THESIS

CALCIUM, TELOMERE LENGTH, AND PARASITISM IN PASSERINES NESTING AT HIGH ELEVATION

Submitted by

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ABSTRACT

CALCIUM, TELOMERE LENGTH, AND PARASITISM IN PASSERINES NESTING AT HIGH ELEVATION

Most organisms are exposed to numerous environmental stressors at various points throughout life, and, through natural selection, organisms' responses to such stressors have been optimized by natural selection for the best fitness outcomes. During the breeding season, wild vertebrates often make a trade-off between current reproduction and self-maintenance when dealing with environmental stressors.

The total cost of reproduction is made up of all of the resources and energy that go into activities related to reproduction (e.g., nest building, finding a mate, foraging for food and nutrients related to offspring production, parental care) that do not go into self-maintenance. The cost of these activities can vary depending on resource availability, where limited resources can increase the cost associated with breeding due to increased energy associated with foraging and competing for the resource. In birds, calcium is a critical resource due to its importance in egg production and offspring development, and low calcium availability often leads to decreased reproductive success.

In my first chapter, I used an experimental approach to assess the effects of supplemental calcium on reproductive parameters of Tree Swallows (*Tachycineta bicolor*) in a high elevation environment. Calcium-supplemented birds in my study area laid more, larger eggs, and had higher hatching success compared to control females. These results provide evidence that calcium availability is a constraint on breeding Tree Swallows at high elevation, perhaps due to

the harsh conditions and concomitantly higher metabolic costs that force a costlier and more intense trade-off between foraging for food or for calcium.

The increase in reproductive parameters for calcium supplemented nests in Chapter 1 highlights a cost associated with calcium foraging that constrains reproduction. For my second chapter, I aimed to better understand how calcium availability affects the cost of reproduction in mother Tree Swallows and offspring by using telomere shortening as a proxy of life stress and lifespan. Telomeres are terminal features of chromosomes consisting of repetitive DNA sequences that shorten with age and stress, and whose length is positively correlated with survival. I used telomere shortening as a proxy for the costs associated with reproduction to better understand life history trade-offs of Tree Swallows at high elevation sites. Similar to Chapter 1, I found that Tree Swallows supplemented with calcium had higher reproductive success, although I also found that supplemented nests had more telomere shortening compared to birds at control nests. These results provide evidence that Tree Swallows supplemented with calcium experience higher reproductive output at the cost of lower expected survival in the form of more telomere shortening.

While investing resources in reproduction may lead to higher reproductive output for the current breeding season, this increase in reproductive success can come with a cost to survivorship. One way that resource allocation can shape survivorship is through investment in immune function. In many systems, however, more species-level and individual-level research is needed on host-parasite relationships before trade-offs between immune function and reproduction can be assessed. For my third chapter, I conducted a survey of avian Haemosporida: blood parasites that include those that cause avian malaria. I surveyed an avian community for haemosporidian parasites in the Colorado Rocky Mountains in order to estimate prevalence and

diversity of blood parasites and to find species-level and individual-level characteristics that influence infection prevalence. I found that open cup nesters have higher blood parasite prevalence than cavity or open cup nesters. Additionally, male Ruby-crowned Kinglets, White-crowned Sparrows, and Wilson's Warblers had a higher prevalence of haemosporidian parasites compared to the other species analyzed, as did Red-breasted Nuthatches, which, like Ruby-crowned Kinglets, have a high body condition index. This chapter presents baseline knowledge of avian blood parasite presence, prevalence, and diversity across avian species in the Colorado Rocky Mountains and adds to our knowledge of host-parasite relationships of blood parasites and their avian hosts.

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CHAPTER 1: CALCIUM SUPPLEMENTATION POSITIVELY AFFECTS TREE SWALLOW REPRODUCTIVE PARAMETERS IN A HIGH ELEVATION ECOSYSTEM

Synopsis

Calcium is a limiting nutrient in many avian systems given its critical importance for egg production and chick growth. High elevations, where harsh conditions increase metabolic and reproductive costs, may exacerbate calcium limits on reproduction. Through an experimental approach, I assessed the effects of supplemental calcium on reproductive parameters of Tree Swallows (*Tachycineta bicolor*) in a high elevation environment. Calcium supplemented birds in my study area laid more, larger eggs, and had higher hatching success compared to control females. My results provide evidence that calcium availability is a constraint on breeding Tree Swallows at high elevation, perhaps due to the harsh conditions and concomitantly higher metabolic costs that force a costlier and more intense trade-off between foraging for food or for calcium.

Introduction

Reproduction in vertebrates requires higher levels of energy and particular nutrients than does self-maintenance (Robbins 1993). Of these specific nutrients, calcium is critical for successful breeding for most vertebrates, and it is especially important for birds; egg production in birds requires 10-15 times as much calcium for developing eggs or embryos compared to similar-sized reptiles and mammals (Simkiss 1967, Klasing 1998).

Calcium carbonate makes up 95% of the dry weight of an avian eggshell (Graveland and Van Gijzen 1994). Additionally, calcium from the eggshell is used for skeletal development of the embryo within the egg (Booth and Seymour 1987, Balkan et al. 2006). As important as

calcium is to reproduction, most passerines cannot maintain long-term skeletal reserves of the nutrient (Pahl et al. 1997). Storage limitations, as well as inadequate amounts of calcium in the diets of insectivorous and granivorous birds, force egg-laying females to supplement their diets with calcium-rich items such as snail shell, grit, and eggshells encountered in the environment (Davies 1977). Calcium requirements in altricial bird species also remain elevated after hatching because of continued skeletal development and parents must feed calcium-rich foods to offspring until fledging (Graveland 1996).

The high demand for calcium throughout reproduction in birds makes calcium availability an important component of habitat quality affecting the reproductive success of most avian species (Graveland and Drent 1997, Sanz 1997, Tilgar et al. 1999, Mänd et al. 2000a, b, Tilgar et al. 2002, Bidwell and Dawson 2005, Dawson and Bidwell 2005, Wilkin et al. 2009, Espín et al. 2016). Most studies reporting calcium-limited reproduction in wild birds attribute their results to soil acidification (Scheuhammer 1991, St. Louis and Breebaart 1991, Graveland and van der Wal 1996, Sanz 1997, Graveland 1998, Reynolds and Perrins 2010). Calcium leaching from the soil due to acid deposition causes a decline in the availability of both calcium in the soil and calcium-rich prey items (Graveland and Van Gijzen 1994). Acidity in the environment can occur naturally via processes such as nitrification and degradation of plant materials, but an increase in anthropogenic activities has accelerated soil acidification in many areas (Graveland and Drent 1997). Deposition of the compounds formed by combustion of fossil fuels via ‘acid rain’ and intensive agriculture is responsible for 80-90% of total soil acidification, and this deposition is implicated as the most important factor in the loss of calcium from soil (Graveland and Drent 1997).

Previous work on Tree Swallows (*Tachycineta bicolor*) shows that the effects of supplemental calcium are equivocal and additional work is needed to refine our understanding of the relationships between calcium and reproduction. While a number of studies have found that calcium has a positive effect on birds breeding in naturally base-poor environments (Tilgar et al. 1999, Mänd et al. 2000a, b, Tilgar et al. 2002), few studies have looked at the effects of calcium supplementation in areas where environmental calcium is available (but see e.g., Johnson and Barclay 1996, Bidwell and Dawson 2005, Dawson and Bidwell 2005). In a study by Johnson and Barclay (1996), reproduction of House Wrens (*Troglodytes aedon*) was not affected by supplemented calcium and the authors concluded that calcium availability was not a factor in reproduction in areas where acidification had not taken place. However, Dawson and Bidwell (2005) showed trends of higher egg masses, thicker eggshells, and larger clutches for calcium supplemented nests than for controls. In another study by Bidwell and Dawson (2005), there was no statistically significant difference in survival or tarsus length between chicks in calcium supplemented versus control nests.

In areas with neutral soil pH, avian reproduction may still be limited by calcium availability due to naturally occurring variation in environmental conditions. Birds breeding at high elevations face a suite of challenging conditions, including colder, often windier, weather, periods of dense fog caused by heavy cloud cover, and precipitation is more likely to be in the form of snow early in the breeding season (Nagy and Grabherr 2009). Johnson et al. (2018) found that Tree Swallows breeding at high elevations (~2500 m) showed delayed reproduction, longer incubation periods, smaller clutches, and smaller eggs compared to those nesting at lower elevations (~1400 m). The discrepancy in reproductive outcomes was attributed to harsh environmental conditions, which caused higher energetic stress on the female in terms of

thermoregulation, increased self-feeding to compensate for increased exertion, and overall fewer resources available to meet these energetic needs (Johnson et al. 2018). If it is the case that females nesting at higher elevation sites face harsher environmental conditions compared to females at low elevation, then high elevation birds may be limited by calcium due to the need to forage for energy over calcium to pay the higher metabolic costs. As natural forms of calcium are expected to become less readily available with increasing acidification in the western United States (Benedict et al. 2013, Ellis et al. 2013), determining whether calcium currently constrains reproduction by birds at high elevation is important so that we can better understand how calcium might limit reproduction in the future.

In this study, I supplemented calcium for breeding Tree Swallows nesting in a high elevation mountain valley that is not considered calcium deficient to determine whether calcium is limiting in this population. I hypothesized that calcium is limiting reproduction in this system because birds may forage to meet energetic needs first and calcium second given the demands of the harsher conditions at higher elevation. Further, I predicted that supplementing calcium to breeding Tree Swallows would lead to increases in reproductive parameters including clutch size, egg volume, and hatching success, relative to controls.

Methods

Study Species

Tree Swallows are migratory insectivores whose range spans North America (Winkler et al. 2011). While Tree Swallows naturally nest in abandoned woodpecker cavities, they seem to prefer artificial nest boxes placed near water and open fields (Robertson and Rendell 1990). Tree Swallows are territorial during the breeding season and both intraspecific and interspecific competition for nesting sites takes place (Lifjeld and Robertson 1992). A typical clutch size is

four to seven eggs, one egg is laid per day, and incubation lasts 14 to 15 days (Stocek 1970). Tree Swallows may also be affected by their relatively short legs and concomitantly low mobility on the ground, making it difficult to forage for calcium-rich items on the substrate (Lifjeld and Robertson 1992).

Study Area

My study took place at the Colorado State University Mountain Campus in Larimer County, Colorado, USA. The study area is located within a mountain valley at an elevation of 2,750 meters (9022 feet). The area has not yet been affected by anthropogenic acid deposition and natural sources of calcium are available (Clow and Sueker 2000, Binkley et al. 2003, Mast et al. 2010).

Study Design

My study took place over five summers (2013-2018), starting in mid-May of each year and continuing through early to mid-August to cover the entire breeding season from nest building to fledging. I built 90 nest boxes and placed them on the landscape in March 2013, before Tree Swallows arrived to breed. In 2017, I increased the number of nest boxes to 200. All boxes were spaced at least 10 m apart in open areas along a stream with nest box openings facing south to southeast. Calcium was supplemented in the form of crushed oyster shell, which is commonly used to supplement domestic poultry and is similar in calcium content to snail shells, the main sources of natural dietary calcium for Tree Swallows (Bidwell and Dawson 2005). I supplemented nest boxes with either oyster shell (treatment) or local soil (control) by placing a handful of material in a tray attached to the roof of the nest boxes. Once I observed a shallow ring of grass in the bottom of a nest box, indicating the start of nest construction, I alternated assignment of nests to the calcium treatment or control as the nests were initiated. The trays were

replaced during the study if damaged or lost and the calcium or control soil supplies were frequently checked and refreshed, if needed. Due to the territoriality of Tree Swallows, the chances that a bird from a control nest consumed oyster shell from an experimental nest were low; I also did not observe this behavior at any time during the study.

I continued supplying the nest boxes with local soil or oyster shell until the nestlings had fledged. Nests were checked and clutch size was recorded every 2-3 days. Individual egg length (mm) and width (mm) measurements were taken using a caliper once a clutch was complete, when no new eggs were in the clutch for two days. I measured both egg length and width to the nearest 0.01 mm three times then calculated the mean. I used the mean values for each egg to calculate egg volume using the formula $V=0.51LW_2$, where L is the length of the egg, W is the width of the egg, and 0.51 is a Tree Swallow species-specific constant (Hoyt 1979). My protocol was reviewed and approved by the Colorado State University Institutional Animal Care and Use Committee (Protocol ID: 17-7309A).

Data Analysis

I evaluated the effects of calcium supplementation on reproductive parameters of nesting Tree Swallows by constructing a set of linear mixed models for each of the three response variables of interest (clutch size, egg volume, and hatching success). Each model included "box" as a random effect, and each candidate model set included a treatment effect model (calcium or control), a year effect model, an additive model including treatment and year, a model for the interaction of treatment and year, and an intercept-only model indicating no effects. I included a year effect model as the study spanned six years during which the weather conditions at my site varied considerably. I also included an additive model for treatment and year to account for possible compounding effects of annual variation in weather conditions and calcium

supplementation. Lastly, I included an interaction effect to account for the influence of calcium availability varying by year. I carried out all analyses in program R v.3.3.2 using the 'lmer' package. I used an information-theoretic approach for model selection and ranking for each of the three model sets (Burnham and Anderson 2002). I considered the model with the lowest AICc value in each set to be best supported by the data. I also calculated ΔAICc values (difference between each model and the top-ranking model) and Akaike weights (w_i; estimates of the probability that the i_{th} model is the best model given the data and the model set) for model selection and inference.

Return rate was very low at my study site and only six birds were recorded as having returned to breed in a later year. For these returning birds, one of the years was randomly selected and the data from that year were included in the analysis so that no single bird appeared in the dataset more than once.

Results

One hundred eighty-eight nests were initiated and monitored over the six years of the study. Sample size varied among years as well as for each analysis due to differences in nest survival over the breeding seasons. In my analysis of clutch size, the treatment model was ranked highest and carried the highest Akaike weight (w_i=0.80) (Table 1.1): supplemented nests had higher mean clutch sizes (mean=5.55 ± 0.12 SE) compared to control nests (mean=5.09 ± 0.10 SE; Figure 1.1). In the egg volume analysis, the additive treatment + year model ranked highest and carried all of the Akaike weight (w_i= 1.0; Table 1.1): supplemented nests had higher mean egg volume (mean = 1918.54 ± 17.04 SE) compared to control nests (mean=1890.70 ± 12.19 SE; Figure 1.2). In the hatching success analysis, the additive treatment + year model carried the highest Akaike weight (w_i=1.0; Table 1.1): a higher proportion of eggs hatched in supplemented

nests (mean=0.81±3.98 SE) compared to control nests (mean=0.61±2.90 SE; Figure 1.3) and this varied over the years or the study.

Discussion

Experimentally supplemented calcium allowed nesting Tree Swallows to produce more, larger eggs per clutch with a higher hatching success than those birds provided with only local soil. My results provide evidence that calcium availability is a limiting factor for breeding Tree Swallows at high elevation, even when calcium is naturally available.

While a variety of other studies have examined the effects of calcium supplementation on reproductive success in birds (e.g., Graveland 1996, Poulin and Brigham 2001, Tilgar 2003, Tilgar et al. 2004, Wilkin et al. 2009, Espín et al. 2016), few have focused on the effect in a non-acidified landscape, and none, to my knowledge, have examined these effects in systems at elevations over 2,000m (my site was at 2750m). Birds breeding at high elevation face a suite of challenges that make living and breeding more metabolically costly than at lower elevations, resulting in relatively lower reproductive success with increasing elevation (Altshuler and Dudley 2006, Nagy and Grabherr 2009, Johnson et al. 2018). Results indicated that increased energetic costs may limit the time birds spend foraging for calcium, thereby imposing a trade-off between foraging for energy or calcium, where energy is a higher priority.

Consistent with such a view, clutch sizes were larger for calcium-supplemented nests compared to controls (Figure 1.1) in this study. Tilgar et al. (2002) also found that calcium-supplemented Great Tits (*Parus major*) breeding in a non-acidified landscape produced more eggs per clutch, and other studies have shown similar trends (Johnson and Barclay 1996, Mänd et al. 2000a). Smaller clutch sizes in control nests may be due to a time constraint associated with calcium foraging. Graveland and Drent (1997) found that Great Tit females on a low

calcium diet spent double the time searching for calcium rich items compared to those on a high calcium diet, and the authors inferred that producing smaller clutches may be an adaptive way to deal with low calcium availability. Here, supplemented Tree Swallows were not constrained by time spent foraging for calcium; therefore, they were able to produce larger clutch sizes than control nests.

