THE ROLE OF DISEASE AND ECTOPARASITES IN THE ECOLOGY OF NESTLING GOLDEN EAGLES

by

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DEDICATION

To my family.

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ABSTRACT

Climate and anthropogenic land use changes can alter biological communities and affect disease infection rates and parasite species distribution and abundance. Management to mitigate the threats of emerging infectious diseases and parasite species requires identifying and understanding factors that influence individual susceptibility within populations. Golden eagles (*Aquila chrysaetos*) in southwestern Idaho face several current and emerging threats, including a landscape-mediated diet shift that has increased the potential for disease infection, and warming temperatures that may increase the distribution and abundance of hematophagous ectoparasites. We examined prevalence of *Trichomonas gallinae* infection in golden eagle nestlings across western North America in 2015 and conducted a detailed study of the risk factors associated with *T. gallinae* infection in southwestern Idaho. We also quantified the abundance of Mexican chicken bug (*Haematosiphon inodorus*; Hemiptera: Cimicidae) in golden eagle nests in southwestern Idaho in 2015 and 2016. We developed a pit fall trap method to measure *H. inodorus* abundance, investigated factors that might affect abundance in nests, tested the 'nest protection' hypothesis that eagles modify nest sites to reduce the effects of ectoparasitism, and measured the physiological effects of ectoparasitism on nestlings.

In our study of *T. gallinae*, we found a 6% infection rate distributed broadly across our western North America study area, with a relatively high *T. gallinae* infection rate, 41%, in Idaho. The probability of *T. gallinae* infection increased as the proportion of

rock pigeons in nestling diet increased. Landscape-level change in southwestern Idaho is related to an increase in eagle diet diversity, and an increase in rock pigeons in nestling diet increased the probability of *T. gallinae* infection.

In our study of *H. inodorus*, we found that eagles reuse less parasitized nests in successive years, and that south-facing nests and nests with later phenology had higher *H. inodorus* abundance. We found support for the 'nest protection' hypothesis. Golden eagles selected gray rabbitbrush as nest material, a plant that has high phenolic concentrations relative to others available on the landscape, and aromatic nest material had a positive effect on nestling hematocrit, suggesting these nest additions reduced the effects of ectoparasitism on nestlings. We found that increased ectoparasitism reduced nestling mass and hematocrit, and increased the probability that nestlings either fledged early or died in the nest. Nestling circulating corticosterone, which may act as a mechanism in the timing of fledging behavior, increased relative to ectoparasite infestation levels.

Our results suggest that the current and emerging threats of disease and ectoparasites have the potential to negatively affect golden eagle productivity in southwestern Idaho. Although our data suggest there is a low incidence of *T. gallinae* infection in golden eagle populations across western North America, shifts in eagle diet, that result from habitat degradation and loss of historical prey resources, have the potential to affect golden eagle nestling survival. In addition, the presence and intensity of ectoparasitism affects the physiological condition of young eagles, and changes to the landscape in southwestern Idaho may reduce the ability of eagles to 'defend' their nests from the effects of ectoparasitism with aromatic plants. Given the projections of current

climate trends, continued monitoring of the effects of disease and ectoparasites on golden eagle populations will be important for future conservation.

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LIST OF ABBREVIATIONS

BSU	Boise State University
LMM	Linear mixed model
GLMM	Generalized linear mixed model
USFWS	United States Fish and Wildlife Service
BLM	Bureau of Land Management
USGS	United States Geological Survey
NCA	Morley Nelson Snake River Birds of Prey National Conservation Area
UCA	Upstream Comparison Area
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction

INTRODUCTION

Climate change and anthropogenic land use change, including agricultural production and urbanization, can promote new interactions between pathogens, vectors, and hosts. Biological impoverishment, as a result of climate change, habitat fragmentation, and the increased human ecological footprint, has resulted in unprecedented disease emergence (Aguirre and Tabor 2008). Moreover, climate change is predicted to precipitate changes in the spatial distributions of species, which have the potential to affect community structure and dynamics (Møller et al. 2013). These changes include the expansion of both parasite species and vector species distributions, which could introduce diseases and threats to host fitness into previously unaffected areas (Cumming and Van Vuuren 2006). Management to mitigate the threats of emerging infectious diseases and parasite species requires an understanding of the drivers and factors that influence the risk of infection and individual susceptibility within populations.

Avian trichomonosis, caused by the parasitic protozoan *Trichomonas gallinae*, has been identified as an emerging infectious disease in avian populations worldwide (Tompkins et al. 2015). An epidemic strain of *T. gallinae*, first discovered in Great Britain in 2005, spread from the United Kingdom along migratory routes with documented cases in Scandinavia (Lawson et al. 2011; Lehikoinen et al. 2013) and the Canadian Maritime Provinces (Forzán et al. 2010) over a two-year period. The epizootic

caused by avian trichomonosis continues to occur in British finch populations (Chi et al. 2013), and has been observed in wild columbid, passerine, and avivorious raptor populations worldwide with six major outbreaks documented since the year 2000 (Tompkins et al. 2015). At least 15 genetic strains of *T. gallinae* are known to infect avian species, and this variation causes differences in susceptibility and virulence (Sansano-Maestre et al. 2009). As a result, *T. gallinae* is increasing recognized as a conservation concern for the management of threatened or endangered species (Real et al. 2000; Bunbury et al. 2007).

Ectoparasites affect their host species in a myriad of ways that include influencing behavior, morphology, survival, and life history traits (Newton 1998), and global increases in temperature and anthropogenic changes in land use may allow parasites to expand their current distributions (Cumming and Van Vuren 2006). One potential example of such range expansion is the Mexican chicken bug (Hemiptera: Cimicidae: *Haematosiphon inodorus*), a relatively new addition to the avian ectoparasite community in southwestern Idaho. The first documentation of *H. inodorus* in Idaho (McFadzen et al. 1996), coincides with a period of significant warming in winter minimum temperatures (Heath et al. 2012), and previous studies have shown the detrimental effects of *H. inodorus* on nestling prairie falcons (*Falco mexicanus*) in Idaho (McFadzen and Marzluff 1996) and other breeding raptors in the Southwest U.S.

The effects of exposure to new or increased rates of disease and parasite infestations in raptors could be compounded by climate-induced habitat degradation or the loss of historical prey resources (Staley and Bonneaud 2015). Thus, documenting and understanding host-parasite interactions, the theme of this thesis, is an important

consideration for the effective conservation and management of threatened species, particularly in a time of increased global change.

The chapters presented in this thesis have been formatted and prepared as manuscripts to be submitted to peer-reviewed journals. Each manuscript will include co-authors, although for the purposes of the thesis, these authors are identified in the 'Acknowledgements' section. Chapter 1, Prevalence and Risk Factors of *Trichomonas gallinae* Infection in Golden Eagle Nestlings in Western North America', is written and formatted for the Journal of Wildlife Diseases. Chapter 2, 'Quantifying Abundance and Identifying Risk Factors that Predict Hematophagous Ectoparasites in Golden Eagle Nests, and a Test of the Nest Protection Hypothesis', is written and formatted for Oikos. Chapter 3, 'The Physiological Effects of Hematophagous Ectoparasites on Golden Eagle Nestlings', is written and formatted for Conservation Physiology.

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PREVALENCE AND RISK FACTORS OF TRICHOMONAS GALLINAE INFECTION IN GOLDEN EAGLE NESTLINGS IN WESTERN NORTH AMERICA

Abstract

Climate and anthropogenic land use changes alter biological communities, which can affect both disease infection rates and virulence. Understanding the risks of infectious disease is important for the conservation of imperiled species. Avian trichomonosis, caused by the protozoan *Trichomonas gallinae*, is an emerging infectious disease that affects bird species worldwide. Columbiformes, particularly rock pigeons (*Columba livia*), are considered the reservoir for the parasite, and raptor species are susceptible to infection via predation of infected birds. Previous studies have shown *T. gallinae* infection rates in nestling raptors are influenced by oral pH and diet; however, no studies have quantified prevalence and identified causal factors of infection in golden eagles (*Aquila chrysaetos*). We examined prevalence of *T. gallinae* infection in golden eagle nestlings across western North America in 2015, and conducted a detailed study of the risk factors associated with *T. gallinae* infection in southwestern Idaho. We found a 6% infection rate of *T. gallinae* distributed broadly across our western North America study areas with a relatively high infection rate, 41%, in Idaho. At our Idaho study area, nestling age did not explain infection probability. However, nestling oral pH became more acidic with age, which has been shown to create a less hospitable environment for *T. gallinae*. The probability of *T. gallinae* infection increased as the proportion of rock

pigeons in nestling diet increased. Landscape-level change in southwestern Idaho is related to an increase in eagle diet diversity, and an increase in rock pigeons in nestling diet increased the probability of *T. gallinae* infection. Although our data suggest there is a low incidence of *T. gallinae* infection in golden eagle populations across western North America, shifts in eagle diet that result from habitat degradation and loss of historical prey resources have the potential to affect golden eagle nestling survival.

Introduction

Changes in climate, land cover, and the distribution of introduced species drive changes in biological communities and can promote new interactions between pathogens, vectors, and hosts (Patz et al. 2000; Zamora-Vilchis et al. 2012). Emerging infectious diseases pose significant threats to wildlife populations, and studies focused on understanding the relationships between patterns of change and the risks of disease will support the conservation of imperiled species (Daszak et al. 2000). Avian trichomonosis, caused by the flagellated protozoan parasite *Trichomonas gallinae*, is an emerging infectious disease affecting avian communities worldwide (Tompkins et al. 2015). In 2005, an outbreak of a clonal strain of *T. gallinae* caused significant declines in populations of two common passerine species, the greenfinch (*Carduelis chloris*) and the chaffinch (*Fringilla coelebs*) in the United Kingdom (Robinson et al. 2010). Significant population declines of common avian species suggest that populations of rare or threatened species could face increased risks from trichomonosis.

Trichomonas gallinae is commonly found in birds of the Columbidae family and the disease has followed the introductions, and subsequent range expansions, of rock pigeons (*Columba livia*) (Stabler 1951). *Trichomonas gallinae* was first described in the

United States in 1934 (Stabler 1954), and was likely introduced to the New World avifauna in the 1600s as European settlers began transporting rock pigeons to North America (Schorger 1951). Wild columbid populations can have variable infection rates of *T. gallinae,* and some individuals can be sub-clinical carriers (Stabler 1954). The parasite has had a significant negative effect on isolated and previously unexposed columbid populations, including the endangered Mauritius pink pigeon (*C. mayeri*), where *T. gallinae* is the primary cause of nestling mortality and a limitation to population growth (Bunbury et al. 2008). *Trichomonas gallinae* primarily affects the upper digestive tract of birds. Clinical signs of infection include caseous lesions in the oropharynx that can lead to starvation or suffocation (Stabler 1947). Vertical transmission of the parasite occurs through protein-rich crop milk which adult columbids feed their offspring (Amin et al. 2014). Transmission can also occur indirectly and horizontally, through communal food and water sources (Stabler 1954; Villanúa et al. 2006). For example, Lennon et al. (2013) found a higher incidence of infection in columbids living on farms that provided supplementary food for game birds than those did not, which suggests that these food sources increase transmission rates. Purple and Gerhold (2015) found that *T. gallinae* can persist in water for up to 18 hours, demonstrating that bird baths are a potential source of parasite transmission, and *T. gallinae* infection rates have been positively correlated with warmer, drier, conditions that effectively limit sources of fresh water and force birds to communally drink from few, potentially contaminated, sources (Bunbury et al. 2007). Although the severity of infection depends on the virulence of specific *T. gallinae* strains and the susceptibility of individual birds, infection rates also likely vary temporally, spatially, and within host species (Stabler 1948; Chi et al. 2013; Girard et al. 2014).

Moreover, warmer temperatures and reduced precipitation, both of which are possible outcomes of future climate change, could increase *T. gallinae* viability and genetic variability, thereby leading to higher incidence and severity of infection in avian species (Rogers et al. 2016).

