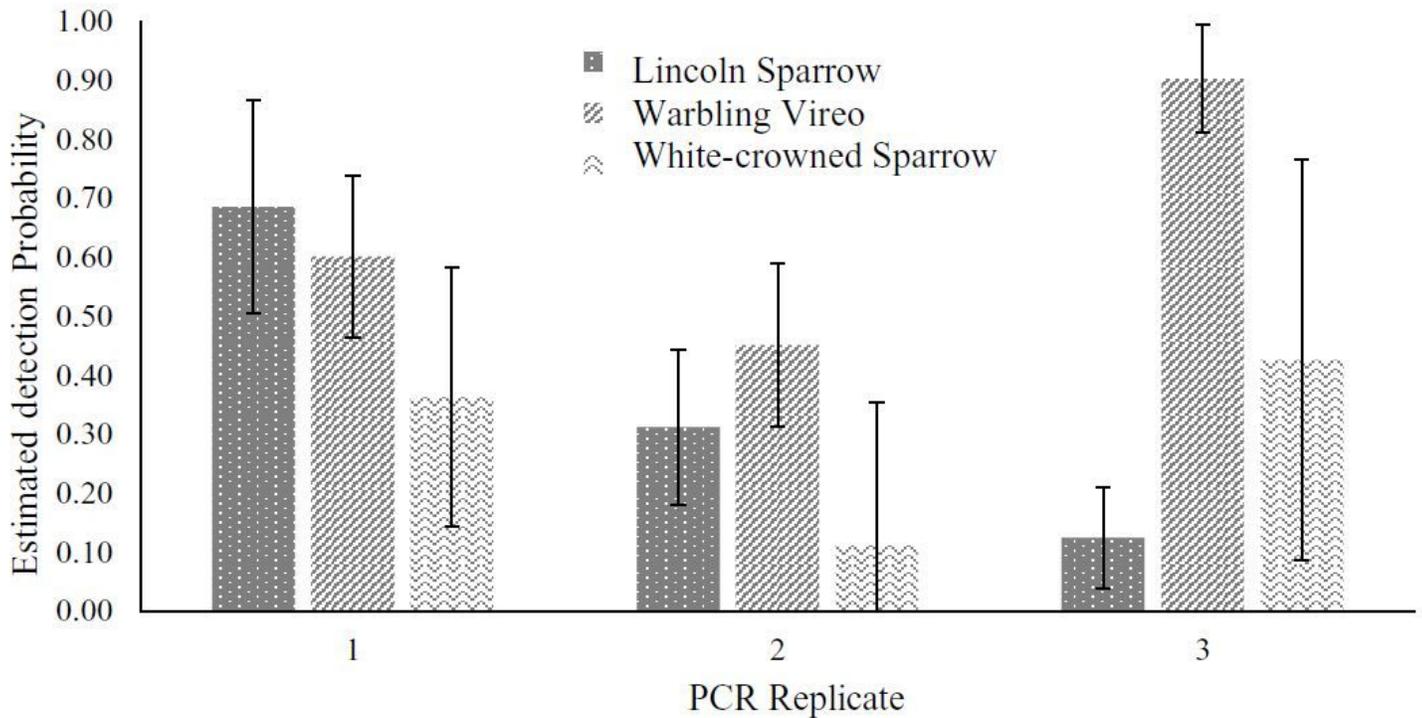


Figure 5

Estimated detection probability ($p \pm SE$) of *Haemoproteus* for each PCR replicate for the Lincoln's Sparrow, Warbling Vireo, and White-crowned Sparrow.

Supplementary Files

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