Similarly, mean egg volume for calcium-supplemented nests was larger, as was the variability in egg volume among years compared to control nests (Figure 1.2). Tilgar et al. (1999) and Mand and Tilgar (2003) demonstrated that calcium supplementation of Pied Flycatchers (*Ficedula hypoleuca*) also led to larger egg volumes, a finding that these authors attributed to increased calcium availability. However, this was not the case in Great Tits (Mand et al. 2000) or Tree Swallows (Bidwell and Dawson 2005) supplemented with calcium. Many calcium supplementation experiments show improvements in only one egg parameter due to a trade-off between more or larger eggs; improvements in both clutch size and egg volume were observed here. Because egg volume is positively correlated with general energy intake (Selma and Houston 1996, Ramsay and Houston 1997, Hogstedt 1981, Williams et al. 1996), supplementing Tree Swallows with calcium may allow birds to spend more time foraging for energy, causing an increase in egg volume for calcium-supplemented birds compared to controls. Therefore, increased egg volume in supplemented nests may be partly due to indirect effects of calcium supplementation. As for the year effect, the decrease in egg volume observed may be attributed to the 50 inches of snowfall in April of 2016 (High Plains Regional Climate Center 2019), leading to high snow cover present in early May when birds were arriving on the breeding grounds and foraging for the energy and resources needed for reproduction.

Hatching success is an important measure when evaluating reproductive success, as eggs in a nest that do not hatch may result in wasted resources on the part of the female. Hatching success in my study was nearly 15% higher in supplemented nests and varied by year (Figure 1.3). My results are similar to Bidwell and Dawson (2005), who found that calcium supplemented nests hatched nearly 20% more eggs than the control nests. Though the direct reason for higher hatching success is unknown, Graveland and Drent (1997) credit lower hatching success to thinner eggshells and shell deformities linked to calcium deficiency. This hypothesis could not be evaluated in my study because I did not measure eggshell thickness. Nonetheless, because skeletal development involves the embryo utilizing calcium from the shell to form its skeleton, increased calcium may translate into a stronger skeleton and enhanced ability of the chick to hatch from the egg. Calcium-supplemented nests showed a large decrease in hatching success in 2017, perhaps due to the 51 inches of snowfall in mid-May of 2017 (High Plains Regional Climate Center 2019), the start of the incubation period for this population.

I conclude that calcium is a limiting factor even in the absence of soil acidification, most likely due to the harsh conditions at high elevations that intensify the trade-off between self-maintenance and reproduction. Because calcium is naturally available at the study site, I expect that acidification will only amplify the constraints on reproduction for birds in this study. Acidification is expected to increase in high elevation areas in the western United States due to excess nitrogen deposition, which continues to increase due to fossil fuel combustion and the production and application of industrial fertilizer (Benedict et al. 2013, Ellis et al. 2013). Thus, I expect growing limits on reproduction for high elevation environments in the future.

Table 1.1 Model set and rankings for groups of models exploring the importance of calcium supplementation ('Calcium') and 'year' on mean clutch size per nest, mean hatching success per nest, and mean egg volume per nest in Tree Swallows at a high-elevation valley in northern Colorado during 2013-2018. The number of parameters (K), -2 log-likelihood (-2LL), and model weights (w_i) are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$). The minimum AICc value for mean clutch size per nest was 649.72, for mean egg volume per nest it was 2260.40, and for mean hatching success per nest it was 2296.42. 'Box' was included as a random effect in each model.

Dependent Variable	**Model**	**K**	**$\Delta AICc_i$**	**-2LL**	**w_i**
Mean Clutch Size per Nest	Calcium	4	0.00	-315.14	0.80
	Calcium + Year	9	3.59	-311.60	0.13
	Intercept	3	5.40	-318.88	0.05
	Calcium * Year	14	10.28	-309.36	0.00
	Year	8	11.26	-316.52	0.00
Mean Egg Volume per Nest	Calcium + Year	9	0.00	-1075.09	1.00
	Calcium * Year	13	31.76	-1095.54	0.00
	Year	8	37.61	-1099.58	0.00
	Calcium	4	75.74	-1122.95	0.00
	Intercept	3	82.01	-1127.13	0.00
Mean Hatching Success per Nest	Calcium + Year	9	0.00	-1093.98	1.00
	Calcium * Year	13	31.71	-1114.39	0.00
	Year	8	37.55	-1118.42	0.00
	Calcium	4	73.95	-1140.93	0.00
	Intercept	3	80.29	-1145.14	0.00

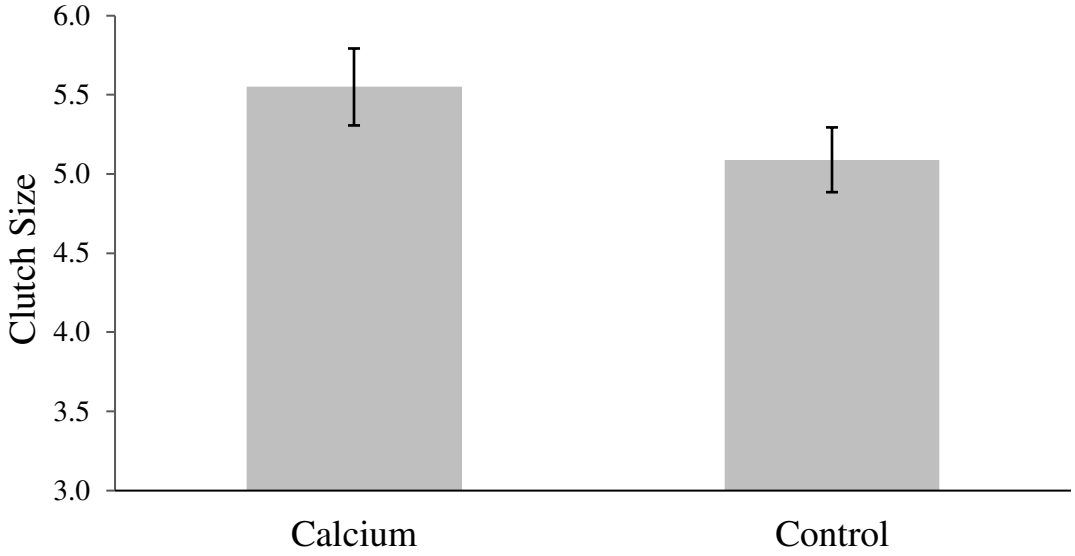

Figure 1.1 Mean (±SE) Tree Swallow clutch size per nest of calcium supplemented (n=95) compared to control nests (n=93) in a high elevation study site in northern Colorado from 2013-2018.

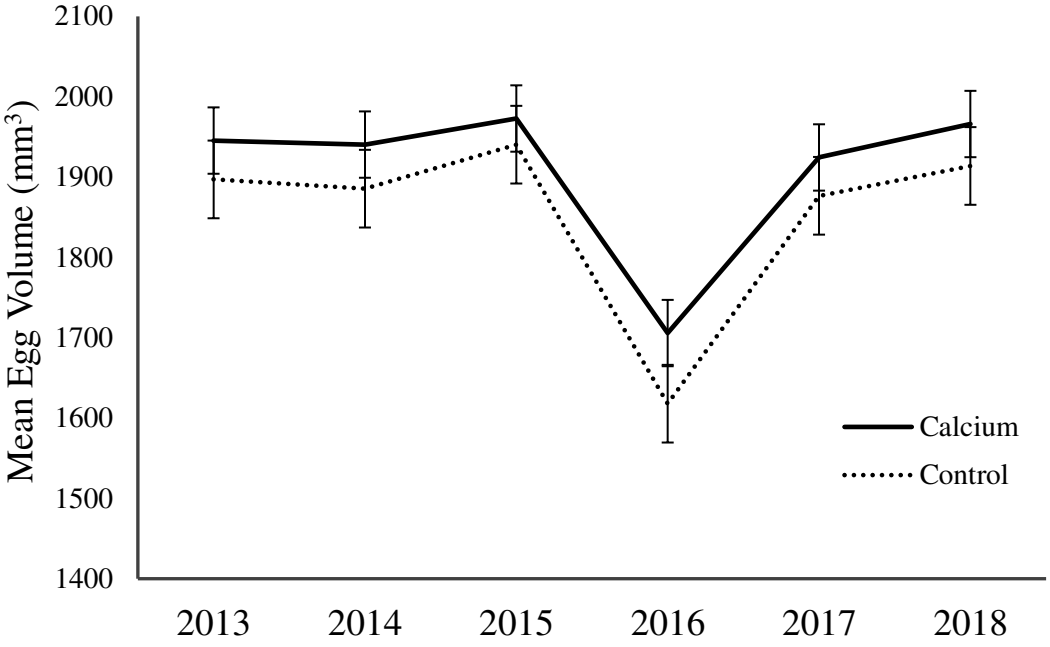

Figure 1.2 Mean (±SE) Tree Swallow egg volume of calcium supplemented (n=95) compared to control nests (n=93) over the course of the six years of the study in a high elevation study site in northern Colorado.

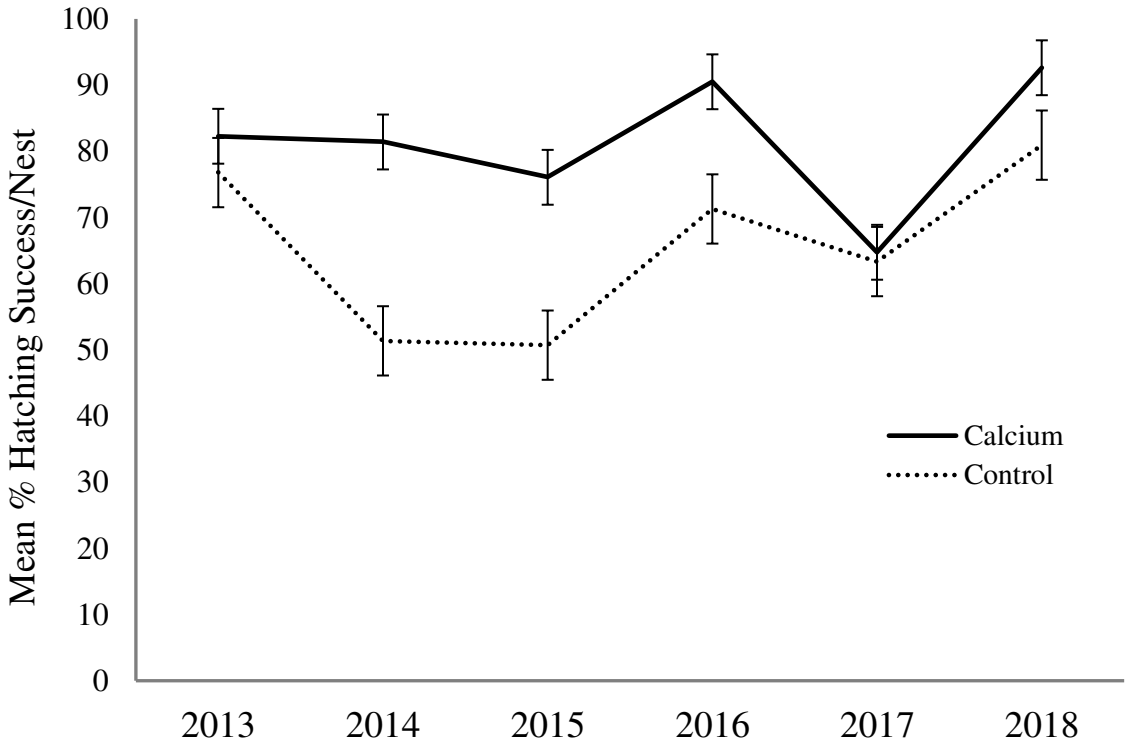

Figure 1.3 Mean (±SE) Tree Swallow hatching success of calcium supplemented (n=95) compared to control nests (n=93) over the course of the six years of the study in a high elevation study site in northern Colorado.

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CHAPTER 2: CALCIUM SUPPLEMENTATION AFFECTS TELOMERE DYNAMICS IN BREEDING TREE SWALLLOWS

Synopsis

Calcium is a limiting nutrient in many avian systems given its critical importance for egg production and chick growth, yet, how calcium availability affects the cost of reproduction in mother Tree Swallows and offspring is unknown. I used telomere shortening as a proxy for the costs associated with reproduction to better understand life history trade-offs in Tree Swallows. Telomeres are terminal features of chromosomes consisting of repetitive DNA sequences that shorten with age and stress and whose length is positively correlated with survival. Through an experimental approach, I assessed the effects of supplemental calcium on reproductive parameters and telomere shortening in breeding Tree Swallows (*Tachycineta bicolor*) in northern Colorado. I found that Tree Swallows supplemented with calcium had higher reproductive success and greater telomere shortening compared to control birds. My results provide evidence that Tree Swallows supplemented with calcium experience higher reproductive output at the cost of lower expected survival, as indicated by higher levels of telomere shortening.

Introduction

Life history theory is built upon the premise that resources, and the time and energy it takes to acquire them, are limiting, and that allocation of these resources to competing functions can result in trade-offs (Stearns 1992). Fundamentally important to the evolution of life history strategies is the cost of reproduction, for which it is assumed that energy and resources allocated to breeding activities are no longer available for self-maintenance. Thus, an increase in

reproductive investment can be linked to a reduction in longevity through reductions in investment towards self-maintenance (Williams 1966, Stearns 1992, Roff 2002).

To better understand the trade-offs between reproductive investment and longevity, the physiological mechanisms that regulate life history trade-offs need to be investigated. Telomeres provide a mechanistic link between life history trade-offs, as telomeres shorten with oxidative stress, and this shortening then negatively affects future survival (Horn et al. 2010, Monaghan 2010, Young 2018). Telomeres are the non-coding, repetitive DNA sequences that cap the ends of eukaryotic chromosomes and protect them from degradation (Blackburn 1991). Because DNA polymerase cannot completely replicate linear DNA at the ends, telomeres shorten with each cell division (Olovnikov 1996). Telomere length can thus vary among individuals of the same age depending on genetic background (Asghar et al. 2011, Horn et al. 2011), oxidative stress (Cattan et al. 2008, Houben et al. 2008), parasitism (Asghar et al. 2015), as well as environmental and social stressors (Beadell et al. 2004, Kotrschal et al. 2007, Gil et al. 2018). Once telomeres shorten to a critical length, further cell division damages coding DNA, leading to an accumulation of dead cells in tissues that cause organ dysfunction and other aging-related negative effects (Campisi et al. 2001). Among vertebrates, telomere shortening is correlated with lifespan, with more telomere shortening occurring near the end of life; therefore, current reproduction may affect the degree of telomere loss and have downstream effects on classic life history traits such as lifespan (Young 2018).

Numerous studies have provided support for a link between reproduction and telomere length, many of which have recently been carried out on birds (e.g., Reichert et al. 2014, Costanzo et al. 2017, Sudyka et al. 2019). Bauch et al. (2013) found that Dunlins *(Calidris alpine)* experience significant telomere loss during the breeding season, and, furthermore, that

the most successful parents (in terms of total offspring produced) had the shortest telomeres. Heidinger et al. (2012) found that captive Zebra Finches *(Taeniopygia guttata)* engaging in reproduction had accelerated telomere loss compared to individuals that did not reproduce. Also in captive Zebra Finches, experimentally increased brood sizes resulted in reduced telomere length for adults at the end of the breeding season compared to control or reduced broods (Reichert et al. 2014).

One costly investment made by breeding animals is foraging for the energy and nutrients needed to produce offspring (Carey 1996). In birds, limited nutrients may be more critical than energy in limiting reproductive success, and calcium is the most important of these nutrients (Burley and Vadehra 1989, Barclay 1994). Calcium is necessary for egg production, as well as embryo and nestling skeletal development (Balkan et al. 2006), though the diets of insectivorous and granivorous birds also tend to contain inadequate amounts of calcium (Graveland and Van Gijzen 1994). Because of the need to find exogenous sources of calcium, many passerines are forced to spend valuable time searching for calcium-rich items during the egg-laying and chick-rearing periods instead of foraging for energy or other important nutrients (Graveland and Berends 1997, Graveland and Drent 1997, Mänd et al. 2000a). In fact, many studies show that supplementing calcium to breeding birds leads to an increase in reproductive success, concluding that calcium is limiting in various avian systems (Graveland and Drent 1997, Tilgar et al. 1999, Mänd et al. 2000a, b, Tilgar et al. 2002, Mand and Tilgar 2003, Bidwell and Dawson 2005, Dawson and Bidwell 2005).

The increases in parameters associated with reproductive success for calcium supplemented nests points to a cost associated with calcium foraging that constrains reproduction. When access to calcium is cost-free, as with calcium supplementation, resources

are allocated to other functions, such as self-maintenance or other activities. The aim of this study was to understand how calcium availability affects the cost of reproduction in mother Tree Swallows and offspring by using telomere shortening as a proxy of life stress and lifespan. To address this, I had two main questions:

Question 1.a - Does calcium supplementation positively affect telomere length in breeding female Tree Swallows?

I hypothesized that mothers of calcium supplemented nests would experience less telomere shortening during the breeding season compared to control nests, as calcium supplementation would alleviate the costs associated with calcium foraging. Very few studies have investigated how factors affecting the cost of reproduction affect telomere length dynamics, although Badas et al. (2016) showed that supplementing breeding Blue Tits (*Cyanistes caeruleus*) with the antioxidants tocopherol (Vitamin E) and methionine (an essential amino acid) resulted in less telomere shortening than in controls, demonstrating a reduction in the cost of reproduction. Aside from telomere length measurements, many studies have shown that factors affecting the current cost of reproduction are linked to changes in survival in the future. Brood size (Nur 1984, Daan et al. 1996, Santos and Nakagawa 2012), immune function (Deerenberg et al. 1997, Cichoń et al. 2001, Hasselquist et al. 2001), food availability (Ramsay and Houston 1997, Eikenaar et al. 2003, Ardia et al. 2006), and energy expenditure (Winkler and Allen 1995) have been shown to influence the trade-off between current reproduction and survival in birds.