Raptors that feed on prey infected with *T. gallinae* are susceptible to infection. Previous studies have found high rates of trichomonosis in raptor populations that experienced habitat loss and associated changes in historical prey populations. Boal et al. (1998) found *T. gallinae* in 85% of urban Cooper's hawk (*Accipiter cooperii*) nestlings in Tucson, Arizona compared to a 9% infection rate in nestlings from a nearby rural population. The infection rate in urban Cooper's hawk nestlings was related to an urban environment where nestling diet consisted of 83% columbids compared to the rural population where columbids were not a readily available prey source (Boal 1997). Similarly, Real et al. (2000) detected *T. gallinae* in 36% of Bonelli's eagle (*Aquila fasciata*) nestlings in northeastern Spain and identified trichomonosis as an important cause of nestling mortality. Palma et al. (2006) found that in the absence of traditional prey items, Bonelli's eagles increased their consumption of rock pigeons and, therefore, risk of infection. All three studies concluded that trichomonosis negatively impacted reproductive success and affected their respective species at the population level (Boal et al. 1998; Real et al. 2000; Palma et al. 2006). Similar studies have described high infection rates in local populations of northern goshawks (*Accipiter gentilis*) in Great Britain (Cooper and Petty 1988) and Poland (Wieliczko et al. 2003) where landscape level changes and encroaching development have caused shifts in traditional diets to include higher proportions of columbids.

In addition to diet, Urban and Mannan (2014) found age-dependent susceptibility to *T. gallinae* infection in Cooper's hawk nestlings in Arizona. Mean nestling oral pH was sufficiently basic (6.83) to create a hospitable environment for *T. gallinae*, which thrives at a pH range of 6.5 to 7.5 (Read 1957). Nestling oral pH became more acidic as young hawks approached fledging and the oral pH of adult Cooper's hawks was over seven times more acidic than that of nestlings, making adults less susceptible to infection (Urban and Mannan 2014). However, it is unclear if all adult raptors have an acidic oral pH strong enough to prevent infection, as *T. gallinae* has been detected in adult bald eagles (*Haliaeetus leucocephalus*) (Stone and Nye 1981).

In western North America, golden eagles (*Aquila chrysaetos*) occupy a wide range of open habitats, including shrub steppe, grasslands, and deserts (Kochert et al. 2002). Golden eagles prey primarily on mammals and, to a lesser extent, on birds and reptiles (Olendorff 1976). The relative importance of prey taxa varies by region, however leporids (e.g., hares and rabbits) are consistently an important prey type and sciurids (e.g., ground squirrels, prairie dogs, and marmots) are an important secondary food source (Bedrosian et al. *in press*).

Southwestern Idaho is home to a dense breeding population of golden eagles along the Snake River Canyon and associated uplands, yet much of the sagebrush steppe community has been degraded and fragmented by anthropogenic features and land conversion to agriculture, energy, and urban development (Leu et al. 2008). Furthermore, overgrazing of native vegetation and the spread of exotic invasive plants has altered historical fire regimes, causing further change to native plant communities (Fleischner 1994). Research on eagle diet from 1971-1981 (hereafter historical diet) in southwestern

Idaho showed that golden eagles preyed primarily on black-tailed jackrabbits (*Lepus californicus*) during the breeding season (Steenhof and Kochert 1988). However, since the early 1980s, the native shrub communities that jackrabbits typically inhabit (Knick and Dyer 1997) have been reduced by more than 50% through the combined effects of wildfire, livestock grazing, drought, and exotic plant species (Kochert and Pellant 1986; U.S. Department of the Interior 1996). Research conducted in southwestern Idaho from 2014-2015 (hereafter current diet) showed that these habitat alterations were correlated with a shift in golden eagle diet composition and diversity (Heath and Kochert 2016). Specifically, the authors report both a decrease in the proportion of jackrabbits in nestling diet and an increase in avian prey, including rock pigeons, which would be expected to increase the probability of *T. gallinae* infection among eagles.

Given the recent and increasingly frequent outbreaks of virulent strains of *T. gallinae* (Robinson et al. 2010; Rogers et al. 2016), anthropogenic changes to western shrubland ecosystems that have altered raptor prey availability (Steenhof et al. 1999), and increased contact between nesting raptors and synanthropic species like rock pigeons (Leu et al. 2008), our objectives were to document the prevalence of *T. gallinae* in nestling golden eagles, identify the risk factors that predict infection, and examine whether exposure risk has changed over time. To meet our objectives, we sampled golden eagle nestlings at breeding sites throughout western North America to assess the prevalence of *T. gallinae* infection over a wide geographic area, and we conducted a detailed study of a golden eagle population in southwestern Idaho to understand the risk factors associated with *T. gallinae* infection. Specifically, we evaluated whether nestling age, oral pH, or diet predicted *T. gallinae* infection rates. Finally, we used historical data

on nestling diet and the presence of oral lesions, which are suggestive of *T. gallinae* infection, to address whether risk of infection has changed over time at our Idaho study area.

Methods

Study Areas

We collected oral swab samples in 2015 from golden eagle nestlings from the Tehachapi Mountains and Mojave Desert in southern California, Butte Valley in northern California, western Nevada, central and eastern Oregon, central and eastern Washington, the west desert mountains of Utah, northwestern Wyoming, northwestern Arizona, eastern New Mexico, western Colorado, southwestern Nebraska, the Seward Peninsula of Alaska, and southwestern Idaho (Figure 1.1). Our study area in Idaho, where we conducted our detailed study of the factors associated with *T. gallinae* infection, was located along the Snake River Canyon in the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA) and the adjacent Upstream Comparison Area (UCA), which extends east from the NCA to Hagerman, Idaho (Figure 1.1, inset). The steep basalt cliffs of the Snake River Canyon provide nesting locations for golden eagles. The surrounding uplands are a mosaic of land cover types and habitats including native sagebrush steppe and salt-desert communities characterized by big sagebrush (*Artemisia tridentata*), shadscale (*Atriplex confertifolia*), gray rabbitbrush (*Ericameria nauseosa*), and disturbed grasslands and rangeland dominated by exotic annuals, native perennial grasses, irrigated agricultural land, and rural and suburban development (U.S. Department of the Interior 1996).

Oral Sampling for *T. gallinae*, DNA sequencing, and nestling pH

During the 2015 golden eagle breeding season (April – June), we swabbed the surface areas of the mouth and upper esophagus of nestling eagles with a sterile, dry, cotton-tipped swab to sample for *T. gallinae*. Swabs were immediately introduced into InPouch TF *Tritrichomonas foetus* test kits (BioMed Diagnostics, White River, OR, USA). For samples collected outside of Idaho, test kits were shipped overnight to Boise State University and incubated at 37°C within 72 hours of sampling. Idaho samples were incubated typically within 12 hours of sampling. After 24 hours of incubation, the InPouch test kit was placed under a compound light microscope and visually inspected at 100X magnification for the presence of *T. gallinae* (Cover et al. 1994). If no live, motile *T. gallinae* were detected on the first examination, we continued to incubate and observe samples every 24 hours for up to 6 days. Samples were recorded as negative for *T. gallinae* if no motile *T. gallinae* were observed within 144 hours (BioMed Diagnostics, 2012). To test if differences in time to incubation affected our ability to detect *T. gallinae*, we collected replicate samples at our Idaho study area and delayed incubation for 24, 48, 72, 96, and 120 hours. In all cases, delayed incubation up to 72 hours did not affect detection of *T. gallinae*. At sampling locations outside of Idaho, we collected samples 1-2 times during the breeding season. At our Idaho study area, we collected samples every 8-10 days during the same period, resulting in 3.9 ± 1.3 (mean ± SD) samples per nestling. Idaho nestlings that developed oral lesions indicative of trichomonosis were treated with a 30 mg dose of Spartrix (Janssen, Brussels, Belgium), an antiprotozoal drug effective at reducing the development of oral lesions, after we completed sampling. We continued to monitor nestlings treated with Spartrix for re-

infection, but subsequent *T. gallinae* infections were not included in our analysis of risk factors.

We performed DNA extractions on a subset of InPouch kits to confirm the presence of *T. gallinae*, identify the strain, and test for false negatives. PCR amplification and sequencing of the ITS1/5.8S/ITS2 ribosomal region was performed using the primers described in Cepicka et al. (2005) at the University of Tennessee-Knoxville. Forward and reverse sequences were assembled and aligned and consensus sequence chromatograms were trimmed and edited by hand using Sequencher 5.3 (Gene Codes Corporation, Ann Arbor, MI, USA). The resultant nucleotide sequences were subjected to a basic local alignment search tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). Additionally, DNA extractions were performed on eight samples collected in Oregon and Utah that were not incubated within 72 hours, but were suspected to contain *T. gallinae* based on the presence of oral lesions or the presence of rock pigeons as prey items during sampling.

We measured the oral pH of 15 nestlings every 10 days throughout the nestling period at the Idaho study area. To take the samples, we held a microelectrode (Cole-Parmer Combination pH Microelectrode BNC, Vernon Hills, IL) under the ventral surface of the tongue until the reading on a digital field meter (Oakton pH Tester 10 BNC, Oakton Instruments, Vernon Hills, IL) stabilized. The microelectrode was stored in a 4.0 pH buffer solution (Aldon Corporation, Avon, NY) during transport in the field and was rinsed with distilled water before use. The microelectrode was allowed to rest in the buffer solution in between sampling events. We calibrated the microelectrode to three points (pH = 4.0, 7.0, and 10.0) at least once a day prior to sampling (Urban and Mannan

2014). The sex of nestlings was determined through DNA analysis of nestling blood samples processed at Purdue University (West Lafayette, IN, USA). All field methods followed protocols approved by the Boise State University Institutional Animal Care and Use Committee (Protocol #006-AC14-007).

Current and Historical Nestling Diet

We used golden eagle nestling diet information collected in 2015 (Heath and Kochert 2016; Dudek, unpublished data) to assess whether the proportion of rock pigeons in the diet predicted risk of *T. gallinae* infection. We assessed nestling diet using a combination of prey remains, pellet analysis, and nest camera images as described in Steenhof and Kochert (1985) and Heath and Kochert (2016). We summarized the frequency of all prey items collected from nest sites to calculate the proportion of rock pigeons in nestling diet. We analyzed historical nestling diet data collected in the NCA and UCA by Steenhof and Kochert (1988) to determine whether the proportion of rock pigeons in nestling diet, and the exposure risk as a result of diet, had changed over time. To make this comparison, we selected 17 eagle territories where nestling diet was assessed in both the historical diet study and the current diet study. Therefore, by comparing the same territories from different time periods, we controlled for territory location within the study area. In the historical diet study, the presence of oral lesions indicative of trichomonosis was documented in field notes (USGS unpublished data).

Data Analysis

We used a generalized linear mixed model (GLMM) with presence and absence of *T. gallinae* as a binomial response variable and nestling age as the predictor variable to examine the association between nestling age and infection. This model included nestling

identity and territory identity as random variables to account for non-independence of samples. We created a linear mixed model to test whether nestling age, sex, or an interaction between age and sex explained oral pH with nestling identity and territory identity as random variables. We used a GLMM with presence and absence of *T. gallinae* as a binomial response variable and the proportion of rock pigeons in nestling diets as the predictor variable to test the association between the proportion of rock pigeons in nestling diet and the probability of *T. gallinae* infection. We used a GLMM with a negative binomial distribution for the response variable, total count of rock pigeons in nestling diet, and an offset for the total prey items cataloged at each nest to compare whether the proportion of pigeons in nestling diet has changed over time (i.e., from the historical study to recent study) at the same 17 territories, which would indicate a change in host exposure to infection. Both of these models included territory identity as a random variable. To explore whether pathogenicity has changed in the system, we used a GLMM with a binomial distribution with the presence or absence of lesions indicative of *T. gallinae* infection as the response variable, and territory identity as a random variable, to examine the interaction between study period (i.e., the historical study or the recent study) and the proportion of pigeons in the diet. All numerical predictors were scaled and centered before analysis. For GLMMs, we created confidence intervals by back-transforming the prediction after adding and removing the standard error. Linear models were created using functions lmer and glmer in the package lme4 (Bates et al. 2015) and function glmmADMB in the package glmmADMB (Fournier et al. 2012). All analyses were performed in R (version 3.2.2, R Core Development Team 2016). Descriptive statistics are reported as mean ± standard deviation.

Results

We collected oral swab samples from 96 eagle nestlings ranging in age from 21 days old to 59 days old, from 62 nests, across 10 western states (not including Idaho). We found incidence of *T. gallinae* infection at four western study areas. *Trichomonas gallinae* was detected in 6.2% (n = 6) of non-Idaho nestlings, and 9.7% (n = 6) of nests outside of Idaho had at least one nestling that developed infection. Positive samples came from Kern and Siskiyou counties in California, Crook and Lake counties in Oregon, and Tooele County, Utah. Overall, the prevalence of *T. gallinae* infection across the western study areas was lower than the Idaho study area. In Idaho, we collected samples from 32 eagle nestlings from 19 nests. These nestlings ranged in age from 7 days old to 63 days old. *Trichomonas gallinae* was detected in 41% (n = 13) of nestlings, and 42% (n = 8) of nests had at least one nestling that developed infection.

We confirmed the presence of *T. gallinae* with DNA extraction through DNA amplification via PCR followed by nucleotide sequencing in 52% (n = 25) samples. Sequence analysis also identified the presence of non-*T. gallinae* protozoans in four samples. Three non-*T. gallinae* showed 100% identity and 100% coverage to *Trichomonas gypaetinii* (Martinez-Diaz et al. 2014) recovered from two Idaho nestlings and one California nestling. To our knowledge, this is the first record of *T. gypaetinii* DNA in wild raptors in North America. *Trichomonas* spp. sequences showed 91-99% identity to ITS genotype B, 74% to genotype C, 90-100% to genotype D, 92% to genotype E, and 98% to genotype L (Gerhold et al. 2008). The remaining non-*T. gallinae* sequence, from one nestling, had a 95% identity and 100% coverage to *Monocercomonas colubrurom*, a protozoan typically found in the guts of reptiles (Richter et al. 2008). PCR

and sequence results of 17 samples had a 94% agreement to microscopy detection of *Trichomonas sp.*; the single false positive via microscopy was *M. colubrurom*. In addition, PCR and sequencing detected *T. gallinae* in 4 (n = 8) samples from Oregon and Utah that were not incubated within 72 hours of collection. No living organisms were detected under the microscope due to delayed incubation, but *T. gallinae* DNA was detected via PCR.

At our Idaho study area, the mean nestling age, for which *T. gallinae* infection was detected in culture from oral swabs, was 23.5 ± 11.0 days old (range 8 – 38 days old). The mean age of detection of oral lesions was 30.3 ± 13.5 days old (range 12 – 49 days old). We observed the development of oral lesions 7.2 ± 7.0 days after detecting presence of *T. gallinae* in culture from oral swabs. Twelve of 13 (92%) nestlings that tested positive for *T. gallinae* in culture subsequently developed oral lesions suggestive of *T. gallinae* infection. In all cases in which we observed oral lesions and administered antiprotozoal medicine, treatment resulted in the disappearance of oral lesions within 8 – 10 days. Moreover, *T. gallinae* was not detected in cultured swabs taken on the subsequent visit. We observed three cases of *T. gallinae* reoccurrence, both in culture and through the presence of oral lesions within 16, 24 and 25 days of initial treatment. All three birds were successfully treated a second time. Two nestlings that tested positive for *T. gallinae* in culture developed small oral lesions just prior to fledging and were left untreated. One nestling presumably fledged successfully, whereas the other was found dead in the nest after its sibling had fledged. Decomposition was too advanced to determine whether oral lesions contributed to this individual's death.

Nestling age did not predict the probability of *T. gallinae* infection ($\chi^2 = 0.3$, $p = 0.58$). Between the ages of 8 and 38 days old, younger nestlings were no more likely to become infected with *T. gallinae* than older nestlings. Oral pH of nestlings decreased as nestlings aged ($\chi^2 = 9.0$, $p = 0.003$, Figure 1.2) and was not related to sex ($\chi^2 = 1.3$, $p = 0.25$). Mean oral pH of nestlings less than 32 days old, when the majority of nestlings first tested positive for *T. gallinae*, was 7.21. Mean oral pH during our last sampling, when nestlings were at least 49 days old, was 6.68. This result indicates that as nestlings approached fledging age, they were still susceptible to developing infection. Although nestling oral pH decreased as nestlings aged, there was no significant relationship between *T. gallinae* infection and oral pH ($\chi^2 = 2.1$, $p = 0.14$).

During the 2015 breeding season, the proportion of rock pigeons in nestling diet predicted *T. gallinae* infection ($\chi^2 = 4.5$, $p = 0.03$, Figure 1.3). As the proportion of rock pigeons in the diet increased, so did the probability of developing *T. gallinae* infection; chance of infection approached 100% when rock pigeons accounted for at least 10% of nestling diet. When we compared the two diet studies, we found that the proportion of rock pigeons in eagle nestling diet increased significantly from the historical study to the current study ($\chi^2 = 7.9$, $p = 0.005$, Figure 1.4). However, we did not find a significant interaction between period and the proportion of rock pigeons in the diet on the probability of developing oral lesions, indicating that parasite pathogenicity within the population has not changed at these sites from the historical study to the current study ($\chi^2 = 1.2$, $p = 0.26$).

Discussion

Parasites like *T. gallinae* negatively affect the survival of raptor nestlings and may become a conservation concern when ecosystem-level changes increase transmission and infection rates. We found evidence of *T. gallinae* infection in golden eagle nestlings in five different study areas across western North America, with a relatively high rate of infection in southwestern Idaho. We used BioMed InPouch test kits and PCR to confirm the presence of *T. gallinae*, and we demonstrate that *T. gallinae* infection in eagle nestlings is treatable with antiprotozoal medicine. Although mortality from *T. gallinae* infection depends on numerous factors including host susceptibility and parasite virulence, previous studies reported high mortality rates in nestling raptors infected with *T. gallinae* (100% - Cooper and Petty 1988; 86% - Real et al. 2000). We treated 11 of 13 nestlings with *T. gallinae* infection with the antiprotozoal medicine Spartrix. Without treatment, we expect that 34% of all eagle nestlings in 2015 (n = 32) would have likely died; however, even with treatment, these 11 nestlings were still at risk of re-infection prior to or soon after fledging.

In Idaho, younger nestlings had a less acidic oral pH, but we found no relationship between *T. gallinae* infection and oral pH. Although oral pH decreased as nestlings aged (see also Urban and Mannan's 2014 study of Cooper's hawks), mean nestling oral pH at 80% of fledgling age was 6.68; which is within the tolerable range for *T. gallinae* (Read 1957). It is unknown if oral pH of golden eagles continues to decrease as fledglings age, but evidence of *T. gallinae* infection in recently fledged golden eagles (Beecham 1970; Kochert 1972) suggests that the oral pH of fledglings may not be acidic enough to prevent infection.

We found a positive association between the probability of *T. gallinae* infection in golden eagle nestlings and the proportion of rock pigeons in nestling diets. Smith et al. (1983) first isolated and positively confirmed the presence of *T. gallinae* in golden eagle nestlings in the NCA, and although large caseous lesions suggestive of *T. gallinae* infection have been noted since the late 1960s (USGS unpublished data), no studies have yet attempted to find an association between golden eagle diet and *T. gallinae* infection rates. Although previous studies have demonstrated a link between the presence of columbids in raptor nestling diets and *T. gallinae* infection, we demonstrate that nestlings whose diet consists of >10% rock pigeons have nearly a 100% chance of infection, indicating that there may be a threshold at which nestlings are more susceptible to infection.

Relatively low rates of infection in other western golden eagle breeding populations might be related to habitat quality and the availability of historical prey populations (e.g., leporids and sciurids) for the meta-population in western North America (Bedrosian et al. *in press*). Columbidae accounted for <5% of prey in any of the 37 individual studies in this meta-population study (B. Bedrosian, pers. comm.), which is below the 10% critical threshold we found predicts *T. gallinae* infection in Idaho. Golden eagles of the Arizona/New Mexico plateau, the Mojave Desert, the Wyoming Basin, the Northern Basin and Range of Oregon, and northern California still primarily prey on leporids and sciurids; however, avian prey have been historically important for breeding eagles in Oregon and northern Utah (Bedrosian et al. *in press*). The upper Columbia Plateau of Washington has undergone a conversion of shrub-steppe habitat to agricultural land, similar to southwestern Idaho, resulting in the reduction of jackrabbit and ground

squirrel populations. Land use change has caused shifts in eagle diet composition and breadth, with a high diversity of avian prey (Watson and Davies 2015). Our sample size from Washington nestlings was low and, although we did not document *T. gallinae*, shifts in diet composition and the presence of rock pigeons in current nestling diet has increased the potential risk of exposure.

Although our data indicate that the incidence of *T. gallinae* infection was higher in Idaho than in other western states, and that it may be influenced by diet, we cannot rule out the possibility that the timing of opportunistic sampling in study areas outside of Idaho may have limited detection of *T. gallinae* at those sites. Many of the samples from other western breeding populations were obtained near the end of the nesting period when young were being banded or fit with satellite transmitters. Given that we documented *T. gallinae* infection developing when nestlings were 8 – 38 days old in Idaho, it is possible that *T. gallinae* could have already caused nestling mortality at nests sampled late in the season, thereby causing us to underreport infection rates at those sites.

Our comparison of historical and current nestling diet at 17 nesting territories found a lack of an interaction between period and the proportion of pigeons in nestling diet for predicting oral lesions. This suggests that parasite pathogenicity within rock pigeon populations may not changed over time, and eagle nestlings are currently as likely to develop *T. gallinae* infection through the consumption of pigeons as they were historically. Although our data suggests *T. gallinae* pathogenicity has not changed, the proportion of rock pigeons in nestling diets in southwestern Idaho has increased from the historical diet study (Steenhof and Kochert 1988) to the current diet study (Heath and Kochert 2016), likely increasing exposure risk for eagle nestlings. Natural fluctuations in

prey abundance likely caused periodic increases in risk of infection for eagle nestlings historically, but the effects of wildfire and the expanding human footprint on current available prey resources in southwestern Idaho has likely increased the level of exposure risk for nestlings with potential negative consequences at a population level.

Our documentation of *T. gypaetinii* represents, to our knowledge, a new geographic distribution for the protozoan, which has previously only been reported in Egyptian vultures (*Neophron percnopterus*) and cinereous vultures (*Aegypius monachus*) in Europe (Martínez-Díaz et al. 2014). We are unsure of the geographical distribution of *T. gypaetinii* in North America. It is unknown if this parasite was introduced recently into North America, and represents a new risk to North American raptor populations, or has been endemic and previously undetected. Given *T. gypaetinii* is morphologically similar to *T. gallinae*, only molecular analysis can distinguish the species, which underscores the importance of integrating classical and molecular analysis of *Trichomonas* spp. We observed the development of oral lesions similar to those caused by *T. gallinae* in two of the three cases of *T. gypaetinii* in our study; however, further laboratory studies are needed to determine the pathogenicity of *T. gypaetinii*.

Our study is the first comprehensive survey to report the prevalence of *T. gallinae* infection in golden eagle nestlings over large parts of their western range. Although *T. gallinae* is present in eagle breeding populations across western North America, factors related to nestling diet may increase risks of infection within local populations. The effects of wildfire and other anthropogenic land use changes on sagebrush steppe habitat and available prey resources (Kochert and Pellant 1986; U.S. Department of the Interior 1996; Steenhof et al. 1999) have caused a shift in eagle nestling diet composition and

diversity (Heath and Kochert 2016), and likely increased the probability of infection and the potential for negative consequences for reproduction at those territories. Future variation in climatic conditions, such as temperature and rainfall, have the potential to affect transmission rates and pathogenicity within pigeon populations (Bunbury et al. 2008; Rogers et al. 2016), which could further change infection rates in eagle populations. Understanding prevalence and the risk factors of disease infection rates and pathogenicity is crucial in developing conservation strategies to manage wildlife populations. Monitoring changes in infections rates and pathogenicity will be important for the future conservation of threatened species like golden eagles.

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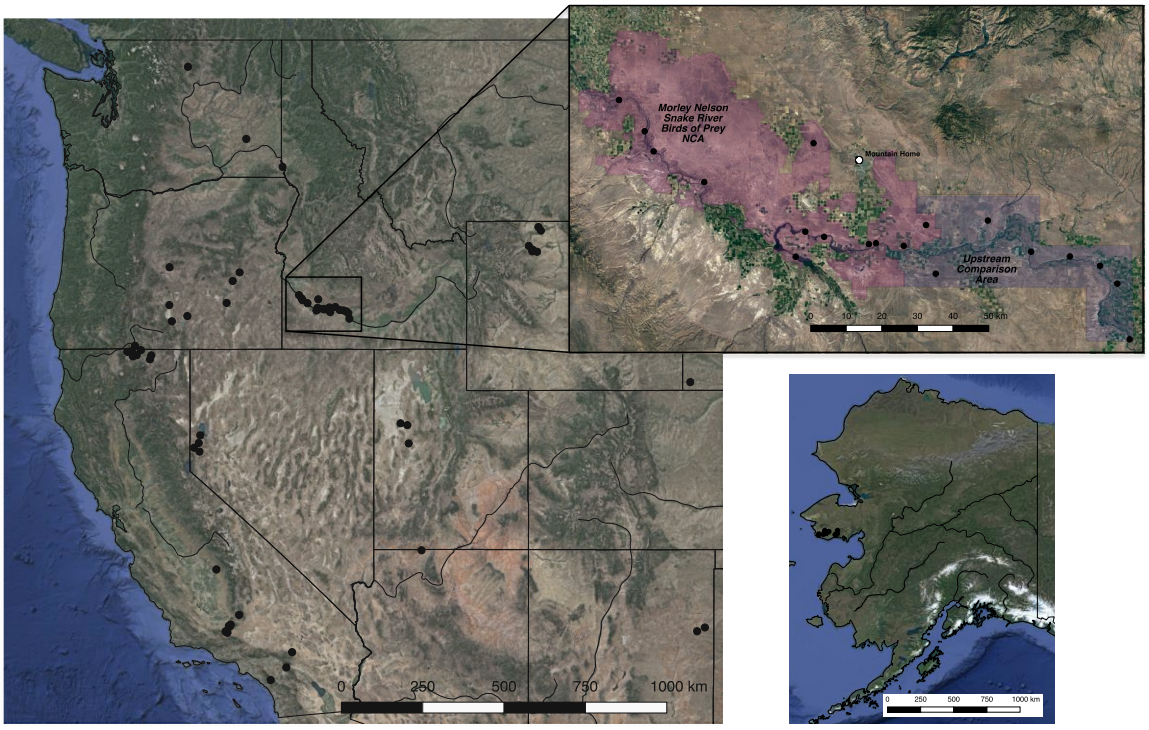

Figure 1.1 Location of sampling locations (black dots) where oral swabs were collected in the western United States to test for *T. gallinae*, including an inset for the study area in southwestern Idaho and the Seward Peninsula in Alaska. Sampling for *T. gallinae* occurred from April-June 2015.