Ardia et al. (2003) increased the cost of reproduction in Tree Swallows by experimentally increasing brood size and found that mothers of enlarged nests showed reduced immune response and survival. Similarly, Winkler and Allen (1995) experimentally increased the cost of

reproduction in Tree Swallows by clipping one-third of the flight feathers of breeding females thereby increasing the energetic demands associated with foraging. Clipped females laid later and smaller clutches, were in poorer body condition, and were less likely to return to breed the following year than controls. Both studies illustrate that the cost of reproduction can be incurred as a reduction in survival.

I reasoned that, if changes in the cost of reproduction affect survival, then changes in the cost of reproduction should also affect telomere shortening. By supplementing Tree Swallow mothers with calcium, the cost of reproduction associated with calcium foraging is reduced, and so the overall cost of reproduction is decreased. If a lowered cost of reproduction leads to improved survival, then the expectation would be less telomere shortening in calcium-supplemented mothers compared to control mothers over the course of the breeding season.

Question 1.b – Do older mothers have increased telomere shortening compared to younger mothers?

I hypothesized that older birds might invest more in reproduction, at the cost of lower expected survival, which I tested by assessing telomere shortening, a well-accepted measure of aging. This hypothesis is in accordance with the Terminal Investment Hypothesis (Williams 1966), which states that older individuals should have lower, if any, chances of reproducing in the future, such that older birds will invest more into reproduction compared to younger birds who should invest more in self-maintenance to keep their probability of future survival and fecundity high (Godfray 1991, Klomp 1970). In looking at phenotypic traits that help predict survival in Tree Swallows, Ouyang et al. (2016) found that older individuals with more experience have greater reproductive investment, shorter telomeres, and a lower probability of returning to the breeding site the next year. Because of the importance of age in telomere length

and telomere shortening, age will be included as a covariate in my analyses to determine if results match the predictions of the Terminal Investment Hypothesis.

Question 2 - Does calcium supplementation positively affect telomere length of nestling Tree Swallows at 12 days-old?

I hypothesized that chicks in control nests would have shorter telomeres at twelve days compared to those in calcium-supplemented nests because the cost of reproduction for supplemented mothers is lowered and, therefore, the cost of reproduction absorbed by the offspring will be less than that absorbed by young in control nests. Many studies have illustrated how exposure to stress, that is any physical or physiological conditions that prompt the activation of the vertebrate "stress response," in the parental generation influences telomere length in offspring (e.g., Buchanan 2000, Asghar et al. 2014, Haussmann and Heidinger 2015). Parental stress can affect offspring telomere length directly through effects on parental germline telomeres pre-fertilization (Haussmann and Heidinger 2015) or indirectly by exposing offspring to stress hormones during pre-natal development or by altering parental behavior during post-natal development (Haussmann and Heidinger 2015).

If control mothers experience a higher cost of reproduction and are exposed to more stress than experimentally supplemented mothers, offspring from control nests should incur a higher cost of reproduction from their parents compared to those in supplemented nests, which will be reflected in the form of shorter telomeres at 12 days-old.

Methods

Study System

I studied the Tree Swallow (*Tachycineta bicolor*), an insectivorous passerine that feeds on the wing and nests near open fields and water sources where flying insects are abundant.

Nesting takes place in empty cavities (e.g., those made by woodpeckers) near water and open areas, but nest boxes are used readily (Robertson and Rendell 1990). A typical clutch size is four to seven eggs, one egg is laid per day, and incubation lasts 14 to 15 days (Stocek 1970). Once hatched, both parents provide care for the altricial nestlings until fledging, which takes 17 to 23 days (Stocek 1970). Tree Swallows are an ideal study species for this experiment due to extensive knowledge about their breeding habits, behavior, physiology, and life history (Jones 2003). Numerous telomere studies have used Tree Swallows as a wild study species (e.g., Haussmann et al. 2003, Haussmann et al. 2005, Ouyang et al. 2016, Belmaker et al. 2018), and the short lifespan of Tree Swallows allows us to observe changes in telomere length more quickly than long-lived organisms (Haussmann et al. 2003). Studying Tree Swallows that use nest boxes also comes with certain logistical advantages, such as the ability to readily and repeatedly trap breeding females and offspring.

My study took place during the summers of 2017 and 2018 at the Colorado State University Mountain Campus located in Larimer County, Colorado, USA (N40.5611, W105.5978). The area is located within a mountain valley at an elevation of 2,750 m, which is the highest elevation of any avian calcium supplementation study (Chapter 1). The area has not yet been affected by anthropogenic acid deposition and natural sources of calcium are available (Clow and Sueker 2000, Binkley et al. 2003, Mast et al. 2010). The nest box trail runs along the edge of a riparian area and consists of 200 nest boxes mounted on t-posts, ~1.5 m above the ground and at a distance of at least 10 m between boxes. A large population of nesting Tree Swallows inhabits this area during the summer and has been the focus of previous studies (Chapter 1).

Study Design and Sampling

Starting in early May of both years, I checked nest boxes daily to detect the start of nest construction. Once nest initiation was detected, indicated by a shallow grass ring at the base of the nest box, I randomly assigned the nest to either the calcium or the control group. At nest initiation, I also captured females at the nest by covering the entrance once the female was inside, or by using a mesh trap door. Once in hand, I banded females and measured tarsus length (cm), wing length (cm), and mass (g). I then classified birds into age categories of one-year old or greater-than-one-year depending on the presence or absence of grey/dull plumage around the beak and eyes (Pyle 1997). After taking measurements, I collected between 10 µl and 30 µl blood samples using brachial venipuncture using an insulin syringe and a 27-gauge needle as suggested by Owen (2011). Blood was stored in a heparinized capillary tube and stored at -20°C until analysis (Criscuolo et al. 2009).

Following blood collection, I supplemented nests with either oyster shell (calcium treatment) or local soil (control), depending on the group assignment, in a tray attached to the roof of the nest box (Figure 2.1). I replaced supplementation trays during the study if damaged or lost and refreshed the calcium or soil supply when needed. I checked nests every day to determine clutch completion once laying began and took individual egg length and width measurements once a clutch was completed. To obtain accurate measures, I took three measurements for both egg length and width to the nearest 0.01 mm and calculated the mean for each egg. I measured egg width at the largest diameter. Egg volume was calculated using the formula $V=0.51LW_2$, where L is the length of the egg, W is the width of the egg, and 0.51 is a species-specific constant (Hoyt 1979); I used the individual-egg mean measures in this formula to calculate volume. Once hatched, I calculated hatching success for each nest as the proportion

of eggs in the clutch that hatched. On day 12 after hatching, blood samples were collected from offspring and again from mothers using the same collection and storage methods described above. At this time, chicks were banded, and tarsus length (cm) and mass (g) were measured. 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 # 17-7304A).

Telomere Length Assay

DNA was isolated from whole blood samples using the DNeasy Blood and Tissue kit (Qiagen, Valencia, California) following the manufacturer's protocol. DNA concentration was measured using a NanoDrop 8000 spectrophotometer (Thermo Scientific). Following the protocol of Criscuolo et al. (2009), telomere length was quantified by quantitative real-time polymerase chain reaction (qPCR). Telomere length was measured as the ratio (T/S) of telomere repeat copy number (T) to a control single gene copy number (S), which was then standardized to a reference sample and expressed as relative telomere length (RTL); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the single copy control gene.

Tree Swallow DNA samples were run in triplicate and a common control sample was run on each plate to facilitate comparison between plates. Quantitative PCR plates included serial dilutions (0.2, 0.4, 2, 10, 30, and 50 ng) of DNA of the same reference bird to generate a standard curve to control for the amplifying efficiency of the qPCR. An independent Tree Swallow sample from my study site (not included in the study) was used as the reference sample rather than a purified commercial sample to avoid potential differences in amplification efficiency between the samples and the standard curve.

Statistical Analysis

I first analyzed egg volume, hatching success, and brood size in relation to calcium treatment to determine whether these reproductive parameters are influenced by calcium supplementation. As clutch and brood size are highly correlated (r= 0.66), I used only brood size in my analysis. I constructed a set of linear mixed models for each of the three response variables of interest. Each model included 'box' as a random effect, and each candidate model set included a treatment effect model (calcium or control), a year effect model, an additive model including treatment and year, an interaction model for treatment and year, and an intercept-only model indicating no effects.

To measure change in RTL of the mother during the breeding season, I calculated D, a measure of temporal RTL shortening adjusted for the regression to the mean, following Kelly and Price (2005) using the equation:

$$D = \rho(X_1 - \bar{X}_1) - (X_2 - \bar{X}_2), \text{ where}$$

$$\rho = \frac{2rs_1s_2}{s_1^2+s_2^2},$$

X_1 is the RTL for mothers at time-point one (pre-breeding), X_2 is the RTL for mothers at time-point two (when chicks were 12 days-old), r is the correlation between X_1 and X_2, s is standard deviation, and s^2 is variance. RTL measurements at both time-points were transformed to a log-normal scale to avoid negative lengths. I then fit D as the response variable in linear mixed effects models.

In analyzing telomere length in mother Tree Swallows, I included 'box' as a random variable in each model, and treatment, age, brood size, year, and their interactions as fixed effects. My model set consisted of all possible additive and interactive combinations of fixed effects.

I also analyzed average chick RTL per nest at day 12 using linear mixed effect models. For chick RTL analysis, I used the average RTL of all of the chicks in a nest as the response variable, with 'box' as a random effect included in each model, and treatment, mother RTL at time-point one, mother age, and year as fixed effects. I included all possible additive and interactive combinations of fixed effects in my model set.

I carried out all analyses in R 3.5.2 using the 'lmer' function in the 'lmer4' package. I used an information-theoretic approach for model selection and ranking (Burnham and Anderson 2002) for each of the three model sets. I considered the model with the lowest AICc value in each set to be best supported by the data. I also calculated ΔAICc (difference between each model and the top-ranking model) and Akaike weights (w_i; estimates of the probability that the i_{th} model is the best given the data and the model set).

Results

Calcium supplementation had a positive effect on reproductive success, as treatment was included in the top model for brood size, egg volume, and hatching success (Table 2.1). Supplemented nests had larger average egg volume (mm^3) per nest (mean=1796.37±37.77 SE) compared to controls (mean=1767.80 ±32.38 SE; Figure 2.2). Average hatching success was also higher in supplemented nests (mean=93.48±2.38 SE) compared to controls (mean=82.94±4.96 SE; Figure 2.3). Lastly, supplemented nests had a larger average brood size (mean=4.86±0.19 SE) compared to control nests (mean=4.0±0.27 SE; Figure 2.4).

In analyzing telomere length in mother Tree Swallows, the 'Treatment' model carried the highest Akaike weight (w_i=0.35; Table 2.2): supplemented nests had higher adjusted telomere shortening (mean=0.078±0.06 SE) compared to controls (mean=-0.06±0.03 SE; Figure 2.5). The second-best model, the 'Age' model, also carried some Akaike weight (w_i=0.31; Table 2.2): one-

year-old mothers had lower adjusted telomere shortening (mean=-0.05±0.04 SE) compared to older mothers (mean=0.03±0.05 SE; Figure 2.6).

The 'Treatment + Mother Pre-breeding RTL' model carried the most Akaike weight (w_i=0.70; Table 2.3) for offspring RTL: calcium supplemented offspring had longer average RTL per nest at 12 days (mean=3.23±0.15 SE) compared to control nests (mean=2.25±0.17 SE; Figure 2.7) and offspring RTL was positively correlated to mother pre-breeding RTL (r=0.67).

Discussion

During avian reproduction, higher levels of stress can be reached through increased parental investment (Alonso-Alvarez et al. 2004, Metcalfe and Alonso-Alvarez 2010, Christie et al. 2012). When reproductive investment exceeds what is sustainable for parents, the costs for longevity become apparent through accelerated telomere shortening (Santos and Nakagawa 2012). Thus, studies increasing brood size in a range of organisms have shown that costly reproductive events have a negative impact on adult lifespan (Reichert et al. 2014) and early life of offspring (Nettle et al. 2013, Boonekamp et al. 2014, Herborn et al. 2014). Traditionally, brood-manipulation studies have reflected the relationship between investment in reproduction and telomere loss; however, whether factors thought to alleviate reproductive costs affect telomere shortening in the wild is unknown. I examined the role that calcium supplementation plays in the relationship between reproductive investment and telomere length in order to better understand how calcium availability influences the trade-off between current reproduction and longevity.

Calcium and reproductive parameters

My results provide evidence that calcium is a limiting factor in Tree Swallow reproduction in a high-elevation, non-acidified system. Experimentally supplemented nests had

increased reproductive productivity in the form of larger average egg volume, higher hatching success, and larger brood size than control nests. These findings are similar to those of Bidwell and Dawson (2005), who reported a trend for higher clutch size and improved hatching success with calcium supplementation in Tree Swallows nesting in a non-acidified environment. Together with results reported in Chapter 1, calcium seemingly is a limiting factor for Tree Swallow reproduction and is advantageous in terms of reproductive output. However, the stress associated with increased reproductive effort comes at a direct cost to the mother via telomere shortening and associated reduced lifespan.

Calcium supplementation and telomere shortening in mother Tree Swallows

Contradictory to my prediction, mother Tree Swallows supplemented with calcium experienced higher degrees of telomere shortening compared to control mothers. On average, supplemented females in my study produced 0.86 additional chicks and suffered an increase of 0.14 in adjusted telomere shortening compared to controls, suggesting that producing and raising more offspring is costly in terms of stress and lifespan.

This increase in telomere shortening indicates a higher cost of breeding for supplemented mothers, likely due to the increased energy and resource expenditure that accompanies raising more offspring. However, brood size was not included in the top model for telomere shortening (though the effect was in the predicted direction), indicating that it was not necessarily the number of offspring produced, but that calcium supplementation caused an increase in brood size above what is optimal for each mother.

Parents should produce the number of offspring that maximizes their overall fitness, meaning that an individual should not produce the maximum number of offspring possible per year, but the maximum number that simultaneously optimizes longevity (Lack 1947, Charnov

and Krebs 1974). This optimum varies with parental condition, as well as with the environmental conditions present during reproduction and has been studied widely (e.g., Murphy et al. 2000, Pettifor et al. 2001, de Heij et al. 2006). Many studies have concluded that reproductive costs are 'investment-dependent,' as they are only apparent once reproductive efforts have surpassed what parents were prepared to sustain (Santos and Nakagawa 2012, Reichert et al. 2014). This is true of my study system, as additional telomere shortening is the cost of reproduction that mother Tree Swallows face after exceeding their optimum clutch size.

Calcium supplementation studies are similar to clutch manipulation experiments in that supplemented mothers were subject to an increased clutch size, and this increase in clutch size came with a higher reproductive cost. Studies that have measured the fitness consequences of variation in clutch or brood size have determined that clutch sizes are optimized at the individual level in many species (e.g., Dijkstra et al. 1990, Stearns 1992, Pettifor et al. 2001). Optimal clutch size is determined by the condition of the individual as well as their breeding environment, therefore variations from the optimum result in increased costs (Boyce and Perrins 1987, Nooker et al. 2005). Most clutch manipulation studies support this idea, as increases in clutch sizes result in higher energy expenditure (Moreno and Sanz 1994, Engstrand et al. 2002) and lower parent and/or offspring survival (De Kogel 1997, Styrsky et al. 2005, de Heij et al. 2006). This was apparent in my study where mothers supplemented with calcium had larger clutches and increased telomere shortening due to higher reproductive costs.

Telomere shortening has been linked to reproductive investment through exposure to oxidative stress (von Zglinicki 2002, Epel et al. 2004, Wiersma et al. 2004, Haussmann and Marchetto 2010). Because reproduction entails high cellular replication, higher investments in reproduction are expected to increase an individual's exposure to oxidative damage through

increased reactive oxygen species or decreased investment into antioxidant defenses, both of which lead to relatively shorter telomeres (Selman et al. 2012, Blount et al. 2016).

Along with calcium supplementation, age class was also an important factor in predicting telomere shortening during the breeding season. Older birds had more telomere shortening (mean=0.03±0.05 SE) compared to year-old birds (mean=-0.05±0.04 SE). Ouyang et al. (2016) also found that Tree Swallow reproductive output increased with age, and that this higher reproductive output coincided with shorter telomeres. My results support the terminal investment hypothesis, as older birds are investing relatively more into reproduction (an average of 4.5 chicks per nest) compared to younger birds (an average of 4.15 chicks per nest), perhaps due to the fewer chances they have to reproduce in the future (Clutton-Brock 1984, Stearns 1992). In the context of life history theory, younger birds may lay smaller clutches and exert less energy in order to keep their probability of future survival and fecundity high (Klomp 1970, Godfray 1991).