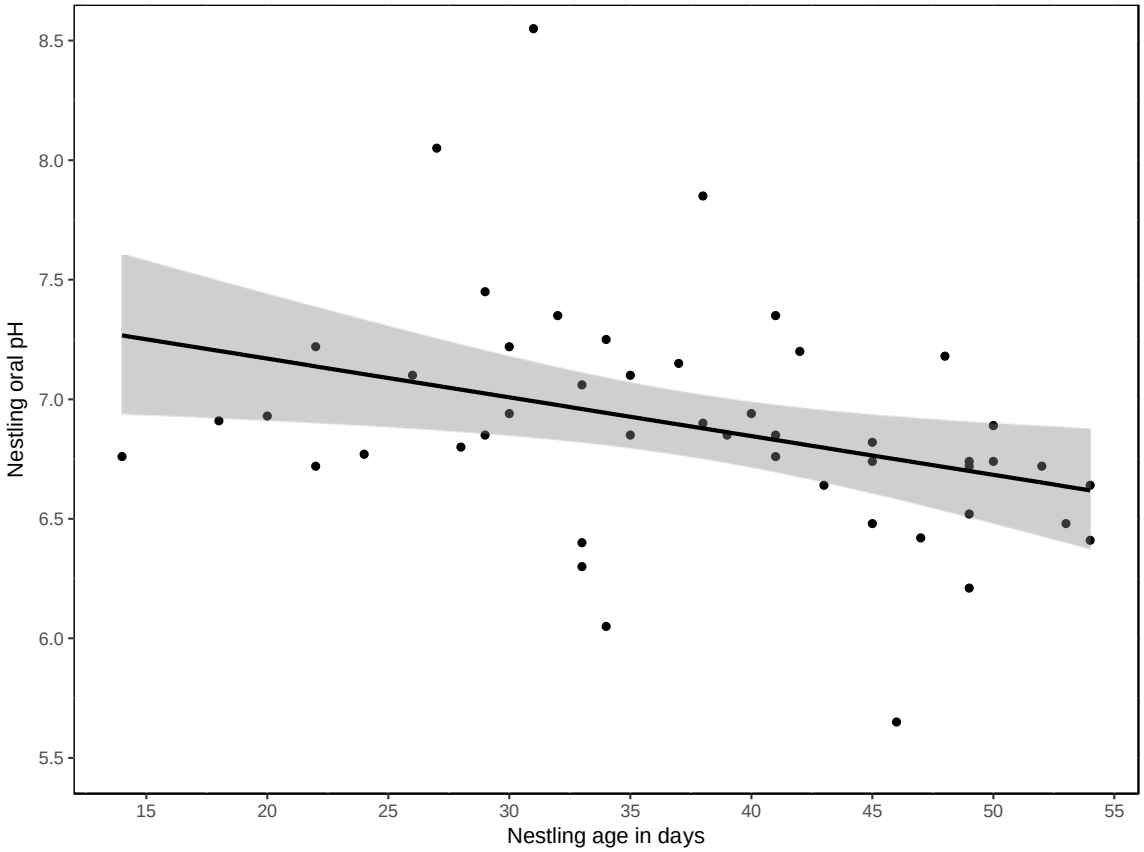

Figure 1.2 Observed oral pH (dark circles) and predicted line (dark line) with associated 95% confidence intervals (solid gray area) of golden eagle nestlings aged between 14 and 54 days old in southwestern Idaho, USA in 2015. Nestling oral pH decreased as nestlings aged ($\chi 2 = 9.2$, $p = 0.003$).

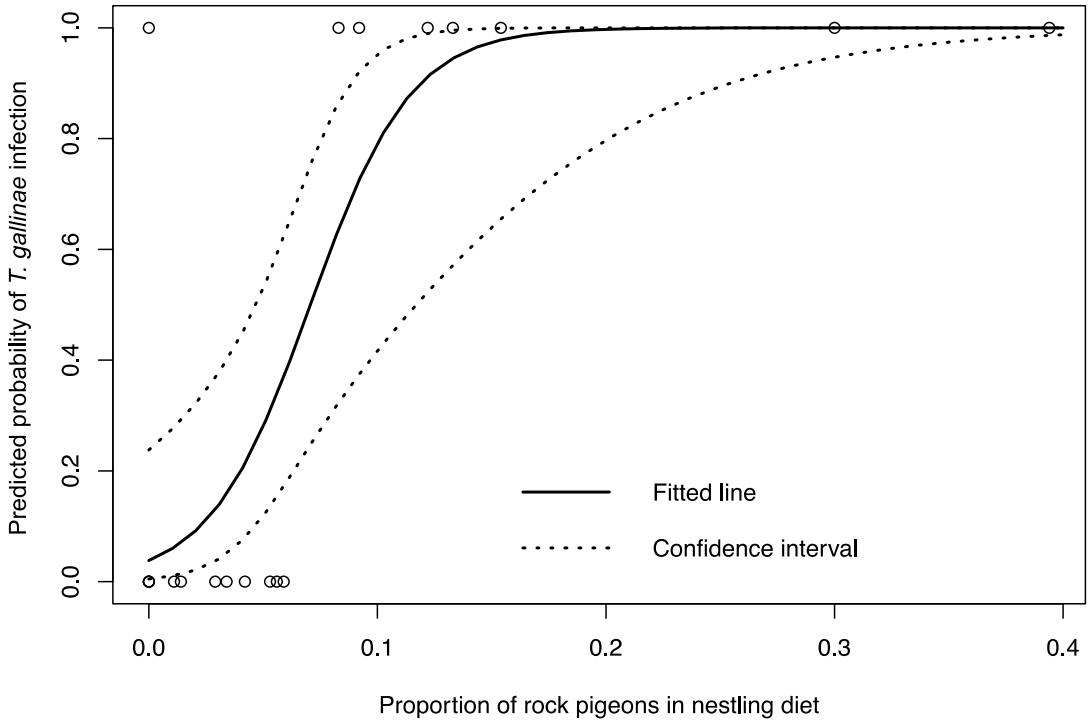

Figure 1.3 Observed occurrence (open circles) and predicted probability (solid line) with associated 95% confidence intervals (dotted lines) of T. gallinae infection in golden eagle nestlings in southwestern Idaho, USA in 2015. As the proportion of rock pigeons in nestling diet increased, so did the probability of T. gallinae infection ($\chi 2$ = 4.5, p = 0.03).

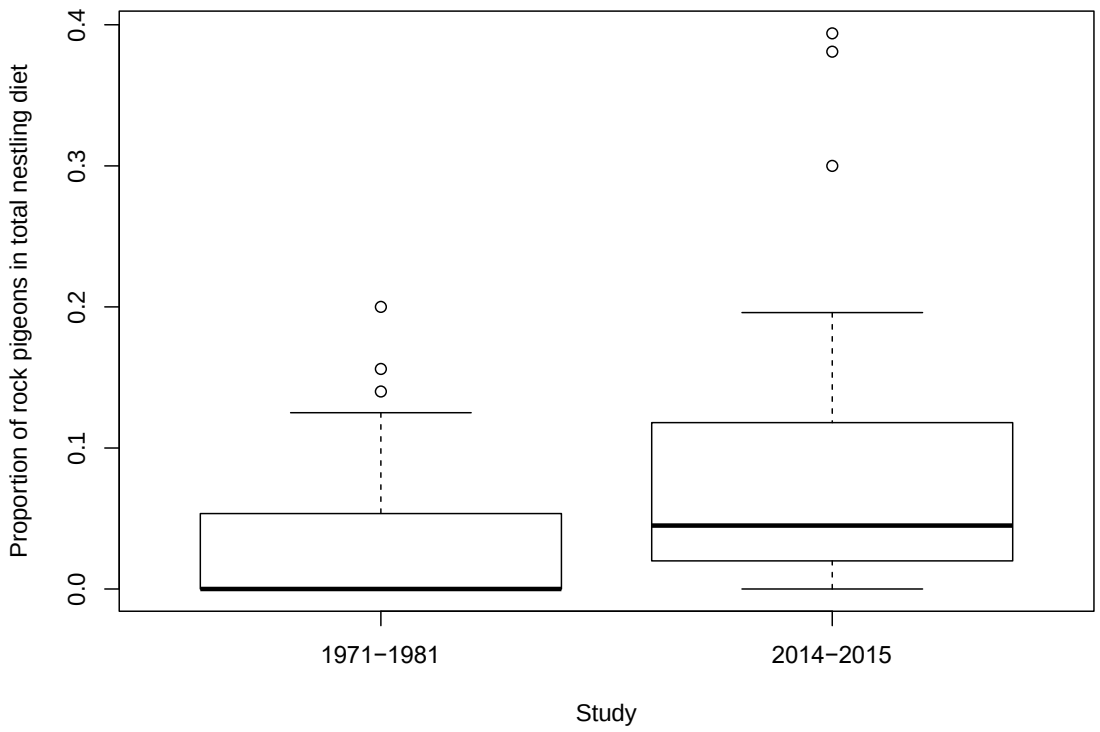

Figure 1.4 Proportion of rock pigeons in golden eagle nestling diets in southwestern Idaho, USA at the same 17 territories during the historical (1971-1981) diet study and the recent (2014-2015) diet study. Bold lines within boxes represent the median, upper and lower limits of the box are the first and third quartiles, whiskers contain 1.5 times the interquartile range, and open circles are outliers. Golden eagle diet in recent years consisted of more rock pigeons, a common vector of T. gallinae ($\chi 2 = 7.9$, $p = 0.005$), suggesting that risk of infection in 2014-2015 was likely higher compared to the period of 1971-1981.

QUANTIFYING ABUNDANCE AND IDENTIFYING RISK FACTORS THAT PREDICT HEMATOPHAGOUS ECTOPARASITES IN GOLDEN EAGLE NESTS, AND A TEST OF THE NEST PROTECTION HYPOTHESIS

Abstract

Changes in climate and land use will likely affect host-parasite interactions through removal of constraints that limit parasite abundance or distribution, or changes in the ability of hosts to deter parasites. Cliff-nesting birds, such as raptors, may be particularly susceptible to ectoparasites during the nesting period because young birds are unable to escape the source of parasites. Assessing ectoparasite load of raptors can be problematic because parasites are often difficult to detect, and few standardized survey methods practical for sampling large raptor nests exist. Our goal was to document the occurrence and quantify the abundance of ectoparasites in golden eagle (*Aquila chrysaetos*) nests during the nesting period, and to focus specifically on factors that might affect Mexican chicken bug (*Haematosiphon inodorus*; Hemiptera: Cimicidae) abundance in nests, such as previous nest use, nest aspect, nearby cliff-nesting species, proportion of aromatic green nest material, and nest phenology. Further, we tested the 'nest protection' hypothesis to determine if the addition of aromatic plants in nests might function to either reduce ectoparasites in the nest or moderate the effects of ectoparasitism on nestlings. Using pitfall traps to estimate the relative abundance of *H. inodorus*, we found that nests that had been used by eagles in the previous three years had lower *H. inodorus* abundance than those that had not been used, south-facing nests had

higher *H. inodorus* abundance than north-facing nests, and eagle pairs that bred later in the season experienced higher *H. inodorus* abundance in their nests. Additionally, our results supported the 'nest protection' hypothesis. Golden eagles preferentially selected gray rabbitbrush, a plant that has high phenolic concentrations relative to others available on the landscape, as nest material. Moreover, use of aromatic nest material was positively associated with nestling hematocrit, which suggests the addition of this material reduced the effects of ectoparasitism on nestlings.

Introduction

Shifts in climate can create conditions that allow for changes in the spatial distributions of species, and these changes have the potential to alter community structure and dynamics (Møller et al. 2013). Insects are particularly responsive to environmental change, and many insect taxa, including parasite species, are expanding their geographic distributions with increasing global temperatures and anthropogenic change (Sánchez-Guillén et al. 2016). Generalist parasites that expand into new areas have the potential to associate with, and adversely affect, new host species or previously unaffected populations of their usual hosts (Cumming and Van Vuren 2006). Given the fitness costs of parasites to their hosts (Brown and Brown 1986; Møller et al. 1990), selection in hosts should favor adaptations to avoid or limit parasite infestations (Goater et al. 2013). In the present study we examine the occurrence of hematophagous ectoparasites in golden eagle (*Aquila chrysaetos*) nests, and whether eagles exhibit nesting behaviors that reduce these infestation rates. In a time of increased global change, understanding host-parasite interactions is essential to the conservation and management of threatened wildlife, including raptors.

Bird nests are contained ecosystems composed of a rich and diverse community of both free-living and parasitic arthropods (Heeb et al. 2000). The abundance of ectoparasitic arthropods within nests is governed by many factors. At a local scale, climate may be an important driver in the occurrence of specific nest ectoparasites (Cumming and Van Vuren 2006), and their densities within nests could be influenced by abiotic factors such as temperature and humidity (Heeb et al. 2000). Indeed, microclimate conditions in nests have been shown to influence ectoparasite infestation in several passerine species. For example, higher ambient temperatures increased ectoparasite load in tree swallow (*Tachycineta bicolor)* nests, likely because warmer temperatures allowed ectoparasites to develop more rapidly (Dawson et al. 2005). Certain ectoparasitic insects, such as *Protocalliphora* spp., spend less time in larval and pupal stages as ambient temperatures increase (Bennett and Whitworth 1991). Abiotic factors such as temperature can be influenced by nest orientation; however, the importance of nest orientation to ectoparasitism remains unclear. George (1959) found that the abundance of ectoparasites in European pied flycatcher (*Ficedula hypoleuca*) nests was highest in south and west-facing (i.e., warmer) nests. In a study of breeding great tits (*Parus major*), Goodenough et al. (2011) reported that south-southwest facing nest boxes were significantly warmer, used less frequently, and were associated with lower offspring condition than nest boxes in other orientations. However, there was no relationship between ectoparasite abundance and orientation-induced differences in nest box temperatures.

Ectoparasite abundance at nests often exerts a strong influence on nest-site choice for many avian species (Loye and Carroll 1998). Continued use of the same nest, or adjacent nests, can increase the abundance of nest ectoparasites and reduce nesting

success (Rothschild and Clay 1952; Bennett and Whitworth 1991). Many passerine birds have been shown to avoid infested nests, and select non-infested nests if a choice is available (Barclay 1988; Loye and Carroll 1998). Many raptor species defend territories that include multiple nests, and the use of alternate nests in successive years has been suggested as a mechanism to avoid ectoparasites (Philips and Dindal 1977; Wimberger 1984; Kochert and Steenhof 2012). For example, Ontiveros et al. (2008) found that dipteran ectoparasites have a negative effect on the breeding success of Bonelli's eagles (*Aquila fasciatus*), and eagle pairs that used alternate nests in successive years had greater breeding success, leading the authors to hypothesize that nest reuse may increase nest ectoparasites with negative fitness consequences.

Breeding phenology may affect exposure to ectoparasites during the nesting period. In temperate climates, earlier nesting pairs may face colder conditions during egg laying and early incubation stages, but breeding early may reduce the number of days nestlings are exposed to the warm conditions that promote ectoparasite activity. Ectoparasite abundance has been shown to increase throughout the breeding season, with negative fitness consequences for late-breeding birds (Brown and Brown 2015). Although relatively little is known about how ectoparasites affect breeding phenology, in passerines the level of parasitism at nests influences decisions of whether to initiate second broods (Møller 1990).

Modifications of nest sites through the manipulation of nest material may reduce the reproductive success, and therefore density, of obligate ectoparasites (Heeb et al. 2000). Many birds that regularly reuse nests add aromatic green plant material to their nests throughout the nesting period (Wimberger 1984; Clark and Mason 1985;

Lambrechts and Dos Santos 2000). One hypothesis for this behavior is the 'nest protection' hypothesis, which holds that phytochemical compounds in plants decrease nest parasites or pathogens and indirectly benefit nestlings (Clark 1991; Scott-Baumann and Morgan 2015). Specifically, secondary plant compounds, such as monoterpenes and phenolics, may disrupt or mask the olfactory and tactile cues ectoparasites use to find their hosts, or inhibit development or reproduction in ectoparasite species (Wimberger 1984; Clark and Mason 1988). Clark and Mason (1988) specified three criteria for the 'nest protection' hypothesis: 1) birds must select plants as nest material (i.e., plants are used at a higher proportion in the nest than they are available in the surrounding environment); 2) selected plants must contain more bioactive compounds relative to other available vegetation; and 3) selected plants must be effective at controlling the abundance, or moderating the effects, of ectoparasites.

The 'nest protection' hypothesis has been studied in several passerine species. For example, Clark and Mason (1985) found that European starlings (*Sturnus vulgaris*) preferentially selected plants with higher amounts of secondary compounds as nest lining, and suggested these materials may act as fumigants against parasites or pathogens. In the laboratory, Clark and Mason (1988) found that certain aromatic plants inhibited the feeding ability of the hematophagous northern fowl mite (*Ornithonysus sylviarum*), and suggested that the addition of aromatic plants in starling nests could mitigate the effects of ectoparasitism and benefit the hematological condition of nestlings. Similarly, Gwinner et al. (2000) experimentally controlled for the presence of aromatic plant material in starling nests. Although ectoparasite abundance was unaffected by treatment, nestlings raised in nests with aromatic material were heavier, had higher hematocrit

levels, and had higher post-fledging survival than nestlings raised in control nests, suggesting that aromatic materials may have mitigated the effects of the ectoparasites.

Many raptor species incorporate aromatic green plants into their nests (Wimberger 1984; Ontiveros et al. 2008; Dykstra et al. 2009). While the use of aromatic green plant material by raptors may signal territory occupancy or aid in nest sanitation by covering prey remains (Newton 1979), there is some support for the 'nest protection' hypothesis as an alternative explanation. For example, Bonelli's eagles in Spain select aromatic plants as nest material, and the amount of aromatic material in the nest is associated with a lower abundance of ectoparasitic blow fly (*Protocalliphora* sp.) larvae and increased breeding success of eagle pairs (Ontiveros et al. 2007). Golden eagles also regularly add green plant material to their nests throughout the breeding season (Watson 2010) and, in southwestern Idaho, native shrub-steppe plants contain aromatic secondary compounds that may interact with nest ectoparasites.

Nests of raptor species provide suitable habitat for an array of arthropods due to the large nest volume and the continued reuse of nests over multiple breeding seasons (Philips and Dindal 1977). Ectoparasites associated with raptors include blood-sucking flies, fleas, ticks, and bugs, as well as feather-feeding lice and mites (Philips 2007). The effects of ectoparasite infestation on raptors vary, but hematophagous ectoparasites may increase the costs associated with reproduction by reducing the development or survival of nestlings (McFadzen and Marzluff 1996).

McFadzen et al. (1996) published the first report of Mexican chicken bugs (*Haematosiphon inodorus*; Hemiptera: Cimicidae) in prairie falcon (*Falco mexicanus*) scrapes in the Morley Nelson Snake River Birds of Prey National Conservation Area

(NCA) in southwestern Idaho, which at the time was the northern-most record for this species. Although heavy infestations of cimicid ectoparasites were noted in golden eagle and other raptor nests in southwestern Idaho during the late 1960s and early 1970s (Hickman 1968; M. Kochert pers. comm.), *H. inodorus* represents a relatively new addition to the ectoparasite community. *Haematosiphon inodorus* live in the nest material of raptors and surrounding cliff walls and, while the biology of the insect is well studied (Lee 1955; Usinger 1966), there is little known about distribution and abundance of *H. inodorus* in relation to nesting eagles in southwestern Idaho.

Nests appear to be a limiting factor for *H. inodorus* and cliff-nesting birds may readily exchange ectoparasites through the successive use or synchronous use of adjacent nests (Wilson and Oliver 1978). Both adult and nymph cimicid bugs have been reported emigrating locally from nearby nests into raptor nests in search of available hosts (Santillán et al. 2009). Although the maximum dispersal distance of *H. inodorus* across cliff systems is unknown, we observed bugs on the canyon rim at least 50 m from occupied nests after eagle nestlings have fledged, indicating significant dispersal ability. Given the close proximity of cliff swallow colonies to eagle nests, and the fact that swallows are already natural hosts for swallow bugs (*Oeciacus vicarious*), a hematophagous ectoparasite in the Cimicidae family, it is possible that cliff swallow nests could serve as an alternative host for *H. inodorus*. Fassbinder-Orth et al. (2013) reported swallow bugs switching hosts from cliff swallows to nearby nesting house sparrows (*Passer domesticus*), which may extend the seasonal activity of the bugs.

Golden eagle nesting ecology has been well studied in the NCA since the late 1960s (Kochert and Steenhof 2012). Although researchers noted the presence of

ectoparasites in golden eagle nests (Hickman 1968; Kochert 1972), there have been no attempts quantify their abundance, understand the factors that predict infestation, or assess the role aromatic green nest material might serve to moderate the effects of ectoparasitism. Therefore, our objectives in the present study were to: 1) document and describe the ectoparasite community in golden eagle nests when nestlings were present; 2) specifically quantify the abundance of *H. inodorus* in eagle nests; 3) evaluate factors that could predict *H. inodorus* abundance such as previous nest use, nest aspect, nearby cliff-nesting species, proportion of aromatic green nest material, and nest phenology; and 4) determine whether eagles select aromatic green nest material during the nesting period, and if so, assess whether these materials reduce *H. inodorus* abundance in the nest, moderate the effects of ectoparasitism on nestlings, or both.

Methods

Study Area

Our study area was located along the Snake River Canyon in the NCA and the adjacent Upstream Comparison Area (UCA), which extends east from the NCA to Hagerman, Idaho (Figure 2.1). The steep basalt cliffs of the Snake River Canyon provide nesting locations for golden eagles. The surrounding uplands are a mosaic of land cover types and habitats that include native shrub-steppe and salt-desert communities characterized by big sagebrush (*Artemisia tridentata*), shadscale (*Atriplex confertifolia*), gray rabbitbrush (*Ericameria nauseosa*), and disturbed grasslands and rangeland dominated by exotic annual grasses, native perennial grasses, irrigated agricultural land, and rural and suburban development (U.S. Department of the Interior 1996).

Field Techniques

From March through July 2016, we monitored 16 golden eagle nesting territories. Most nesting territories in our study area have been monitored for more than four decades (Kochert and Steenhof 2012). We visited study nests every 10 days beginning when nestlings were approximately 21 days old, the age at which young eagles are able to thermoregulate (Kochert et al. 2002), until nestlings were approximately 51 days old. On the first nest visit, we placed SenSci ActivVolcano Bed Bug Detector® (SenSci, Lawrenceville, NJ, USA) traps in nests. These devices are small pitfall traps with a scent lure designed to attract and capture common bed bugs (*Cimex lectularius*). We placed three traps in each nest approximately 10 cm beneath the top surface of nest material. Traps were placed along the nest edge that abutted the cliff face and covered with a small cardboard 'tent' that prevented nest material from falling into the trap. Photographs with mapped locations of the traps aided the recovery of traps during the next visit. Traps were collected during each subsequent visit and replaced with fresh traps. Collected traps were placed individually in sealable plastic bags and stored in the freezer until the end of the season when trap contents were evaluated.

In addition to collecting *H. inodorus* abundance data through the use of traps, we grouped levels of infestation into three categories based on visual observations of adult and nymph bugs in the nest and on nestlings: (1) no infestation: no bugs observed in the nest material or on nestlings; (2) low infestation: 1 – 10 bugs observed in total; or (3) high infestation: >10 bugs observed. Visual categorical rankings of infestation were made independent of trap counts. Twice during the nestling period, when nestlings were 4 and 7 weeks old, we used a 25-gauge needle to withdraw blood from the brachial vein of

nestlings to measure nestling hematocrit. Blood was drawn into two heparinized capillary tubes and, within eight hours, was centrifuged for 6 minutes at 10,000 rpm. We recorded the proportion of packed red blood cells in the total blood volume and used the mean of two values to represent hematocrit for each nestling during each visit. All field methods followed protocols approved by the Boise State University Institutional Animal Care and Use Committee (Protocol #006-AC14-007).