The difference in telomere shortening between age groups may also be due to differences in telomere restoration. Telomerase is a ribonucleoprotein capable of elongating telomeres, though the enzyme's activity varies with maximum lifespan in most animals (Haussmann et al. 2004). Bird species with long maximum lifespans tend to show telomerase activity throughout life, though short-lived species show a decline in telomerase activity with age, the biggest decline occurring shortly after early development (Haussmann et al. 2007). Tree Swallows sharply down-regulate telomerase activity before adulthood, though telomerase activity in bone marrow of Tree Swallows is significantly lower in older birds compared to hatchlings or one-year-old birds (Haussmann et al. 2007). Therefore, the suppression of telomerase in older Tree Swallows may also account for more telomere shortening.

Calcium supplementation and telomere length of nestling Tree Swallows

The cost of reproduction in birds can manifest in terms of parental survival, future reproduction, and/or offspring survival. The cost of reproduction may be observed as an effect on offspring (i.e., early-life telomere length), especially when parental effort is increased (Linden and Moller 1989, Martin 2004, Pettifor 1993, Santos and Nakagawa 2012, Knowles et al. 2010).

Calcium supplementation and pre-breeding RTL of the mother were the most important factors in determining offspring telomere length in my study. Even though mothers in experimentally supplemented nests showed increased reproductive output at the cost of faster rates of telomere shortening, their offspring had longer average RTL compared to control chicks. RTL of chicks in supplemented nests at 12 days old (mean=3.23±0.15 SE) was longer compared to that of control nests (mean=2.25±0.17 SE).

It may be that calcium supplemented mothers are bearing the extra cost associated with increased reproductive investment, leaving their offspring to reap the developmental benefits of increased calcium. In other calcium supplementation studies that did not measure telomere length, offspring benefited from excess calcium, even when their parents experienced increased reproductive success. Dawson and Bidwell (2005) found that supplementing calcium to breeding Tree Swallows not only led to larger clutch sizes, but also had a positive effect on offspring growth rate. Similarly, Tilgar et al. (2002) found that supplementing nesting Great Tits with calcium also led to larger clutch and brood sizes, while simultaneously increasing the average tarsus length of offspring. Previous supplementation studies have credited increases in the number of offspring and offspring growth to an overall *reduction* in cost of reproduction. Here however, I provide evidence to the contrary; that is, calcium supplementation *increased* the cost

and/or stress of reproduction, resulting in increased telomere shortening in supplemented mothers.

In a study directly manipulating brood size, nestlings and parents in enlarged nests had shorter telomere lengths and more telomere shortening compared to control and reduced nests (Costanzo et al. 2017), pointing to calcium as the reason for increased offspring growth and survival, even with increased nestling competition. In my study, supplemented mothers suffered the adverse effects of increased productivity on their own survival, although with a concurrent increase in the potential longevity of their offspring. At only 12 days-old, offspring in calcium supplemented nests had longer telomeres, and thus the expectation of longer lifespans, compared to offspring in control nests.

The RTL of chicks was also dependent on pre-breeding telomere length of the mothers. In birds, studies of telomere length inheritance have found stronger correlations between mothers and offspring than between fathers and offspring (Horn et al. 2011, Asghar et al. 2014, Reichert et al. 2015), such that maternal effects might provide better explanations for offspring telomere length. Germline telomeres are more vulnerable to oxidative stress than somatic tissues, and so are more susceptible to telomere erosion (Metcalfe and Alonso-Alvarez 2010). Because telomere length is maternally inherited, stress exposure of the mother influences the telomere length inherited by the offspring (Metcalfe and Alonso-Alvarez 2010).

Conclusion

Many brood and clutch manipulation experiments have concluded that birds raising enlarged broods suffer the costs of reproduction via telomere shortening (Bauch et al. 2013, Reichert et al. 2014). However, most of these studies were either cross-sectional or looked at

yearly shortening of telomere length. The studies that were longitudinal either focused on long-lived species or used captive populations of short-lived species.

My study is novel in that telomere length was measured before and after breeding, allowing evaluation of calcium supplementation on telomere shortening exclusively during the breeding period. Carrying out my study on a wild, short-lived species also allowed me to better understand telomere length dynamics for a species with a fast pace of life, under natural conditions. In addition, no other study has used calcium supplementation as a means of testing the link between reproductive investment and telomere length. While previous calcium supplementation studies concluded that excess calcium increases reproductive output and simultaneously reduced the cost of reproduction, I measured that cost and found that supplemental calcium increased reproductive output at the expense of lower expected survival as assessed by greater telomere loss in the mothers.

My results also provide further support for using telomere length dynamics to elucidate constraints on life history trade-offs. Future work should continue to use telomeres to better understand classic life history trade-offs in various species and under differing environmental conditions given how telomere shortening is highly correlated with stress and lifespan and can be used as a proxy for costs associated with different life history traits.

Table 2.1 Model set and rankings for the top five models of each group exploring the importance of calcium supplementation ('Calcium'), 'Age,' and 'Year' on mean clutch size per nest, mean egg volume per nest, and mean hatching success per nest in Tree Swallows at a high-elevation valley in northern Colorado during 2017-2018. The number of parameters (K), -2 log-likelihood (-2LL), and model weights (w_i) are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$).

	Model	**K**	**ΔAICc$_i$**	**-2LL**	**w_i**
Brood Size	Calcium* Age + Box	6	0.00	-45.26	0.58
	Calcium + Age + Box	5	0.67	-46.99	0.42
	Calcium* Year + Box	6	11.93	-51.23	0.00
	Calcium + Box	4	21.71	-58.81	0.00
	Calcium + Year + Box	5	22.56	-57.90	0.00
Egg Volume	Calcium* Year + Box	6	0.00	-228.26	0.99
	Age + Year + Box	5	10.62	-234.96	0.00
	Calcium + Year + Box	5	10.83	-235.07	0.00
	Calcium* Age + Box	6	14.27	-235.40	0.00
	Year + Box	4	17.06	-239.49	0.00
Hatching Success	Calcium* Year + Box	6	0.00	1.78	0.34
	Calcium + Box	4	0.45	-1.15	0.27
	Age + Box	4	1.21	-1.53	0.19
	Calcium + Year + Box	5	2.66	-0.94	0.09
	Year + Box	4	3.90	-2.87	0.05

Table 2.2 Model set and rankings for the top ten models exploring the importance of calcium supplementation ('Calcium'), 'Age,' 'Year,' and brood size ('Brood') on telomere shorteningsp in breeding female Tree Swallows nesting in northern Colorado during 2017-2018. The number of parameters (K), -2 log-likelihood (-2LL), and model weights (w_i) are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$).

Model	K	$\Delta AICc_i$	-2LL	w_i
Calcium + Box	5	0.00	13.97	0.35
Age + Box	5	1.44	13.25	0.31
Year + Box	5	2.33	12.81	0.23
Brood + Box	5	3.09	12.45	0.06
Treatment + Age + Box	6	7.89	11.35	0.02
Treatment + Year + Box	6	8.77	10.91	0.02
Age + Brood + Box	6	9.14	10.72	0.01
Treatment + Brood + Box	6	9.49	10.57	0.00
Age + Year + Box	6	10.10	10.24	0.00
Treatment* Year + Box	7	13.70	9.83	0.00

Table 2.3 Model set and rankings for groups of models exploring the importance of calcium supplementation on telomere length in nestling Tree Swallows.

Model	K	Δ_i	-2LL	w_i
Treatment + Mother RTL + Box	5	0.00	-48.47	0.70
Treatment*Mother RTL + Box	6	3.35	-48.43	0.13
Treatment + Mother RTL + Age + Box	6	4.08	-48.80	0.09
Treatment + Year + Box	5	4.54	-50.34	0.07
Mother TL + Box	4	11.66	-55.15	0.00
Mother TL + Age + Box	5	15.18	-55.66	0.00
Mother TL*Age + Box	6	16.22	-54.87	0.00
Treatment + Box	4	16.56	-57.60	0.00
Treatment + Age + Box	5	19.45	-57.80	0.00
Treatment*Age + Box	6	19.61	-56.56	0.00

Figure 2.1. Adult Tree Swallow entering experimental nest box. The white tray attached to the roof of the nest box contained either crushed oyster shell (the calcium supplement) or soil (control).

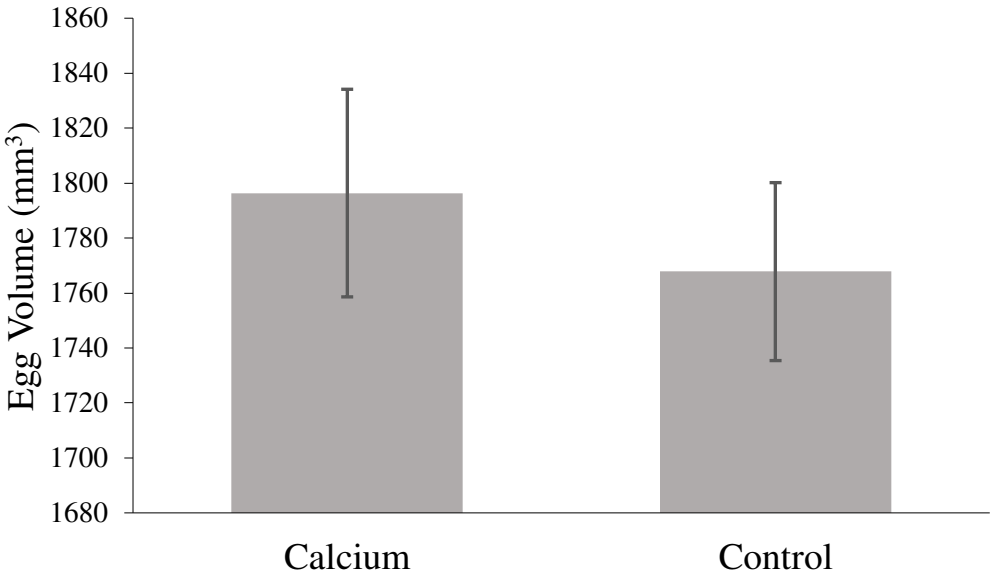

Figure 2.2 Mean (±SE) egg volume of calcium supplemented (n=22) versus control nests (n=26) for Tree Swallows nesting in Northern Colorado.

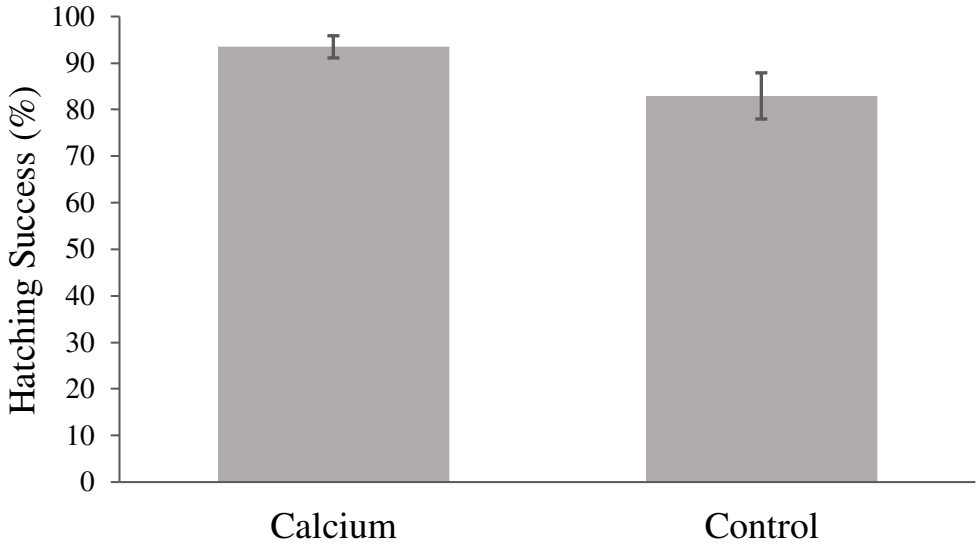

Figure 2.3 Mean (±SE) hatching success of calcium supplemented (n=22) versus control nests (n=26) for Tree Swallows nesting in Northern Colorado.

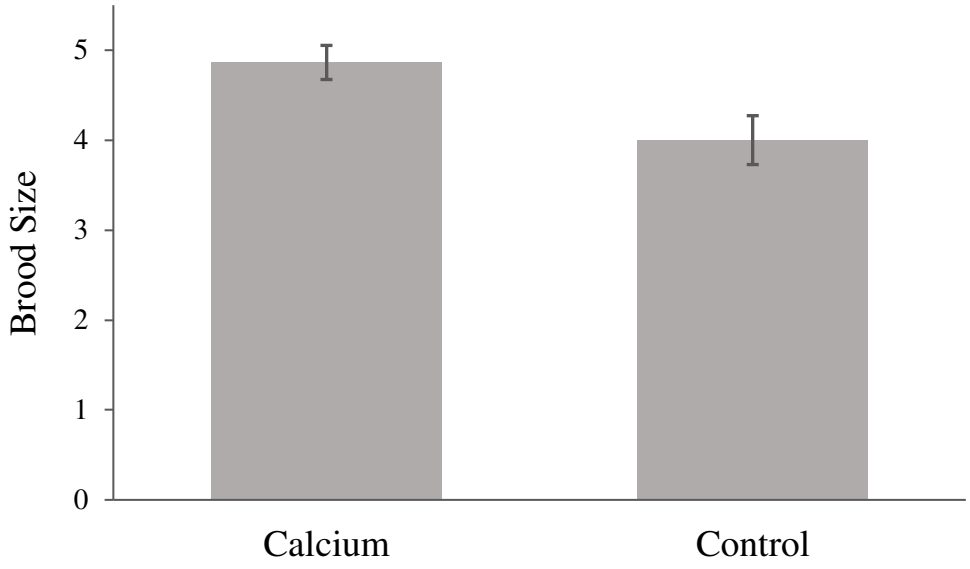

Figure 2.4 Mean (±SE) brood size per nest of calcium (n=22) versus control nests (n=26) nests for Tree Swallows nesting in Northern Colorado.

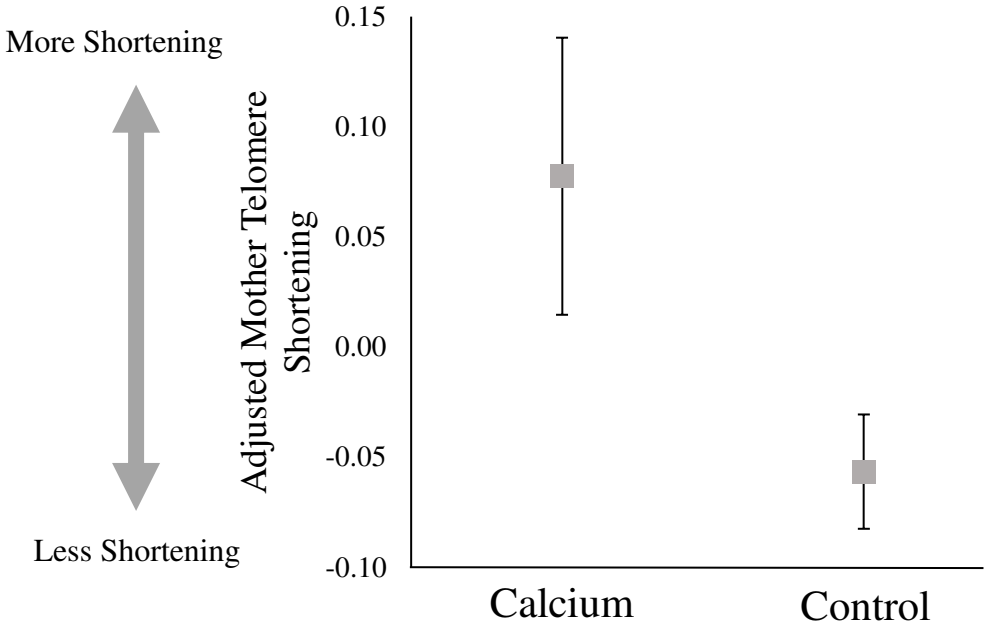

Figure 2.5 Mother Tree Swallow telomere shortening adjusted for the regression to the mean for calcium (n=22) versus control nests (n=26) when calcium was experimentally supplemented in 2017 and 2018 in northern Colorado, . Adjustment for regression to the mean scales the mean of the data to zero.