We obtained historical occupancy data on each study territory from U.S. Geological Survey records at the Snake River Field Station to determine the year each nest was last used by an eagle pair, termed 'previous nest use'. During nest entries, we measured nest aspect as the magnetic azimuth of a line radiating directly away from the center of the nest. We conducted surveys of the surrounding cliff-nesting community by analyzing photographs taken from observation points approximately 400 m away from occupied nests. From each photograph we estimated a 50 m buffer around the nest in all directions and counted the number of cliff swallow nests that were located within that buffer. We collected photographs 3 m directly above the surface of eagle nests and analyzed the images with the Program SamplePoint (Booth et al. 2015) to determine the proportion of the nest surface covered by aromatic green plants during each visit. We used nest material collections and additional reference photographs to identify green plant species in each image. For each nest visit, we pooled the focal plant species with known aromatic compounds (i.e., big sagebrush, gray rabbitbrush, green rabbitbrush (*Chrysothamnus viscidiflorus*) and spiny hop sage (*Grayia spinosa*)) in order to calculate the proportion of the nest surface covered by aromatic green plants. We conducted 100 m line-intercept transects within a 1 km radius around nest sites to determine cover of each

focal shrub within each eagle territory (Floyd and Anderson 1987). We used ArcMap 10.2 to create 1 km radius buffers around each nest, identified areas dominated by shrubs with the 1 km radius using the National Land Cover Database (Homer et al. 2015), and randomly generated transect origin points within these shrub areas. We calculated the percent shrub cover within the 1 km radius of each nest, and conducted a line intercept transect for every 5% of shrub cover (157,079 m^2) within the 1 km radius.

Plant Chemical Analysis

We measured the phenolic compounds of the four focal aromatic plant species observed and collected from nests. We collected fresh clippings of plant vegetative growth from within 1 km buffers of occupied 2016 nests and assessed total phenolic content using colorimetric assays of whole leaf extracts. Samples (0.5 g wet weight) were extracted for two 3-minute periods in 10 mL GC-grade methanol in a sonicating water bath and filtered through glass wool. An adapted Folin-Ciocalteu assay (Ainsworth and Gillespie 2007) was used, where samples were diluted with methanol (1:3) to fit within the standard curve. Gallic acid (#92-6-15, Acros Organics) diluted in methanol was used as a standard (0 to 2900 μM). For each sample extract and standard, 20 μl of the dilution was pipetted in triplicate into 96-well plates. Next, 100 μl of 10% Folin-Ciocalteu reagent was added to each well, mixed gently, and 80 μl of 700 μM (7.5%) sodium carbonate was added and mixed. Plates were allowed to incubate at room temperature for 2 hours, and then were shaken on the plate reader (BioTek Synergy MX multi-mode plate reader) for 60 seconds before reading at an absorbance of 765 nm at room temperature.

Statistical Analysis

We used a generalized linear mixed model (GLMM) with a negative binomial distribution to compare categorical infestation levels to absolute pitfall trap counts. We found parameter estimates of each infestation level were not contained within the 95% confidence intervals of other levels, thus each rank was significantly different. This model had an offset for the number of trap days. Territory identity was included as a random variable. Because this approach validated our use of trap counts to assess *H. inodorus* abundance, we used trap counts as the response variable in subsequent analyses. We used multiple GLMM with negative binomial distributions to assess the influence of previous nest use, nest aspect, neighboring cliff swallow nests, proportion of aromatic green nest material, nest phenology, and sampling date (to control for the time of year samples were collected) on *H. inodorus* abundance. Models had an offset for the number of trap days. Territory identity was included as a random variable in all models. We created a categorical variable for the previous nest use by grouping nests into two categories: used in the previous three years prior to 2016 or not used in the previous three years. We selected three years because closely related swallow bugs have been found to survive in cliff swallow nests not used by swallows for three consecutive years (Loye 1985), so we predicted that nests reused within three years would have higher *H. inodorus* abundance. We created a categorical variable for nest aspect by grouping nests into two categories: north-facing and south-facing. Nest phenology was represented by the estimated hatch date of an egg at each nest. If there was more than one egg at a nest, we used the median estimated hatch date of all eggs. All numerical predictors were scaled and centered before analysis. We conducted an exploratory analysis of covariates

predicting *H. inodorus* abundance by fitting a full model that included all covariates and then sequentially eliminating the covariate with the lowest effect size and largest variance until no additional covariate could be eliminated without leading to an increase in Akaike's information criterion (AIC; Pagano and Arnold 2009). Models with $\Delta AIC < 2$ were considered to have the most support and variables with 85% confidence intervals that did not overlap zero were biologically informative (Arnold 2010).

Unfortunately, we did not have *H. inodorus* abundance data for each blood-sampling event to determine the effect of abundance on nestling hematocrit. Instead, we used categorical rankings of infestation levels to assess the effects of *H. inodorus* on nestling hematocrit. We used linear mixed models and an AIC model selection approach to assess the effects of the proportion of aromatic green nest material and *H. inodorus* infestation level on nestling hematocrit levels. To account for the association between proportion of aromatic green nest material and *H. inodorus* infestation level, we used a GLMM to generate the residuals of aromatic plant-adjusted infestation level and used the residuals in the model. We evaluated models using AIC and considered models with the lowest AIC score to have the most support given the data.

Aromatic green plant selection was measured by Manly's selectivity index (MSI) design III, which computes selectivity for each pair of eagles, to calculate the selection of the four focal aromatic shrub species based on use (i.e., brought to the nest as nest material) vs. presence (i.e., availability of shrubs within a 1 km radius of the nest site). MSI allows the testing of preference vs. avoidance and tests differences between selection ratios. MSI was calculated across all territories and was evaluated using 85% confidence intervals. Shrub species with test statistics and confidence intervals > 1 were

selected and < 1 were avoided (Manly et al. 2002). All analyses were performed in R (version 3.2.2, R Core Development Team 2016). Linear models were created using functions lmer and glmer in the package lme4 (Bates et al. 2015) and function glmmADMB in the package glmmADMB (Fournier et al. 2012). Descriptive statistics are reported as mean ± standard deviation.

Results

Ten insect families representing six orders, as well as spiders, ticks, and a scorpion, were collected from the pitfall traps placed within our 16 golden eagle nests (Table 2.1). Most of the arthropods collected were either larval or adult scavenging Coleopterans that feed primarily on decaying prey remains and detritus in the nest. The two most common ectoparasites of eagle nestlings were blow flies (Diptera: Calliphoridae) and *H. inodorus*. Myiasis, caused by blowflies, was observed typically within the first 5 weeks in the nestling period, at which point larvae exited nestlings, pupated, and emerged as adult flies. We observed myiasis in 9 of 26 (35%) nestlings from 7 of 16 (44%) nests. *Haematosiphon inodorus* were detected in 14 of 16 eagle nests while nestlings were present, and in one additional nest after the nestlings fledged. Although median date of first *H. inodorus* detection in the nest was 12 May, dates of first detection ranged substantially (21 April – 22 May). We collected 2,712 *H. inodorus* from pitfall traps in nests, 85.8% (n = 2,327) of which were nymphs. Pit fall traps had been moved deep into a cliff alcove at one nest, likely by a resident woodrat (*Neotoma sp.*). Traps at this nest did not capture a representative sample of *H. inodorus* abundance and were thus removed from subsequent analyses. Relative *H. inodorus* abundance at the remaining 15 nests varied by nest, and ranged from 0.03 - 13.5 bugs trap^{-1} night^{-1}. These

values were consistent with the categorical infestation levels we assigned during each visit (Figure 2.2). Although abundance of *H. inodorus* at nests generally increased throughout the nesting period, fluctuations in bug populations caused this trend to be uninformative because the confidence intervals of the model overlapped zero (β = 0.09, CI = -0.2, 0.3).

Twelve nests in our study had been used previously by golden eagle nesting pairs within the last 1 – 10 years. Additionally, three study nests were 'new' to the long-term study, and had not been used by eagles in at least 40 years. Eight nests had been used within 3 years and the remaining seven had not been used in >3 years. Nest aspect varied among nests; nine nests faced southeast, south, or southwest, and six nests faced northwest, north, or northeast. Hatch dates at study nests averaged 4 April ± 7.3 days (range 28 March – 20 April). Number of cliff swallow nests within a 50 m buffer of eagle nests averaged 24.5 ± 29.1 (range 0 – 342). Mean proportion of the nest surface covered in aromatic green material during a single visit was 0.04 ± 0.05 (range 0 – 0.16).

The top model for explaining *H. inodorus* abundance included previous nest use, nest aspect, and nest phenology (Table 2.2). Nests reused in within the previous three years were less infested than nests that had not been used in the previous years (β = -2.3, CI = -3.9, -0.6). These results were opposite of our prediction that recent nest use would lead to increased *H. inodorus* abundance. We found higher *H. inodorus* abundance in south-facing nests compared to north-facing nests (β = 1.7, CI = 0.2, 3.3). Nests with a median hatch date later in the season had higher *H. inodorus* abundance than nests with earlier hatch dates (β = 0.9, CI = 0.2, 1.7). The second-most supported model (ΔAIC) contained the same variables as the top model plus the variable 'proportion of aromatic

green nest material'. Aromatic green nest material tended to reduce *H. inodorus* abundance (β = -0.4), but the 85% CI included zero suggesting the direction of the effect is unreliable (CI = -0.8, 0.04).

Green nest material in nests was represented primarily by the four focal aromatic shrub species: big sagebrush, gray rabbitbrush, green rabbitbrush, and spiny hop sage. These shrubs accounted for 72% of all green plant material observed in eagle nests. The proportion of land covered by shrubs within 1 km of eagle nests was variable (0.46 ± 0.25). Seven native shrub species were found within shrub areas, and included the four aromatic shrubs identified earlier plus shadscale, horsebrush (*Tetradymia canescens*), golden currant (*Ribes aureum*). Eagles used gray rabbitbrush for their nests in greater proportion than the proportion available within a 1 km radius of the nests (W_i = 2.53, CI = 1.2, 3.8; Figure 2.3), and used big sagebrush less frequently compared to its availability (W_i = 0.18, CI = 0.02, 0.4). Both green rabbitbrush (W_i = 1.37, CI = 0.9, 1.9) and spiny hop sage (W_i = 1.59, CI = -0.5, 3.7) were used in proportion to their availability on the landscape. In terms of total phenolic content, both gray rabbitbrush and green rabbitbrush had relatively high concentrations of phenolics (325,443 AUC and 287,614 AUC, respectively), which reflect potential insecticidal properties of these species. By contrast, big sagebrush and spiny hop sage contained relatively lower phenolic concentrations (99,533 AUC and 26,650 AUC, respectively).

Mean nestling hematocrit during the nestling period was 0.28 ± 0.06 (range 0.12 – 0.46). Nestling hematocrit was best explained by both *H. inodorus* infestation level and proportion of aromatic green nest material (Table 2.3). *Haematosiphon inodorus* infestation level had a negative effect on nestling hematocrit (β = -0.03, CI = -0.04, -

0.007); nestlings in most infested nests had significantly lower hematocrit levels than nestlings in nests with low or no *H. inodorus* infestation. By contrast, the proportion of aromatic green material in nests had a positive effect on nestling hematocrit ($\beta = 0.004$, CI = 0.0009, 0.008, Figure 2.5). These results suggest that even at nests with high *H. inodorus* infestation, aromatic green plants had a positive effect on nestling health, consistent with the predictions of the 'nest protection' hypothesis.

Discussion

Ectoparasites can impair growth and survival and represent a significant threat to the fitness of raptor nestlings of many species (Delannoy and Cruz 1991; Smith et al. 1998). Severe infestations of ectoparasites can reduce nestling mass and hematocrit levels, and repeated biting can lead to hemorrhages, muscle weakness, and chronic stress of nestlings (McFadzen and Marzluff 1996; Justice-Allen et al. 2016). The presence of *H. inodorus* in raptor nests has been documented previously (Platt 1975; Grubb et al. 1986; McFadzen and Marzluff 1996); however, no studies have yet attempted to quantify abundance or identify the factors that predict abundance at nests. We documented a diverse arthropod fauna in golden eagle nests in southwestern Idaho and developed the use of pit fall traps as an efficient method to measure the relative abundance of nest ectoparasites. Using this method, we show specific nest reuse, aspects, and the timing of breeding phenology influenced *H. inodorus* abundance in nests. Aromatic green nest material tended to be negatively associated with *H. inodorus* abundance and had a positive effect on nestling hematocrit. Our results suggest that eagles modify nests through the addition of aromatic green plant material to moderate the effects of *H. inodorus* ectoparasitism on the potential health (e.g., hematocrit) of their offspring.

Raptor nests are a microcosm for arthropod communities that include both free-living and parasitic species (Heeb et al. 2000). Within golden eagle nests we found a diversity of arthropods, primarily scavenging beetles, but also leaf beetles, plume moths, ants, grasshoppers, ticks, spiders, and a scorpion. Of the scavenging beetles, dermestids, histerids, clerids, and staphylinids were relatively common. Dermestidae beetles are important for the decomposition of animal prey remains in raptor nests (Philips and Dindal 1977), but when abundant, larvae may prey on nestlings (Rothschild and Clay 1952). Beetles in the families Histeridae, Cleridae, and Staphylinidae are scavengers that feed on prey remains, but also may prey on fleas and other insects and, therefore, could be important in determining the abundance of nest ectoparasites (Philips and Dindal 1977).

Although many arthropods associated with the nests of eagles, and other raptor species, are obligate scavengers, we documented two ectoparasites, blow flies and *H. inodorus,* on golden eagle nestlings. Blow fly larvae parasitize raptor nestlings by entering the body near the nares, ears, wing pits and leg pits. Although White (1963) and (Kochert 1972) reported nestling mortality associated with blow fly infestations, most incidents of myiasis are not fatal (Sargent 1938; M. Kochert pers. comm.). We observed myiasis in 35% of nestlings, however, lesions caused by blow fly larvae were small and healed before nestlings were 35 days old.

Haematosiphon inodorus was the second-most numerous type of insect collected from pitfall traps after scavenging beetle larvae, although abundance of *H. inodorus* varied considerably among nests. We found eagles reuse nests with lower *H. inodorus* abundance, potentially to avoid selecting nests with high ectoparasite loads. Reusing

nests with low levels of infestation may be a behavioral response to avoid or reduce the negative effects of ectoparasitism on the survival and condition of offspring (Moore 2002). Ectoparasite abundance exerts strong influence on nest selection in many avian species (Loye and Carroll 1998). For example, colonially nesting cliff swallows tend to avoid nesting in areas with previously high ectoparasite abundance (Brown and Brown 1991) and barn swallows (*Hirundo rustica*) that reuse nests appear able to assess parasite load and select old nests that are not parasitized (Barclay 1988).

In addition to previous nest use, nest aspect and breeding phenology explained *H. inodorus* abundance in golden eagle nests. South-facing nests had higher *H. inodorus* abundance relative to north-facing nests, suggesting that a southern aspect, which may create warmer nest microclimates (Goodenough et al. 2011), had a positive effect on *H. inodorus* populations. Local spring and summer temperatures and humidity at nests can be a function of orientation and many ectoparasitic insects spend less time in larval and pupal stages as ambient temperatures increase (Bennett and Whitworth 1991). Further, our results suggest eagle pairs that breed later in the season are more likely to experience high *H. inodorus* abundance than eagles that breed earlier in the season. Our results could be explained by an increase in warm days, as the season progresses, that nestlings spend in the nest prior to fledge. Warmer temperatures in late spring may allow *H. inodorus* populations to increase in abundance while nestlings are present in the nest. Brown and Brown (2015) demonstrated that swallow bugs increase throughout the breeding season, increasing the cost of parasitism for later nesting birds.

How *H. inodorus* reached southwestern Idaho and how populations continue to move through the cliff ecosystem of the Snake River Canyon is unknown. Many of the

known avian hosts of *H. inodorus* nest over ranges that exceed the current distribution of the parasite. Although climate is likely a limiting factor in the geographical distribution of *H. inodorus*, the proximity of cliff shelters could also be an important factor that governs local distributions. *Haematosiphon inodorus* are highly mobile and are rarely found on raptors away from nest sites (Lee 1955), but the extent of their dispersal movements to find new hosts is not known. Wilson and Oliver (1978) found the bugs on a turkey vulture (*Cathartes aura*) away from a nest site early in the breeding season, suggesting bugs may be transported from nests by hosts when raptors are investigating nest sites prior to breeding. Loye (1985) suggested swallow bugs may be transported on adult swallows to colonize new colonies located several miles away, but also observed bugs crawling across the cliff faces towards adjacent nests. Nearby cliff-nesting species, such as prairie falcons, common ravens (*Corvus corax*), cliff swallows, and rock pigeons (*Columba livia*), are likely come into contact with *H. inodorus*, and may also facilitate the life history and distribution of the ectoparasite (Santillán et al. 2009). Additionally, *H. inodorus* may be brought to eagle nests along with avian prey, such as rock pigeons and prairie falcons (Philips and Dindal 1977).

Negative effects of ectoparasites on host fitness can drive the evolution of a wide variety of host defenses that involve a range of nest maintenance behaviors, which include the addition of aromatic green nest material. We found that eagles selected nest material with high phenolic concentrations, and while these plants were not associated with lower abundance of *H. inodorus* in nests, higher proportions of aromatic material had a positive effect on nestling hematocrit values. Thus our results suggest that the addition of aromatic plants in nests by golden eagles may not directly reduce

ectoparasites in nests, but may instead disrupt the cues these ectoparasites use to locate hosts, inhibit feeding by ectoparasites, or delay reproduction or development of the ectoparasites (Clark and Mason 1988). Additionally, Mennerat et al. (2009) suggested aromatic plants might stimulate host immune systems to the physiological benefit of eagle nestlings. Developing young birds face a trade-off between growth and immune function (Brommer 2004), therefore mechanisms that improve immune function could improve nestling growth rates and hematocrit.

Although our results suggest addition of aromatic plants to nests may benefit golden eagle nestlings, availability of these shrubs for nest material has been adversely affected by human activity. Across the American West, sagebrush steppe communities have been degraded and fragmented by anthropogenic activities (Fleischner 1994; Leu et al. 2008). Within the NCA, wildfire, livestock grazing, drought, and the spread of exotic invasive plants (U.S. Department of the Interior 1996) have reduced shrub availability within eagle territories and surrounding areas, which could reduce the ability of eagle pairs to add beneficial shrubs to nests during the nesting period to moderate ectoparasitism. Future landscape-level change that reduces shrub cover in sagebrush steppe ecosystems may indirectly affect the degree of ectoparasitism experienced by eagle nestlings.

Although parasites are not generally among the most important limiting factors for avian populations, costs of parasitism are important to individual hosts when they lower reproductive success or the probability of survival (Newton 1998). Climate change will likely produce a range of effects on the distribution and abundance of parasite species (Stange and Ayres 2010), but, in addition to changes in climate, we show that

landscape-level land cover change that limits the availability of shrubs has the potential to affect the health of golden eagle nestlings in southwestern Idaho. In Chapter 3, we document the physiological effects of *H. inodorus* ectoparasitism on the condition and development of eagle nestlings. Given the negative impacts of *H. inodorus* infestation on nestling mass, hematocrit, and circulating corticosterone, it is important to understand the factors associated with *H. inodorus* abundance. This is the first study to quantify the abundance of *H. inodorus* in golden eagle nests throughout the breeding season, assess factors that predict infestation, and test the conditions of the 'nest protection' hypothesis. In a time of increased global change, it will be essential to continue to monitor and study the relationships between host-parasite interactions in this system for the conservation of golden eagle populations.

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Table 2.1 List of arthropods, life stages, and the proportion of nests containing each family or order of arthropod in 16 golden eagle nests with nestlings, April – June 2016 in southwestern Idaho, USA. Life stages are represented as: *l* – larva, *n* – nymph, *p* – pupa, *a* – adult).

Order	Family	Life Stages	Proportion of Nests
Coleoptera	Dermestidae	l, p, a	1.00
	Histeridae	l, p, a	0.94
	Cleridae	a	0.19
	Staphylinidae	a	0.06
	Chrysomelidae	a	0.06
Hemiptera	Cimicidae	n, a	0.94
Diptera	Calliphoridae	l, p, a	0.25
Hymenoptera	Formicidae	a	0.25
Lepidoptera	Pterophoridae	a	0.06
Orthoptera	Acrididae	a	0.06
Acari		a	0.31
Araneae		a	0.25
Scorpiones		a	0.06

Table 2.2 Candidate models, number of parameters (*K*), delta AIC (ΔAIC), and model weights (AICwi) used to explain cimicid abundance per trap day in 15 golden eagle nests in southwestern Idaho, USA during the 2016 breeding season. See results for effect estimates and confidence intervals.

Model	*K*	ΔAIC	AICw_i
nest_use + nest_aspect + nest_phenology[a]	4	0	0.34
nest_use + nest_aspect + nest_phenology + proportion_aromatic	5	0.4	0.27
intercept	1	0.8	0.23
nest_use + nest_aspect + nest_phenology + proportion_aromatic + neighboring_cliff_swallows	6	2.2	0.11
nest_use + nest_aspect + nest_phenology + proportion_aromatic + neighboring_cliff_swallows + sampling_date	7	3.9	0.05

[a]AIC of top model = 358.0

Table 2.3 Candidate models, number of parameters (*K*), delta AIC (ΔAIC), and model weights (AICw_i) for models used to explain nestling hematocrit in 15 golden eagle nests in southwestern Idaho, USA during the 2016 breeding season. See results for effect estimates and confidence intervals.

Model	*K*	ΔAIC	AICw_i
proportion_aromatic + infestation_level	6	0	0.43
infestation_level	5	0.76	0.30
proportion_aromatic	5	1.36	0.22
intercept	4	4.13	0.05

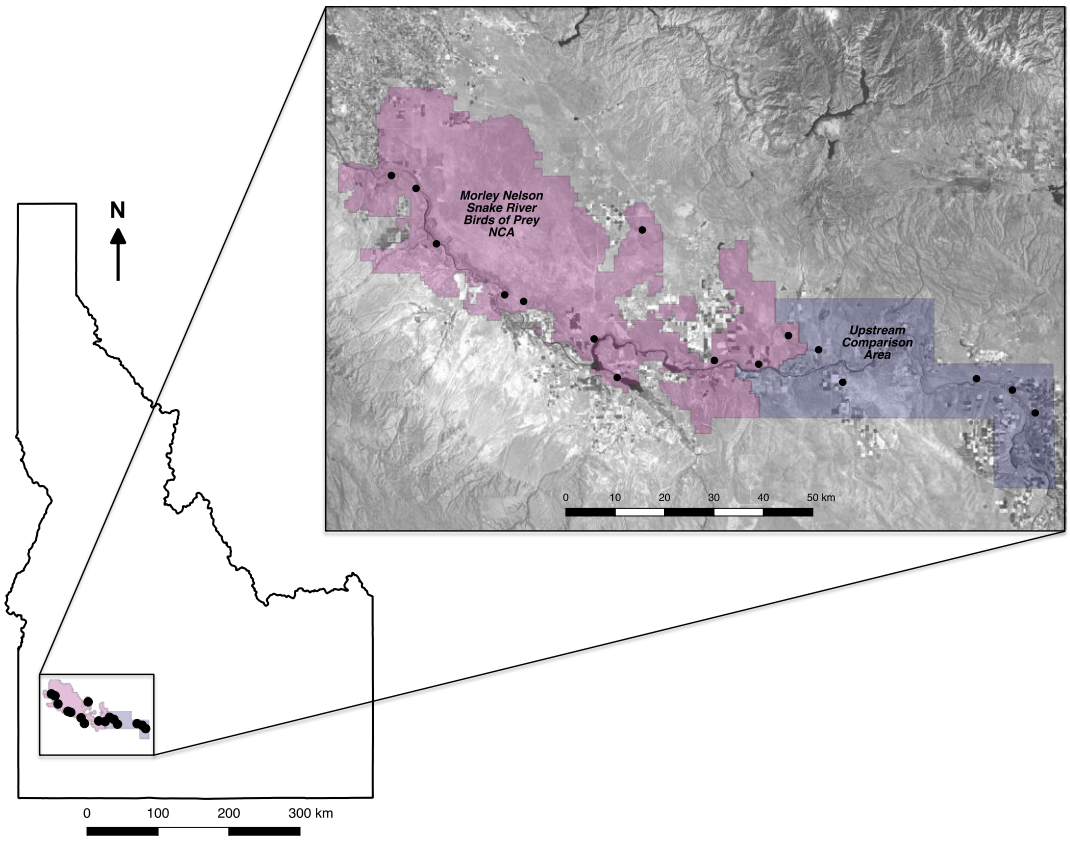

Figure 2.1 Location of 15 golden eagle nests for the *H. inodorus* abundance study nests in the Morley Nelson Snake River Birds of Prey National Conservation Area and Upstream Comparison Area, Idaho, USA in 2016.