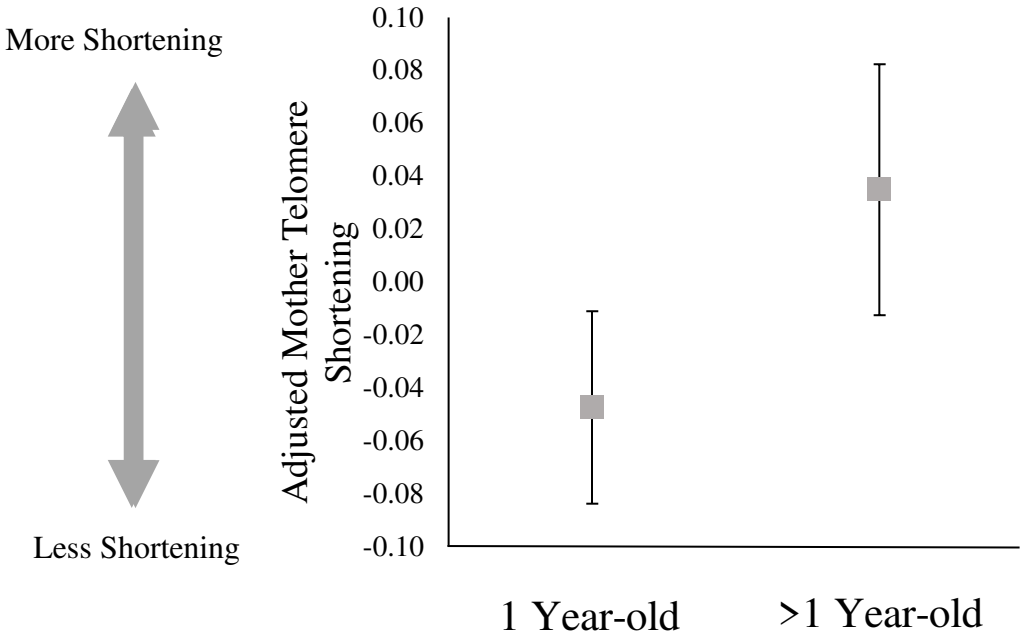

Figure 2.6 Mother Tree Swallow telomere shortening adjusted for the regression to the mean for one-year-old (n=29) and older than one-year-old birds (n=19). Adjustment for regression to the mean scales the mean of the data to zero.

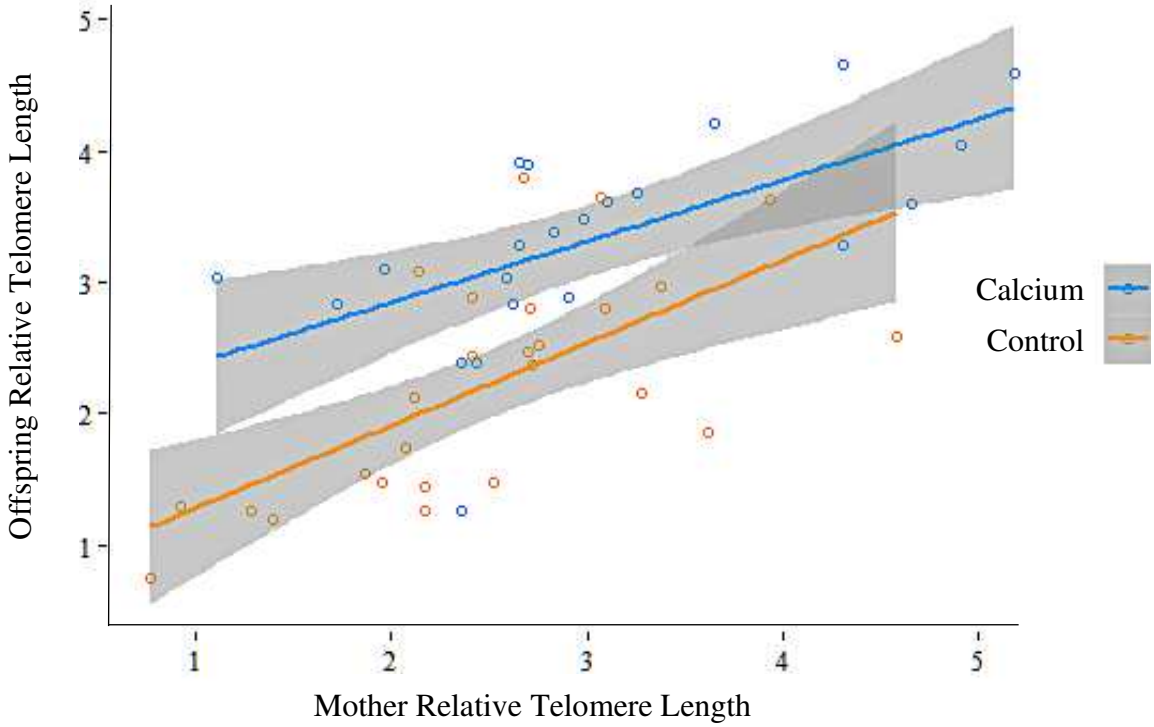

Figure 2.7 Relative telomere length of nestling Tree Swallows at 12 days old between calcium (n=22) and control nests (n=26) in relation to relative telomere length of mothers during pre-breeding.

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CHAPTER 3: SEX AND NEST TYPE INFLUENCE AVIAN BLOOD PARASITE PREVALENCE IN A HIGH ELEVATION BIRD COMMUNITY

Synopsis

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 avian blood parasite distributions, baseline knowledge and continued monitoring of the prevalence and diversity of these parasites is needed. Using an occupancy modeling framework, I 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. I estimated prevalence and diversity of avian haemosporidia across 24 bird species, detecting 39 parasite lineages. I found that open cup nesters have higher *Haemoproteus* prevalence than cavity or ground nesters. Additionally, I found that male Ruby-crowned Kinglets, White-crowned Sparrows, and Wilson's Warblers have higher *Haemoproteus* prevalence compared to other host species, and that Red-breasted Nuthatches and Ruby-crowned Kinglets with a high body condition index also have higher *Haemoproteus* prevalence compared to individuals with a lower condition index. My study presents baseline knowledge of haemosporidian parasite presence, prevalence, and diversity across avian species in the Colorado Rocky Mountains and adds to our knowledge of host-parasite relationships of blood parasites and their avian hosts.

Introduction

Parasitism is an important driver of ecological and evolutionary processes (Tompkins and Begon 1999, Schmid Hempel 2011) as parasites may regulate host population size (Hudson et al. 2006), affect species interactions (Ricklefs 2010), and create selection pressures in wild populations (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 – haemosporidia – are distributed worldwide and infect a number of vertebrates, including mammals (Witsenburg et al. 2012), reptiles, 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). My study took place during the breeding season, allowing me 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 my study site is located. Many parasites have elevation limits because of the constraints of lower temperatures

at higher elevations, though distributions are expanding with climate change as transmission of most vector-borne parasites may be enhanced by higher ambient temperatures (Lindsay and Birley 1996). 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 blood parasites at lower elevations in northeast Colorado, but the absence of these species at higher elevations is likely the reason avian blood parasites have not yet established in areas such as Rocky Mountain National Park (Eisen et al. 2008). Little research has been done on the distribution of haemosporidian parasites in Colorado, especially in high elevation communities.

Across species, factors such as nest type and migration strategy can explain variations 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 Hoye 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 (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.

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 current levels of haemosporidian parasite infection in host populations to aid in temporal studies of parasite diversity and to evaluate changes in prevalence of infection. Using an occupancy approach, I 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 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 my 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 between various individual and species characteristics. My hypotheses related to my second objective regarding specific host and environmental predictor variables are presented in Table 3.1.

Methods

Study system

My 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 my knowledge.

Data collection

I collected data during the summers of 2017 and 2018. The field portion of my study began in early June when birds begin breeding and continued through the end of the breeding season, around mid-August. I 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 µl 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

Haemosporidian parasite infection prevalence and parasite diversity were assessed using molecular techniques. A 2 mm hole punch of blood-soaked strip with approximately 15 µL of blood was taken from a Nobuto Blood Filter Strip for each sample and DNA was extracted using the Qiaquick DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA), following the

manufacturer's dried blood spot protocol. Extracted DNA was stored at -20 °C prior to screening. An aliquot of each extract was screened for parasite presence using a nested polymerase chain reaction (PCR) protocol outlined in Hellgren et al. (2004) to amplify a segment of mitochondrial DNA (mtDNA) from the cytochrome *b* gene. 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 50°C, and 45 seconds at 72°C for 20 cycles. The samples were then 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 µL) 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 µl using illustra PureTaq Ready-To-Go™ beads (GE Healthcare) with freeze-dried, pre-formulated reagents. We ran 5 µl 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 µl contained 0.25 µl BigDye™ (Thermo Fisher Scientific), 2.275 µl sequencing buffer, 1 µl of each nested secondary PCR primer (HaemF and HaemR2), 1 µl of PCR product, and 5.475 µl molecular grade ddH2O. Cycle sequencing was conducted at 94 ^{o}C for 2 min; 40 cycles of amplification at 85 ^{o}C for 10 s; 53 ^{o}C for 10 s and 60 ^{o}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 DNA sequencer. 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

I 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, the probability of parasite detection was estimated along with the proportion of individuals infected with blood parasites, corrected for detection probability. I 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 (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 my 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.

I carried out analyses for *Haemoproteus* and *Plasmodium* parasite prevalence separately. I 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, I used the random effects model in Program MARK to incorporate heterogeneity beyond my predictions in each model. My model set consisted of all possible combinations of predictor variables (Table 3.1) and I used an information-theoretic approach for model ranking and selection (Burnham and Anderson 2002). I 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, I captured 232 birds, and in 2018, I captured 206 birds. Of the 438 birds captured, 180 were males, 206 were females, and for the remainder sex could not be determined. I captured 24 hatch-year birds, 135 second-year birds, 241 after second-year birds, and I could not determine age in the remaining birds. Body condition indices (mass:tarsus) ranged from 0.20 to 25.71 g/mm. I collected molecular data from a total of 437 birds belonging to 24 species over the two years of the study (Table 3.2).

Haemosporidian Parasite Diversity

In total, I detected 10 *Plasmodium* and 29 *Haemoproteus* cytochrome b lineages. Thirty-three lineages had a 100% match to current lineages deposited in the Malavi database (Table 3.2), and the other 6 lineages were considered new lineages. 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 lincolnii*). The greatest number of lineages 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

I detected *Haemoproteus* parasites in at least one PCR run in 109 birds out of the 437 in the study, a naïve *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 3.3). 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, I 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 *Haemoproteu*s 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 3.4). 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 3.4). 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

I detected *Plasmodium* parasites in at least one PCR run in 23 out of 437 birds, which is a total naïve *Plasmodium* prevalence of 5.3%. When analyzing all species together, I detected no heterogeneity using a random effects model. I found no predictor variable with a cumulative variable AICc weight of at least 0.5, and therefore none of my 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 found to be 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 Colorado are little studied. Within the MalAvi database, only one study (Marzal et al. 2011) reported sampling birds in Colorado. Because of this limited amount of data, the factors influencing avian haemosporidian parasite infection in the Colorado Rocky Mountains are 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, I 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 haemosporidian parasite lineages 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*. Using an occupancy-modelling framework to account for imperfect detection of avian blood parasites, I found that nest type is an important species-level factor influencing *Haemoproteus* parasitism at my study site, with open cup nesters having a higher prevalence compared to cavity and ground nesters. I 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

Genetic 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 3.2). *Plasmodium* lineages are considered as generalists, while *Haemoproteus* lineages are generally considered to be host specific (Hellgren et al. 2009). However, I identified *Haemoproteus* in a wider range of bird species than *Plasmodium*, and also found that *Haemoproteus* parasites were more prevalent overall.

The small sample sizes obtained for many host species limit me from determining host specificity of the obtained lineages; however, some patterns were still apparent and most of these centered on the family Turdidae. The 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), however, most detections have occurred in the Thrush family. Accordingly, American Robins at my study site had the highest proportion of TURDUS2 detections compared to other species. As this lineage was found in numerous species at my study site, American Robins may be acting as a reservoir for this 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 my study, individuals from both of these species were found to be positive for the TUMIG07 lineage, along with six other species. 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 my 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 my 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 my 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 exposed to vectors.

Overall *Haemoproteus* prevalence also displayed marked variation between years in my study, with 2018 having a higher overall prevalence compared to 2017 (Figure 3.1). Annual variation in avian blood parasite prevalence is common and has been found in many studies (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 if such patterns 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 found to be positive for the parasite. Low abundance of *Plasmodium* parasites and vectors 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 my 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 my prediction, my 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 susceptibility of each sex to infections (Loye and Zuk 1991). For example, testosterone has immunosuppressive effects in many species, leading to increased 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 my failure to find an association between parasite prevalence and sex in the other seven species that I 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 coronate*), and the Great Tit (*Parus majo*r; 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 greater body condition. The eight other species analyzed in my 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).

I found no relationship between *Haemoproteus* prevalence and individual-level traits (sex, age, BCI) in the American Robin, Mountain Chickadee (*Poecile gambeli*), Pine Siskin (*Spinus pinus*), or Dark-eyed Junco (*Junco hyemalis*), contrary to my hypotheses. My results suggest that the individual-level traits examined in this study may not be important predictors of *Haemoproteus* infection across species. However, I 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 I 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). *Plasmodium* analyses based on blood samples have been known to underestimate prevalence because *Plasmodium* enters latent, exoerythrocytic phases during chronic infection

and may even be absent in the blood stream (Valkiunas 2004) such that sampling peripheral blood may not allow for detection of all true infections.

Conclusion

My 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. Prevalence of avian haemosporidian parasites and the factors influencing infection in the Colorado Rocky Mountains are largely unknown, though my 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. My study is the only avian blood parasite survey conducted in the Colorado Rocky Mountains, though additional research in this area examining host-parasite relationships would help to determine whether potential changes in vector communities or parasite distributions may pose a threat to resident avian populations.

Table 3.1 Positive (+), negative (-), or quadratic (Q) predicted associations, and explanation, of each predictor variable with haemosporidian parasite prevalence and detection probability. BCI is body condition index defined as body mass:tarsus length. PCR is polymerase chain reaction.

Parameter(s)	Predictor variable	Predicted direction	Explanation
Occupancy probability (ψ)	Age	Q	For adult birds, prevalence of blood parasites decreases with age due to increased immunocompetence, until old age in which immunosenescence occurs and is linked to a higher probability of infection (Hammers et al. 2016).
	Sex (female)	+	The cost of reproduction is higher in females, decreasing immunocompetence and making females more susceptible to haemosporidian infection (Bichet et al. 2014).
	BCI (mass (g): tarsus length (mm))	-	Individuals in good condition have stronger immunocompetence and are better able to fight infections (Gonzalez et al. 1999, Roulin 2015).
	Migration status	+	The energetic cost of migration increases with distance, and likely affects immunocompetence (Owen and Moore 2008).
	Nest type (open nest)	+	Open nests are more exposed to mosquito vectors compared to closed nests (Beveroth et al. 2006).
Detection probability (*p*)	PCR run	+	Differences in results of PCR runs may occur due to variation in parasitemia between samples, with higher parasitemia associated with higher probability of detection (Hellgren et al. 2004)

Table 3.2 Host bird species sampled, number of individuals sampled in each host species, raw number of *Plasmodium* and *Haemoproteus* detections for each host species, and the haemosporidian lineages detected in each host species.

Bird species	# Sampled individuals	# *Plasmodium* infections detected	# *Haemoproteus* infections detected	Molecular lineages
American Robin *(Turdus migratorius)*	28	2	10	H_JUHYE03, H_TURDUS2, H_PHYBOR04, H_VIGIL07, H_TUMIG07, H_TUMIG08, H_POEATR01, H_DENADE01, P_LAIRI01
American Three-toed Woodpecker *(Picoides dorsalis)*	1	0	0	
Brown Creeper *(Certhia americana)*	7	0	1	H_SISKIN1
Chipping Sparrow *(Spizella passerina)*	2	0	1	H_VIGIL07
Cordilleran Flycatcher *(Empidonax occidentalis)*	20	1	1	H_PHYBOR04, P_PADOM11
Dark-eyed Junco *(Junco hyemalis)*	31	1	6	H_VIOLI05, H_SISKIN1, H_TURDUS2, P_WW3
Golden-crowned Kinglet *(Regulus satrapa)*	2	1	1	H_MELCAR01, P_LAIRI01
Green-tailed Towhee *(Pipilo chlorurus)*	1	0	0	
Hairy Woodpecker *(Dryobates villosus)*	3	0	0	
Hermit Thrush *(Catharus guttatus)*	5	0	2	H_JUHYE03, H_TUMIG07
House Wren *(Troglodytes aedon)*	6	0	1	H_PHYBOR04, H_GYMSAL01
Lincoln's Sparrow *(Melospiza lincolnii)*	63	4	13	H_GYMSAL01, H_TURDUS2, H_VIGIL07, H_MELCAR01, H_TUMIG08, H_PHYBOR04, H_DENADE01, H_TUMIG06, P_CATUST06, P_PADOM11, P_WW3, P_BAEBICO2
MacGillvray's Warbler *(Geothlypis tolmiei)*	6	0	1	H_PHYBOR01

Mountain Chickadee *(Poecile gambeli)*	22	1	4	H_SISKIN1, H_TURDUS2, H_PHYBOR01, P_WW3
Northern Flicker *(Colaptes auratus)*	5	0	2	H_GYMSAL01, H_ZOCAP08, H_SISKIN1, H_MELCAR01
Pine Siskin *(Spinus pinus)*	45	1	8	H_TABI10, H_JUHYE03, H_SISKIN1, H_PASILI01, H_TURDUS2, H_MELICAR01, P_PADOM11
Red-breasted Nuthatch *(Sitta canadensis)*	24	0	3	H_SISKIN1_Haemoproteus_tartakovskyi, H_VIGIL07
Red-naped Sapsucker *(Sphyrapicus nuchalis)*	6	0	1	H_TUMIG07
Ruby-crowned Kinglet *(Regulus calendula)*	23	0	5	H_SISKIN1, H_ZOCAP08, H_TURDUS2, H_TUMIG06, H_TUMIG07, H_VIGIL07
Tree Swallow *(Tachycineta bicolor)*	12	0	2	H_GYMSAL01, H_TUMIG07
Warbling Vireo *(Vireo gilvus)*	22	3	14	H_TUMIG07, H_TUPHI01, H_PHYBOR04, H_TUMIG08, H_SETAUD08, H_TABIO2, H_VIGIL07, H_VIGIL01, H_PERCAN07, H_MELCAR01, P_PADOM11, P_BAEBICO2
White-crowned Sparrow *(Zonotrichia eucophrys)*	47	2	13	H_SISKIN1, H_TUMIG07, CATUST16, H_PHYBOR04, H_TUMIG06, H_TABIO2, H_MELCAR01, H_TURDUS2, H_VIGIL01, H_DENEDE01, P_MELMEL02, P_GEOTRI09
Wilson's Warbler *(Cardellina pusilla)*	51	5	15	H_TURDUS2, H_PHYBOR04, H_SISKIN1, H_MELCAR01, H_TUMIG07, H_VIGIL07, H_GRBRU02, H_VIGIL01, H_DENADE01, H_LAIRI01, P_CATUST06, P_GEOTRI09, P_GASAN01
Yellow-rumped warbler *(Setophaga coronata)*	11	2	4	H_TABI10, H_VIGIL07, P_BAEBICO2

Table 3.3 Cumulative AICc variable weights for each variable considered in the all-species analysis for *Haemoproteus* prevalence. Those variables with a cumulative weight greater than 0.5 (shaded gray) are considered important. ψ variables are those considered when evaluating prevalence. The p variables are those considered when evaluating detection probability. 'PCR run' indicates the 3 PCR replicates carried out for each sample. 'Nest' indicates nest type (open, ground, or cavity). BCI is body condition index defined as the ration of body mass (g) to tarsus length (mm) for each individual.

Variable	Variable Weight
ψ(sex)	0.21
ψ(age)	0.05
ψ(nest)	0.54
ψ(year)	0.85
ψ(BCI)	0.27
p(PCR run)	0.99

Table 3.4 Cumulative variable AICc weights for each variable considered in each species-specific analysis for *Haemoproteus* prevalence. Variables with a cumulative variable AICc weight greater than 0.5 (shaded gray) are considered important. Cells with a dash indicate that models would not converge with the inclusion of that variable. ψ variables are those considered when evaluating prevalence. 'p' variables are those considered when evaluating detection probability. 'PCR run' indicates the 3 PCR replicates carried out for each sample. 'Nest' indicates nest type (open, ground, or cavity). BCI is body condition index defined as the ratio of body mass (g) to tarsus length (mm) for each individual.

Species	**Cumulative Variable Weights**				
	ψ(sex)	**ψ(age)**	**ψ(year)**	**ψ(BCI)**	**p(PCR replicate)**
American Robin	0.33	0.09	0.26	0.32	0.14
Lincoln Sparrow	0.29	0.25	0.27	0.26	0.96
Mountain Chickadee	----	----	0.22	0.23	0.06
Pine Siskin	0.27	0.25	0.34	0.39	0.24
Red-breasted Nuthatch	0.07	----	----	0.69	----
Ruby-crowned Kinglet	0.57	----	----	0.84	----
Dark-eyed Junco	0.35	0.05	0.03	0.21	0.30
Warbling Vireo	0.33	0.04	----	0.38	0.30
White-crowned Sparrow	0.54	0.10	0.40	0.35	0.50
Wilson's Warbler	0.51	0.07	0.42	0.25	0.08

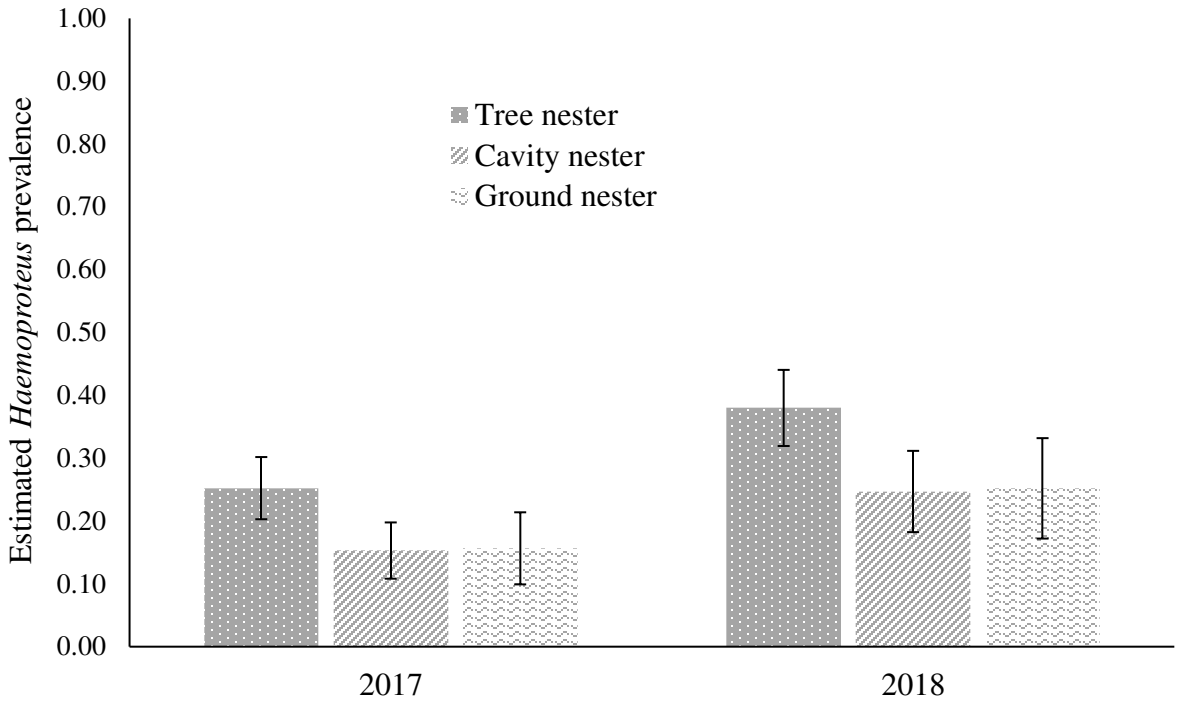

Figure 3.1 Estimated *Haemoproteus* prevalence (± SE) for each nest type (tree, cavity, and ground) between each study year in a bird community in the Colorado Rocky Mountains.

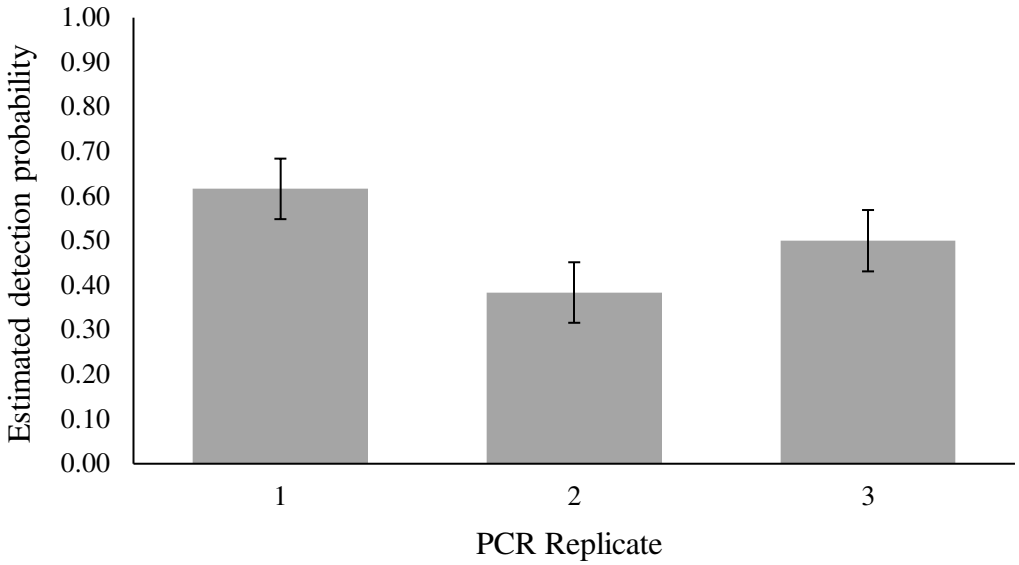

Figure 3.2 Estimated detection probability ($p \pm$ SE) of avian *Haemoproteus* for each PCR replicate in the all-species analysis.

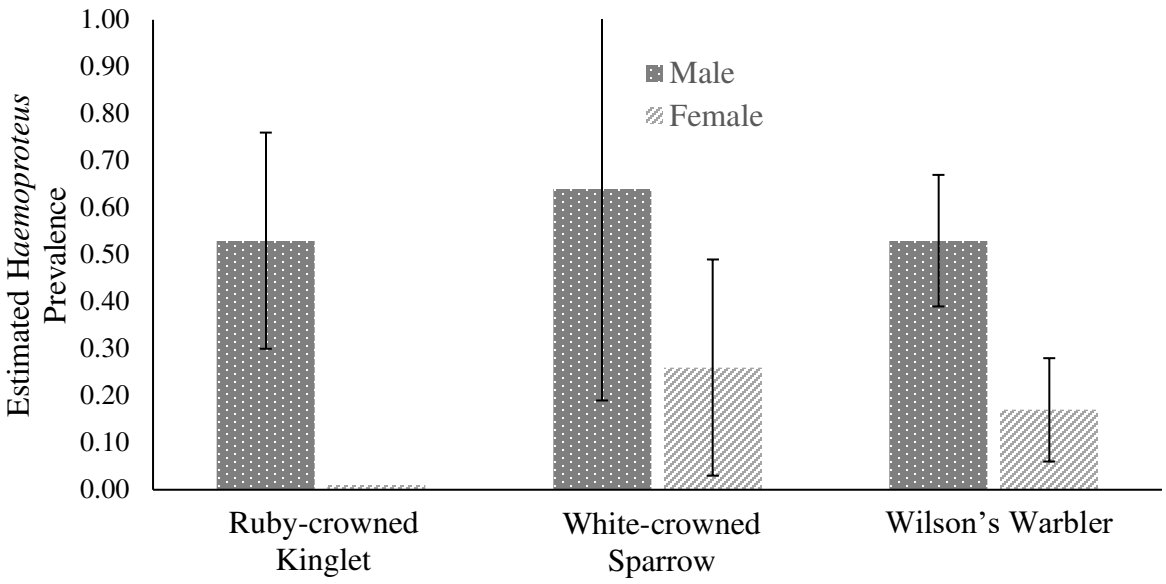

Figure 3.3 Estimated *Haemoproteus* prevalence (± SE) for male and female Ruby-crowned Kinglet, White-crowned Sparrow, and Wilson's Warbler. Note that no female Ruby-crowned Kinglets were positive in this study.

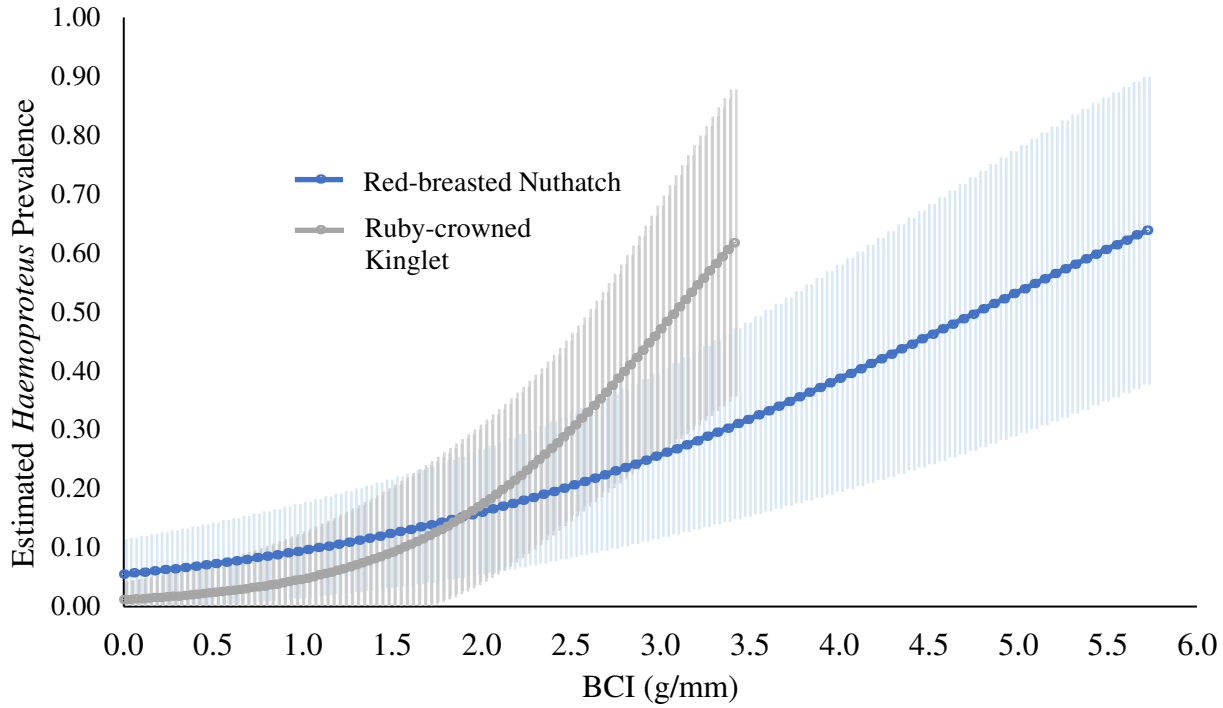

Figure 3.4 Estimated *Haemoproteus* prevalence (± SE) and body condition index for the Red-breasted Nuthatch and the Ruby-crowned Kinglet. BCI is a ratio of body mass (g) to tarsus length (mm) for each individual.

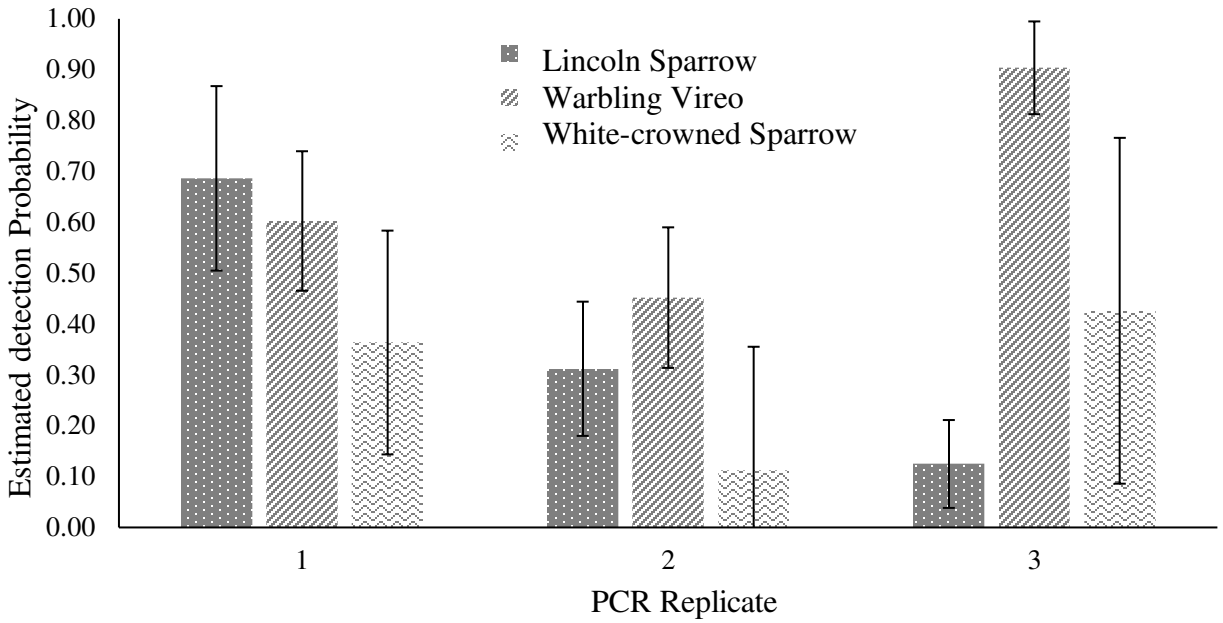

Figure 3.5 Estimated detection probability ($p \pm$ SE) of *Haemoproteus* for each PCR replicate for the Lincoln's Sparrow, Warbling Vireo, and White-crowned Sparrow.

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Appendix 3.1: Model Sets and rankings for the all-species and each individual species analysis exploring the importance of factors affecting the detection probability (p) and prevalence (Ψ) of Haemoproteus blood parasites at a high-elevation valley in northern Colorado during 2017-2018.

Table A3.1.1 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites across all host species captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. 'Nest' indicates nest type (open, ground, or cavity). BCI is body condition index defined as the ration of body mass (g) to tarsus length (mm) for each individual. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(PCR run) + ψ (nest+year)	7	0.00	0.30	891.71
σ(.) + p(PCR run) + ψ (year)	6	1.58	0.13	895.35
σ(.) + p(PCR run) + ψ (year+migration)	7	3.41	0.05	895.12
σ(.) + p(PCR run) + ψ (sex+year)	7	3.63	0.05	895.34
σ(.) + p(PCR run) + ψ (year+BCI)	7	3.64	0.05	895.35
σ(.) + p(PCR run) + ψ (sex+nest+year)	9	4.16	0.04	891.70
σ(.) + p(PCR run) + ψ (nest+year+BCI)	9	4.16	0.04	891.71
σ(.) + p(PCR run) + ψ (nest+year+migration)	9	4.16	0.04	891.71
σ(.) + p(PCR run) + ψ(BCI)	6	5.07	0.02	898.84
σ(.) + p(PCR run) + ψ(nest+BCI)	8	5.30	0.02	894.94
σ(.) + p(PCR run) + ψ(sex+year+migration)	8	5.49	0.02	895.12
σ(.) + p(PCR run) + ψ(year+BCI+migration)	8	5.49	0.02	895.12
σ(.) + p(PCR run) + ψ(sex+year+BCI)	8	5.70	0.02	895.33
σ(.) + p(PCR run) + ψ(.)	5	6.15	0.01	901.98
σ(.) + p(PCR run) + ψ(sex+nest+year+BCI)	10	6.25	0.01	891.70
σ(.) + p(PCR run) + ψ(sex+nest+year+migration)	10	6.25	0.01	891.70
σ(.) + p(PCR run) + ψ(nest+year+BCI+migration)	10	6.25	0.01	891.71
σ(.) + p(PCR run) + ψ(BCI+migration)	7	6.30	0.01	898.01
σ(.) + p(PCR run) + ψ(nest)	7	6.34	0.01	898.05

σ(.) + p(PCR run) + ψ(sex+BCI)	7	7.01	0.01	898.72
σ(.) + p(PCR run) + ψ(age+year)	9	7.04	0.01	894.59
σ(.) + p(PCR run) + ψ(nest+BCI+migration)	9	7.18	0.01	894.73
σ(.) + p(PCR run) + ψ(sex+nest+BCI)	9	7.36	0.01	894.91
σ(.) + p(PCR run) + ψ(age+nest+year)	11	7.43	0.01	890.78
σ(.) + p(PCR run) + ψ(sex+year+BCI+migration)	9	7.57	0.01	895.12
σ(.) + p(PCR run) + ψ(migration)	6	7.64	0.01	901.42
σ(.) + p(PCR run) + ψ(sex)	6	8.03	0.01	901.80
σ(.) + p(PCR run) + ψ(sex+BCI+migration)	8	8.33	0.00	897.97
σ(.) + p(PCR run) + ψ(nest+migration)	8	8.35	0.00	897.98
σ(.) + p(PCR run) + ψ(sex+nest)	8	8.35	0.00	897.98
σ(.) + p(PCR run) + ψ(sex+nest+year+BCI+migration)	11	8.35	0.00	891.70
σ(.) + p(.) + ψ(year)	3	8.45	0.00	908.37
σ(.) + p(PCR run) + ψ(age+year+migration)	10	8.95	0.00	894.40
σ(.) + p(PCR run) + ψ(sex+age+year)	10	9.13	0.00	894.58
σ(.) + p(PCR run) + ψ(age+year+BCI)	10	9.13	0.00	894.58
σ(.) + p(PCR run) + ψ(sex+nest+BCI+migration)	10	9.26	0.00	894.71
σ(.) + p(PCR run) + ψ(sex+age+nest+year)	12	9.54	0.00	890.78
σ(.) + p(PCR run) + ψ(age+nest+year+migration)	12	9.54	0.00	890.78
σ(.) + p(PCR run) + ψ(age+nest+year+BCI)	12	9.55	0.00	890.78
σ(.) + p(PCR run) + ψ(sex+migration)	7	9.61	0.00	901.32
σ(.) + p(.) + ψ(year+migration)	4	10.26	0.00	908.14
σ(.) + p(PCR run) + ψ(sex+nest+migration)	9	10.38	0.00	897.93
σ(.) + p(.) + ψ(sex+year)	4	10.47	0.00	908.35
σ(.) + p(PCR run) + ψ(age+BCI)	9	10.78	0.00	898.33
σ(.) + p(.) + ψ(nest+year)	6	10.95	0.00	904.72
σ(.) + p(PCR run) + ψ(age+nest+BCI)	11	11.01	0.00	894.35
σ(.) + p(PCR run) + ψ(sex+age+year+migration)	11	11.05	0.00	894.40
σ(.) + p(PCR run) + ψ(age+year+BCI+migration)	11	11.05	0.00	894.40

σ(.) + p(PCR run) + ψ(sex+age+year+BCI)	11	11.22	0.00	894.57
σ(.) + p(PCR run) + ψ(sex+age+nest+year+migration)	13	11.67	0.00	890.78
σ(.) + p(PCR run) + ψ(sex+age+nest+year+BCI)	13	11.67	0.00	890.78
σ(.) + p(PCR run) + ψ(age+nest+year+BCI+migration)	13	11.67	0.00	890.78
σ(.) + p(PCR run) + ψ(age)	8	11.75	0.00	901.38
σ(.) + p(PCR run) + ψ(age+nest)	10	12.00	0.00	897.46
σ(.) + p(PCR run) + ψ(age+BCI+migration)	10	12.17	0.00	897.63
σ(.) + p(.) + ψ(year+BCI)	5	12.53	0.00	908.36
σ(.) + p(PCR run) + ψ(sex+age+BCI)	10	12.76	0.00	898.21
σ(.) + p(PCR run) + ψ(age+nest+BCI+migration)	12	12.97	0.00	894.20
σ(.) + p(.) + ψ(sex+nest+year)	7	13.01	0.00	904.72
σ(.) + p(.) + ψ(nest+year+BCI)	7	13.01	0.00	904.72
σ(.) + p(.) + ψ(nest+year+migration)	7	13.01	0.00	904.72
σ(.) + p(PCR run) + ψ(sex+age+nest+BCI)	12	13.08	0.00	894.32
σ(.) + p(PCR run) + ψ(sex+age+year+BCI+migration)	12	13.16	0.00	894.40
σ(.) + p(PCR run) + ψ(age+migration)	9	13.43	0.00	900.98
σ(.) + p(PCR run) + ψ(sex+age)	9	13.65	0.00	901.20
σ(.) + p(PCR run) + ψ(sex+age+nest+year+BCI+migration)	14	13.80	0.00	890.77
σ(.) + p(.) + ψ(BCI)	4	13.98	0.00	911.86
σ(.) + p(PCR run) + ψ(sex+age+nest)	11	14.03	0.00	897.38
σ(.) + p(PCR run) + ψ(age+nest+migration)	11	14.08	0.00	897.43
σ(.) + p(.) + ψ(nest+BCI)	6	14.17	0.00	907.95
σ(.) + p(PCR run) + ψ(sex+age+BCI+migration)	11	14.23	0.00	897.58
σ(.) + p(.) + ψ(sex+year+migration)	6	14.36	0.00	908.13
σ(.) + p(.) + ψ(year+BCI+migration)	6	14.36	0.00	908.13
σ(.) + p(.) + ψ(sex+year+BCI)	6	14.57	0.00	908.35
σ(.) + p(.) + ψ(.)	3	15.08	0.00	914.99
σ(.) + p(PCR run) + ψ(sex+age+nest+BCI+migration)	13	15.08	0.00	894.19
σ(.) + p(.) +ψ(sex+nest+year+BCI)	8	15.08	0.00	904.72

σ(.) + p(.) + ψ(sex+nest+year+migration)	8	15.08	0.00	904.72
σ(.) + p(.) + ψ(nest+year+BCI+migration)	8	15.09	0.00	904.72
σ(.) + p(.) + ψ(BCI+migration)	5	15.19	0.00	911.02
σ(.) + p(.) + ψ(nest)	5	15.24	0.00	911.07
σ(.) + p(PCR run) + ψ(sex+age+migration)	10	15.42	0.00	900.87
σ(.) + p(.) + ψ(age+year)	7	15.90	0.00	907.60
σ(.) + p(.) +ψ(sex+BCI)	5	15.91	0.00	911.74
σ(.) + p(.) + ψ(nest+BCI+migration)	7	16.03	0.00	907.74
σ(.) + p(PCR run) + ψ(sex+age+nest+migration)	12	16.13	0.00	897.37
σ(.) + p(.) + ψ(sex+nest+BCI)	7	16.21	0.00	907.92
σ(.) + p(.) +ψ(age+nest+year)	9	16.25	0.00	903.80
σ(.) + p(.) +ψ(sex+year+BCI+migration)	7	16.42	0.00	908.13
σ(.) + p(.) + ψ(migration)	4	16.55	0.00	914.43
σ(.) + p(.) +ψ(sex)	4	16.94	0.00	914.82
σ(.) + p(.) +ψ(sex+nest+year+BCI+migration)	9	17.17	0.00	904.72
σ(.) + p(.) + ψ(sex+BCI+migration)	6	17.21	0.00	910.98
σ(.) + p(.) + ψ(nest+migration)	6	17.22	0.00	910.99
σ(.) + p(.) + ψ(sex+nest)	6	17.22	0.00	911.00
σ(.) + p(.) + ψ(age+year+migration)	8	17.78	0.00	907.41
σ(.) + p(.) + ψ(sex+age+year)	8	17.96	0.00	907.59
σ(.) + p(.) + ψ(age+year+BCI)	8	17.96	0.00	907.60
σ(.) + p(.) + ψ(sex+nest+BCI+migration)	8	18.09	0.00	907.73
σ(.) + p(.) + ψ(sex+age+nest+year)	10	18.34	0.00	903.79
σ(.) + p(.) + ψ(age+nest+year+migration)	10	18.34	0.00	903.79
σ(.) + p(.) + ψ(age+nest+year+BCI)	10	18.34	0.00	903.79
σ(.) + p(.) + ψ(sex+migration)	5	18.51	0.00	914.34
σ(.) + p(.) + ψ(sex+nest+migration)	7	19.23	0.00	910.94
σ(.) + p(.) + ψ(age+BCI)	7	19.64	0.00	911.35
σ(.) + p(.) + ψ(age+nest+BCI)	9	19.82	0.00	907.37

σ(.) + p(.) + ψ(sex+age+year+migration)	9	19.86	0.00	907.41
σ(.) + p(.) + ψ(age+year+BCI+migration)	9	19.87	0.00	907.41
σ(.) + p(.) + ψ(sex+age+year+BCI)	9	20.04	0.00	907.59
σ(.) + p(.) + ψ(sex+age+nest+year+migration)	11	20.44	0.00	903.79
σ(.) + p(.) + ψ(sex+age+nest+year+BCI)	11	20.44	0.00	903.79
σ(.) + p(.) + ψ(age+nest+year+BCI+migration)	11	20.44	0.00	903.79
σ(.) + p(.) + ψ(age)	6	20.62	0.00	914.40
σ(.) + p(.) + ψ(age+nest)	8	20.84	0.00	910.47
σ(.) + p(.) + ψ(age+BCI+migration)	8	21.01	0.00	910.64
σ(.) + p(.) + ψ(sex+age+BCI)	8	21.59	0.00	911.22
σ(.) + p(.) + ψ(age+nest+BCI+migration)	10	21.76	0.00	907.22
σ(.) + p(.) + ψ(sex+age+nest+BCI)	10	21.88	0.00	907.33
σ(.) + p(.) + ψ(sex+age+year+BCI+migration)	10	21.96	0.00	907.41
σ(.) + p(.) + ψ(age+migration)	7	22.28	0.00	913.99
σ(.) + p(.) + ψ(sex+age)	7	22.51	0.00	914.22
σ(.) + p(.) + ψ(sex+age+nest+year+BCI+migration)	12	22.55	0.00	903.79
σ(.) + p(.) + ψ(sex+age+nest)	9	22.85	0.00	910.40
σ(.) + p(.) + ψ(age+nest+migration)	9	22.90	0.00	910.45
σ(.) + p(.) + ψ(sex+age+BCI+migration)	9	23.04	0.00	910.59
σ(.) + p(.) + ψ(sex+age+nest+BCI+migration)	11	23.85	0.00	907.20
σ(.) + p(.) + ψ(sex+age+migration)	8	24.25	0.00	913.88
σ(.) + p(.) + ψ(sex+age+nest+migration)	10	24.93	0.00	910.38

Table A3.1.2 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in American Robins captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set (Δ**AICc**$_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(.) + ψ(.)	3	0.00	0.33	104.05
σ(.) + p(.) + ψ(sex)	4	1.66	0.15	103.23
σ(.) + p(.) + ψ(BCI)	4	1.66	0.15	103.24
σ(.) + p(.) + ψ(year)	4	2.44	0.10	104.01
σ(.) + p(.) + ψ(sex+BCI)	5	3.28	0.06	102.23
σ(.) + p(.) + ψ(year+BCI)	5	3.34	0.06	102.29
σ(.) + p(.) + ψ(sex+year)	5	4.27	0.04	103.22
σ(.) +p(PCR run) + ψ(.)	5	4.90	0.03	103.85
σ(.) + p(.) + ψ(sex+year+BCI)	6	5.61	0.02	101.79
σ(.) + p(PCR run) + ψ(sex)	6	6.86	0.01	103.04
σ(.) + p(run) + ψ(BCI)	6	6.87	0.01	103.04
σ(.) + p(.) + ψ(age)	6	6.90	0.01	103.07
σ(.) + p(PCR run) + ψ(year)	6	7.64	0.01	103.81
σ(.) + p(PCR run) + ψ(sex+BCI)	7	8.81	0.00	102.03
σ(.) + p(PCR run) + ψ(year+BCI)	7	8.88	0.00	102.10
σ(.) + p(.) + ψ(sex+age)	7	9.35	0.00	102.57
σ(.) + p(.) + ψ(age+BCI)	7	9.41	0.00	102.63
σ(.) + p(.) + ψ(age+year)	7	9.78	0.00	103.00
σ(.) + p(PCR run) + ψ(sex+year)	7	9.81	0.00	103.02
σ(.) + p(PCR run) + ψ(sex+year+BCI)	8	11.52	0.00	101.59
σ(.) + p(.) + ψ(age+year+BCI)	8	11.67	0.00	101.74
σ(.) + p(.) + ψ(sex+age+BCI)	8	11.86	0.00	101.94
σ(.) + p(.) + ψ(sex+age+year)	8	12.50	0.00	102.57

σ(.) + p(PCR run) + ψ(age)	8	12.80	0.00	102.88
σ(.) + p(.) + ψ(sex+age+year+BCI)	9	14.75	0.00	101.47
σ(.) + p(PCR run) + ψ(sex+age)	9	15.66	0.00	102.37
σ(.) + p(PCR run) + ψ(age+BCI)	9	15.72	0.00	102.43
σ(.) + p(PCR run) + ψ(age+year)	9	16.08	0.00	102.80
σ(.) + p(PCR run) + ψ(age+year+BCI)	10	18.41	0.00	101.54
σ(.) + p(PCR run) + ψ(sex+age+BCI)	10	18.61	0.00	101.74
σ(.) + p(PCR run) + ψ(sex+age+year)	10	19.24	0.00	102.37
σ(.) + p(PCR run) + ψ(sex+age+year+BCI)	11	21.99	0.00	101.27

Table A3.1.3 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in Lincoln's Sparrows captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(PCR run) + ψ(.)	4	0.00	0.27	102.29
σ(.) + p(PCR run) + ψ(sex)	5	1.56	0.12	101.51
σ(.) + p(PCR run) + ψ(year)	5	2.04	0.10	101.99
σ(.) + p(PCR run) + ψ(BCI)	5	2.10	0.10	102.06
σ(.) + p(PCR run) + ψ(age)	7	2.29	0.09	97.33
σ(.) + p(PCR run)+ψ(age+year)	8	3.65	0.04	96.10
σ(.) + p(PCR run) + ψ(sex+year)	6	3.67	0.04	101.21
σ(.) + p(PCR run) + ψ(age+BCI)	8	3.68	0.04	96.14
σ(.) + p(PCR run) + ψ(sex+BCI)	6	3.72	0.04	101.26
σ(.) + p(PCR run) + ψ(year+BCI)	6	4.45	0.03	101.98
σ(.) + p(PCR run) + ψ(sex+age)	8	4.61	0.03	97.07
σ(.) + p(PCR run) + ψ(sex+age+year)	9	6.11	0.01	95.89
σ(.) + p(PCR run) + ψ(sex+age+BCI)	9	6.13	0.01	95.91
σ(.) + p(PCR run) + ψ(sex+year+BCI)	7	6.17	0.01	101.21
σ(.) + p(PCR run) + ψ(age+year+BCI)	9	6.31	0.01	96.09
σ(.) + p(.) + ψ(.)	2	6.57	0.01	113.32
σ(.) + p(.) + ψ(sex)	3	7.98	0.01	112.54
σ(.) + p(.) + ψ(age)	5	8.40	0.00	108.36
σ(.) + p(.) + ψ(year)	3	8.46	0.00	113.02
σ(.) + p(.) + ψ(BCI)	3	8.53	0.00	113.08
σ(.) + p(PCR run) + ψ(sex+age+year+BCI)	10	8.86	0.00	95.87
σ(.) + p(.) + ψ(age+year)	6	9.67	0.00	107.21
σ(.) + p(.) + ψ(age+BCI)	6	9.74	0.00	107.27

σ(.) + p(.) + ψ(sex+year)	4	9.94	0.00	112.24
σ(.) + p(.) + ψ(sex+BCI)	4	9.99	0.00	112.28
σ(.) + p(.) + ψ(sex+age)	6	10.62	0.00	108.16
σ(.) + p(.) + ψ(year+BCI)	4	10.72	0.00	113.01
σ(.) + p(.) + ψ(sex+age+year)	7	11.99	0.00	107.03
σ(.) + p(.) + ψ(sex+age+BCI)	7	12.05	0.00	107.09
σ(.) + p(.) + ψ(age+year+BCI)	7	12.16	0.00	107.21
σ(.) + p(.) + ψ(sex+year+BCI)	5	12.28	0.00	112.23
σ(.) + p(.) + ψ(sex+age+year+BCI)	8	14.58	0.00	107.03

Table A3.1.4 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in Mountain Chickadees captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(.) + ψ(.)	2	0.00	0.55	35.57
σ(.) + p(.) + ψ(BCI)	3	2.23	0.18	35.01
σ(.) + p(.) + ψ(year)	3	2.30	0.17	35.08
σ(.) + p(.) + ψ(year + BCI)	4	5.24	0.04	34.85
σ(.) + p(PCR run) + ψ(.)	4	5.35	0.04	34.97
σ(.) + p(PCR run) + ψ(BCI)	5	8.40	0.01	34.40
σ(.) + p(PCR run) + ψ(year)	5	8.48	0.01	34.47
σ(.) + p(PCR run)+ψ(year+BCI)	6	12.43	0.00	34.24

Table A3.1.5 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in Pine Siskins captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(.) + ψ(.)	2	0.00	0.14	57.14
σ(.) + p(.) + ψ(BCI)	3	0.29	0.12	55.09
σ(.) + p(.) + ψ(year)	3	0.57	0.11	55.37
σ(.) + p(.) + ψ(age)	3	1.76	0.06	56.56
σ(.) + p(.) + ψ(sex+BCI)	4	1.88	0.06	54.20
σ(.) + p(PCR run) + ψ(.)	4	1.98	0.05	54.30
σ(.) + p(.) + ψ(sex)	3	2.29	0.05	57.09
σ(.) + p(.) + ψ(sex+year)	4	2.36	0.04	54.69
σ(.) + p(PCR run) + ψ(BCI)	5	2.55	0.04	52.24
σ(.) + p(.) + ψ(age+BCI)	4	2.60	0.04	54.93
σ(.) + p(.) + ψ(BCI+year)	4	2.76	0.04	55.08
σ(.) + p(PCR run) + ψ(year)	5	2.83	0.03	52.53
σ(.) + p(.) + ψ(age+year)	4	2.83	0.03	55.16
σ(.) + p(PCR run) + ψ(age)	5	4.02	0.02	53.72
σ(.) + p(.) + ψ(sex+age)	4	4.08	0.02	56.41
σ(.) + p(.) + ψ(sex+age+BCI)	5	4.38	0.02	54.08
σ(.) + p(PCR run) + ψ(sex+BCI)	6	4.45	0.02	51.37
σ(.) + p(.) + ψ(sex+BCI+year)	5	4.50	0.01	54.20
σ(.) + p(PCR run) + ψ(sex)	5	4.55	0.01	54.25
σ(.) + p(.) + ψ(sex+age+year)	5	4.76	0.01	54.46
σ(.) + p(PCR run) + ψ(sex+year)	6	4.91	0.01	51.83
σ(.) + p(PCR run) + ψ(age+BCI)	6	5.17	0.01	52.08
σ(.) + p(.) + ψ(age+BCI+year)	5	5.23	0.01	54.92

σ(.) + p(PCR run) + ψ(BCI+year)	6	5.33	0.01	52.24
σ(.) + p(PCR run) + ψ(age+year)	6	5.39	0.01	52.31
σ(.) + p(PCR run) + ψ(sex+age)	6	6.64	0.01	53.56
σ(.) + p(.) + ψ(sex+age+BCI+year)	6	7.16	0.00	54.08
σ(.) + p(.) + ψ(sex+age+BCI)	7	7.28	0.00	51.24
σ(.) + p(PCR run) + ψ(sex+BCI+year)	7	7.41	0.00	51.37
σ(.) + p(PCR run) + ψ(sex+age+year)	7	7.63	0.00	51.60
σ(.) + p(PCR run) + ψ(age+BCI+year)	7	8.12	0.00	52.08
σ(.) + p(PCR run) + ψ(sex+age+BCI+year)	8	10.42	0.00	51.24

Table A3.1.6 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in Red-breasted Nuthatches captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(.) + ψ(BCI)	3	0.00	0.69	31.50
σ(.) + p(.) + ψ(.)	2	2.21	0.24	36.42
σ(.) + p(.) + ψ(sex)	3	4.90	0.07	36.40

Table A3.1.7 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in Ruby-crowned Kinglets captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.)+p(.)+ψ(.)+BCI+sex	4	0.00	0.52	26.97
σ(.)+p(.)+ψ(.)+BCI	3	0.95	0.32	31.01
σ(.)+p(.)+ψ(.)	2	3.20	0.10	36.01
σ(.)+p(.)+ψ(.)+sex	3	4.50	0.05	34.56

Table A3.1.8 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in Dark-eyed Juncos captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set ($\Delta AICc_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(.) + ψ(.)	3	0.00	0.35	70.94
σ(.) + p(.) + ψ(sex)	4	1.32	0.18	69.61
σ(.) + p(PCR run) + ψ(.)	5	1.59	0.16	67.03
σ(.) + p(.) + ψ(BCI)	4	2.65	0.09	70.94
σ(.) + p(PCR run) + ψ(sex)	6	3.36	0.07	65.69
σ(.) + p(.) + ψ(sex+BCI)	5	3.96	0.05	69.39
σ(.) + p(PCR run) + ψ(BCI)	6	4.69	0.03	67.03
σ(.) + p(PCR run) + ψ(age)	6	6.21	0.02	68.54
σ(.) + p(PCR run) + ψ(sex+BCI)	7	6.51	0.01	65.47
σ(.) + p(.) + ψ(sex+age+BCI)	7	7.26	0.01	66.22
σ(.) + p(.) + ψ(sex+age)	7	8.43	0.01	67.39
σ(.) + p(.) + ψ(age+year+BCI)	7	8.74	0.00	67.70
σ(.) + p(.) + ψ(age+BCI)	7	9.30	0.00	68.26
σ(.) + p(PCR run) + ψ(age)	8	9.33	0.00	64.62
σ(.) + p(.) + ψ(sex+age+year+BCI)	9	11.03	0.00	62.30
σ(.) + p(PCR run) + ψ(sex+age)	9	12.22	0.00	63.48
σ(.) + p(PCR run) + ψ(age+BCI)	9	13.08	0.00	64.34
σ(.) + p(PCR run) + ψ(age+year)	7	14.40	0.00	73.37
σ(.) + p(PCR run) + ψ(sex+age+BCI)	10	15.45	0.00	62.28
σ(.) + p(PCR run) + ψ(sex+age+year)	8	15.97	0.00	71.26
σ(.) + p(PCR run) + ψ(sex+age+year+BCI)	11	16.39	0.00	58.33
σ(.) + p(PCR run) + ψ(year)	4	16.46	0.00	84.76
σ(.) + p(PCR run) + ψ(age+year+BCI)	10	16.96	0.00	63.79

σ(.) + p(PCR run) + ψ(sex+year)	5	18.47	0.00	83.90
σ(.) + p(PCR run) + ψ(year+BCI)	5	19.27	0.00	84.71
σ(.) + p(PCR run) + ψ(age+year)	9	19.36	0.00	70.63
σ(.) + p(PCR run) + ψ(year)	6	20.43	0.00	82.76
σ(.) + p(PCR run) + ψ(sex+year+BCI)	6	21.57	0.00	83.90
σ(.) + p(PCR run) + ψ(sex+age+year)	10	21.69	0.00	68.52
σ(.) + p(PCR run) + ψ(sex+year)	7	22.95	0.00	81.91
σ(.) + p(PCR run) + ψ(year+BCI)	7	23.75	0.00	82.71
σ(.) + p(PCR run) + ψ(sex+year+BCI)	8	26.62	0.00	81.91

Table A3.1.9 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in Warbling Vireos captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set (Δ**AICc**$_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(.) + ψ(.)	2	0.96	0.15	78.29
σ(.) + p(PCR run) + ψ(BCI)	5	1.33	0.13	69.54
σ(.) + p(.) + ψ(BCI)	3	1.54	0.12	76.16
σ(.) + p(PCR run) + ψ(sex)	5	1.63	0.11	69.85
σ(.) + p(.) + ψ(sex)	3	1.90	0.10	76.52
σ(.) + p(.) + ψ(sex+BCI)	4	2.79	0.06	74.40
σ(.) + p(PCR run) + ψ(sex+BCI)	6	3.29	0.05	67.65
σ(.) + p(.) + ψ(age)	4	6.48	0.01	78.09
σ(.) + p(.) + ψ(age+BCI)	6	6.65	0.01	71.01
σ(.) + p(.) + ψ(age)	6	7.04	0.01	71.40
σ(.) + p(.) + ψ(sex+age)	5	8.25	0.00	76.46
σ(.) + p(PCR run) + ψ(age+BCI)	7	8.27	0.00	68.23
σ(.) + p(.) + ψ(sex+age+BCI)	6	8.85	0.00	73.21
σ(.) + p(PCR run) + ψ(sex+age)	7	9.82	0.00	69.78
σ(.) + p(PCR run) + ψ(sex+age+BCI)	8	11.69	0.00	66.57

Table A3.1.10 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in White-crowned Sparrows captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set (Δ**AICc**$_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(.) + ψ(sex+BCI)	5	0.41	0.10	112.04
σ(.) + p(PCR run) + ψ(sex+year)	7	0.46	0.10	106.79
σ(.) + p(.) + ψ(sex+year)	5	0.86	0.08	112.48
σ(.) + p(PCR run) + ψ(sex)	6	1.17	0.07	110.21
σ(.) + p(PCR run) + ψ(year)	6	1.37	0.06	110.40
σ(.) + p(PCR PCR run) + ψ(.)	5	1.48	0.06	113.11
σ(.) + p(PCR run) + ψ(BCI)	6	1.51	0.06	110.55
σ(.) + p(.) + ψ(sex)	4	1.74	0.05	115.84
σ(.) + p(.) + ψ(year)	4	1.96	0.05	116.06
σ(.) + p(.) + ψ(BCI)	4	2.18	0.04	116.29
σ(.) + p(.) + ψ(.)	3	2.30	0.04	118.77
σ(.) + p(PCR run) + ψ(sex+year+BCI)	8	2.80	0.03	106.28
σ(.) + p(.) + ψ(sex+year+BCI)	6	2.95	0.03	111.98
σ(.) + p(.) + ψ(sex+age+BCI)	7	3.82	0.02	110.15
σ(.) + p(PCR run) + ψ(sex+age+BCI)	9	3.92	0.02	104.41
σ(.) + p(PCR run) + ψ(year+BCI)	7	4.03	0.02	110.36
σ(.) + p(.) + ψ(year+BCI)	5	4.39	0.01	116.02
σ(.) + p(.) + ψ(sex+age+year)	7	5.23	0.01	111.55
σ(.) + p(PCR run) + ψ(sex+age+year+BCI)	9	5.34	0.01	105.83
σ(.) + p(PCR run) + ψ(age)	7	5.42	0.01	105.83
σ(.) + p(.) + ψ(age)	5	5.78	0.01	117.40
σ(.) + p(PCR run) + ψ(sex+age)	8	5.87	0.01	109.34
σ(.) + p(.) + ψ(sex+age)	6	5.91	0.01	114.94

σ(.) + p(PCR run) + ψ(age+BCI)	8	6.58	0.00	110.06
σ(.) + p(PCR run) + ψ(age+year)	8	6.69	0.00	110.17
σ(.) + p(.) + ψ(age+BCI)	6	6.76	0.00	115.80
σ(.) + p(.) + ψ(age+year)	6	6.77	0.00	115.80
σ(.) + p(.) + ψ(sex+age+year+BCI)	8	7.50	0.00	110.98
σ(.) + p(PCR run) + ψ(sex+age+year+BCI)	10	7.91	0.00	105.26
σ(.) + p(.) + ψ(age+year+BCI)	7	9.43	0.00	115.75
σ(.) + p(PCR run) + ψ(age+year+BCI)	9	9.54	0.00	110.03

Table A3.1.11 Model set and rankings exploring the importance of factors affecting the detection probability (p) and prevalence (ψ) of *Haemoproteus* blood parasites in Wilson's Warblers captured and sampled at a high-elevation valley in northern Colorado during 2017-2018. 'PCR run' indicates the 3 PCR replicates carried out for each sample. The number of parameters (K), model weights (w_i), and deviance are shown for each model and the models are ranked by their AICc differences relative to the best model in the set (**ΔAICc**$_i$). Sigma (σ) was a random effect included in every model to account for unmodeled heterogeneity.

Model	K	ΔAICc	w_i	Deviance
σ(.) + p(.) + ψ(sex+year)	5	0.01	0.19	101.85
σ(.) + p(.) + ψ(sex)	4	0.59	0.15	104.98
σ(.) + p(.) + ψ(year)	4	1.57	0.09	105.97
σ(.) + p(.) + ψ(BCI)	4	1.66	0.08	106.06
σ(.) + p(.) + ψ(.)	3	2.10	0.07	108.92
σ(.) + p(.) + ψ(sex+year+BCI)	6	2.69	0.05	101.84
σ(.) + p(.) + ψ(year+BCI)	5	4.09	0.03	105.93
σ(.) + p(PCR run) + ψ(sex+BCI)	7	4.89	0.02	101.20
σ(.) + p(PCR run) + ψ(sex+year)	7	4.90	0.02	101.21
σ(.) + p(PCR run) + ψ(sex)	6	5.19	0.01	104.35
σ(.) + p(.) + ψ(sex+age+BCI)	7	5.35	0.01	101.66
σ(.) + p(.) + ψ(sex+age+year)	7	5.38	0.01	101.69
σ(.) + p(.) + ψ(sex+age)	6	5.65	0.01	104.80
σ(.) + p(PCR run) + ψ(year)	6	6.18	0.01	105.33
σ(.) + p(PCR run) + ψ(BCI)	6	6.27	0.01	105.42
σ(.) + p(PCR run) + ψ(.)	5	6.44	0.01	108.28
σ(.) + p(.) + ψ(age+year)	6	6.77	0.01	105.92
σ(.) + p(.) + ψ(age+BCI)	6	6.85	0.01	106.01
σ(.) + p(.) + ψ(age)	5	6.94	0.01	108.78
σ(.) + p(PCR run) + ψ(sex+year+BCI)	8	7.89	0.00	101.19
σ(.) + p(.) + ψ(sex+age+year+BCI)	8	8.35	0.00	101.66
σ(.) + p(PCR run) + ψ(year+BCI)	7	8.98	0.00	105.29
σ(.) + p(.) + ψ(age+year+BCI)	7	9.59	0.00	105.90

σ(.) + p(PCR run) + ψ(sex+age)	7	10.85	0.00	104.16
σ(.) + p(PCR run) + ψ(sex+age+BCI)	9	10.88	0.00	101.01
σ(.) + p(PCR run) + ψ(sex+age+year)	9	10.92	0.00	101.05
σ(.) + p(PCR run) + ψ(age)	7	11.83	0.00	108.14
σ(.) + p(PCR run) + ψ(age+year)	8	11.97	0.00	105.28
σ(.) + p(PCR run) + ψ(age+BCI)	8	12.06	0.00	105.37
σ(.) + p(PCR run) + ψ(sex+age+year+BCI)	10	14.26	0.00	101.01
σ(.) + p(PCR run) + ψ(age+year+BCI)	9	15.13	0.00	105.26