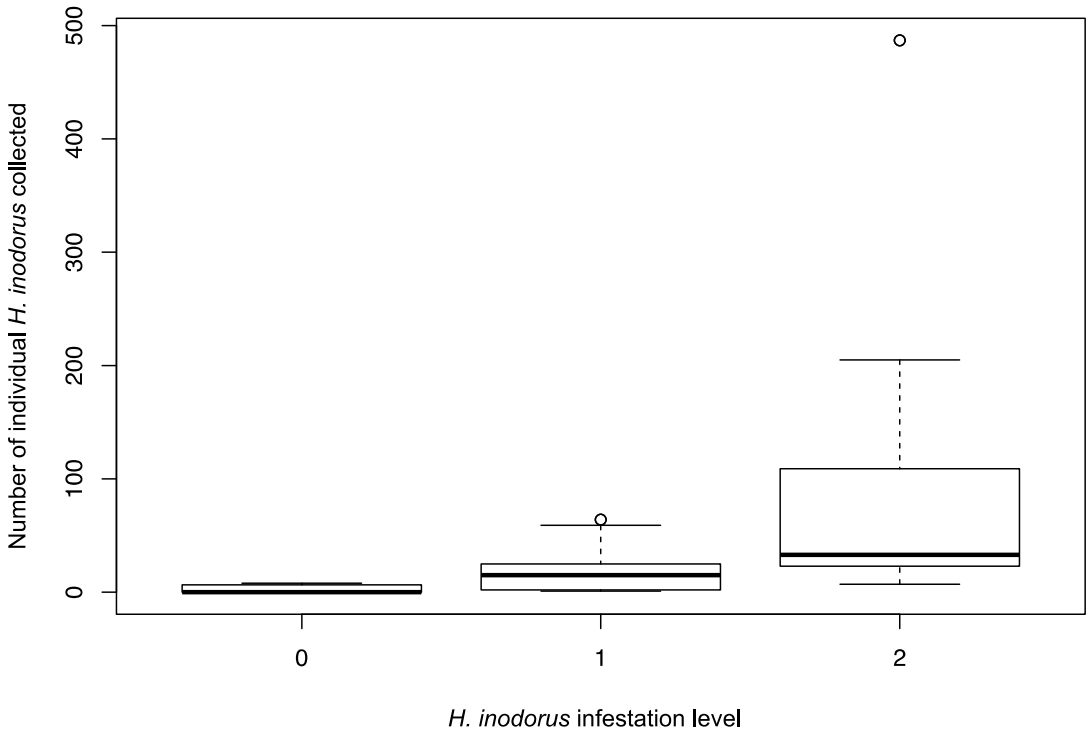

Figure 2.2 Comparison of H. inodorus abundance determined from pitfall traps with H. inodorus infestation level based on visual assessments in 15 golden eagle nests in southwestern Idaho, USA in 2016. Bold lines within boxes represent the median, upper and lower limits of the box are the first and third quartiles, whiskers contain 1.5 times the interquartile range, and open circles are outliers. Parameter estimates of each infestation level were not contained within the 95% confidence intervals of other levels, thus each rank was significantly different.

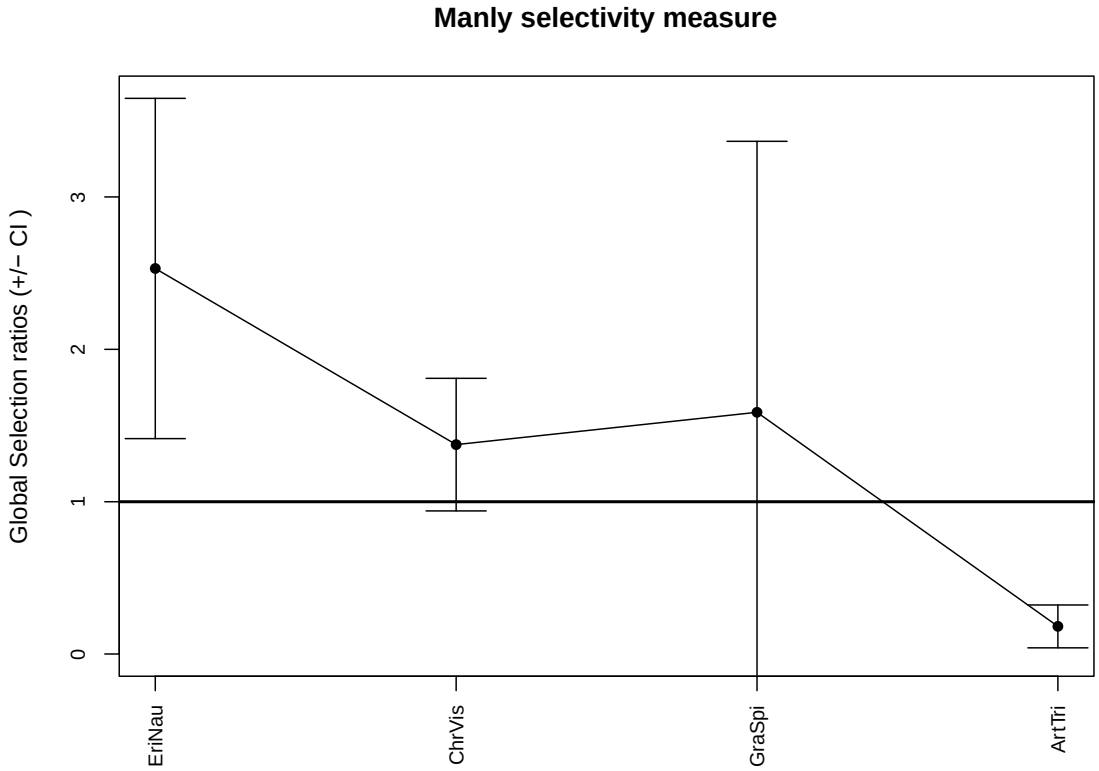

Figure 2.3 Manly's selectivity index (design III) for four aromatic shrub species used by golden eagles in southwestern Idaho, USA in 2016. Selectivity index for shrubs used in 16 nests, and found on the landscape within a 1 km radius of nests. Aromatic shrubs include gray rabbitbrush (EriNau), green rabbitbrush (ChrVis), spiny hop sage (GraSpi), and big sagebrush (ArtTri). Where index is larger than the value 1, selectivity for that shrub in the nest is greater than its availability on the landscape. Error bars indicate 85% confidence intervals.

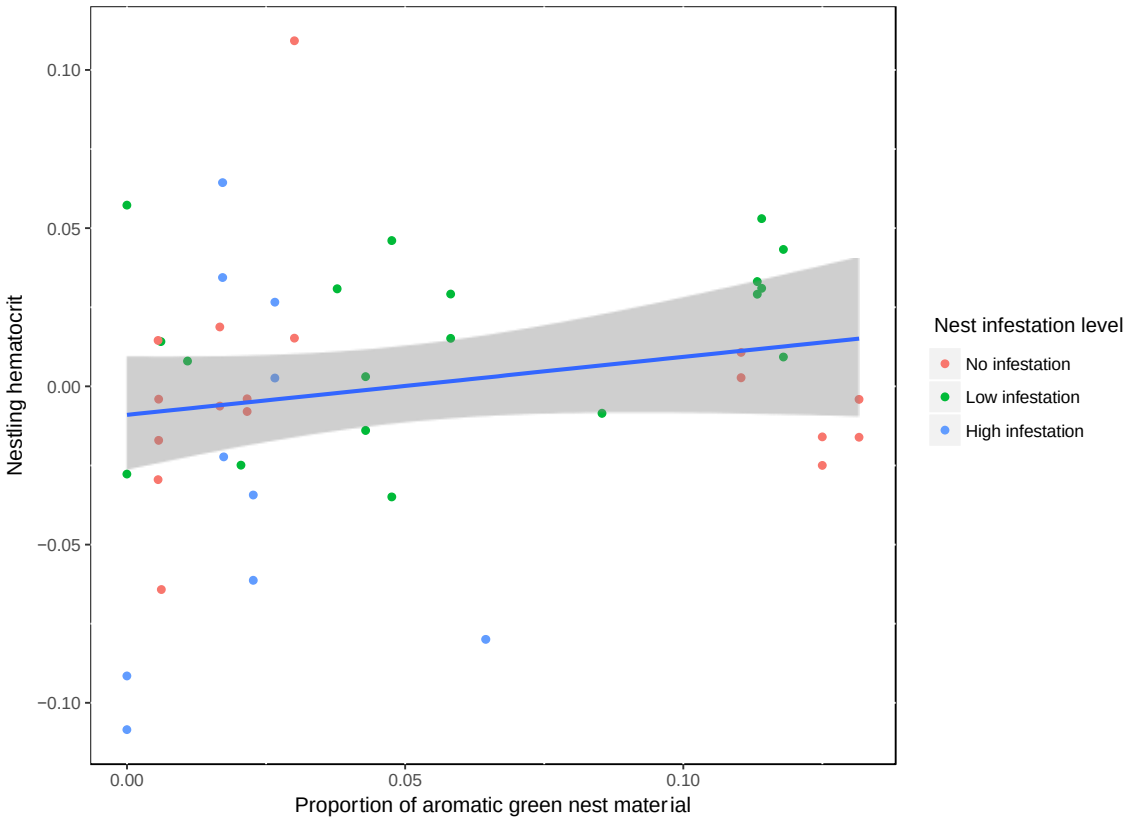

Figure 2.4 Observed nestling hematocrit at different nest infestation levels (colored circles, scaled to remove the effect of infestation level), predicted hematocrit (blue line) and associated 85% confidence intervals (filled gray area) at 16 nests with no, low, and high infestation levels in southwestern Idaho, USA in 2016. The proportion of aromatic green nest material in golden eagle nests had a positive effect on nestling hematocrit (β = 0.004, CI = 0.0009, 0.008).

PHYSIOLOGICAL EFFECTS OF HEMATOPHAGOUS ECTOPARASITES ON GOLDEN EAGLE NESTLINGS

Abstract

Hematophagous ectoparasites can have direct effects on animals by increasing energetic costs through the reduction of resources for growth, development, and survival. Prolonged ectoparasitism has been associated with the release of glucocorticoid hormones, which facilitate short-term survival at the expense of body condition and development, particularly in long-lived species. We studied the physiological effects of Mexican chicken bugs (*Haematosiphon inodorus*; Hemiptera: Cimicidae) on golden eagle (*Aquila chrysaetos*) nestlings in southwestern Idaho to understand the costs associated with ectoparasitism. Golden eagles are a widespread but uncommon raptor species facing threats across their North American range. Our goal was to assess the costs associated with ectoparasite infestation through an examination of nestling mass, hematocrit levels, and timing of fledging. In addition, we validated an ELISA assay to describe circulating corticosterone concentrations in eagle nestlings, and we investigated the physiological impact of ectoparasitism on nestling corticosterone because elevated corticosterone during development may influence the timing of fledging behavior with negative consequences for young eagles. We found that increased ectoparasitism reduced nestling mass and hematocrit and increased the probability that nestlings either fledged early or died in the nest. Relative ectoparasite infestation levels predicted circulating concentrations of corticosterone in eagle nestlings, and heavily parasitized nestlings had

higher corticosterone levels compared to non-parasitized nestlings. This is the first report of circulating corticosterone concentrations for golden eagles, and we show the presence and intensity of ectoparasitism affects the physiological condition of young eagles. Understanding the effects of ectoparasites on golden eagle productivity may be important for the conservation of local breeding populations severely impacted by ectoparasites.

Introduction

Avian populations are vulnerable to a variety of hematophagous ectoparasites that can reduce the reproductive success of their hosts by limiting health, growth, and the survival of nestlings (Brown and Brown 1986; Møller 1993). The nestling period in birds is energetically expensive with rapid structural growth and maturation of physiological systems. Ectoparasite infestation can impact development by competing with hosts for resources that could otherwise be used for growth and maintenance (Pryor and Casto 2015). Nest-bound nestlings in cliff nests may be particularly susceptible to ectoparasitism because of their limited ability to evade parasites living in the nest. In addition, exposure to ecological stressors, like ectoparasitism, can trigger a stress response through the release of glucocorticoid hormones (Raouf et al. 2006). Corticosterone is the primary avian stress hormone, and increased levels of circulating corticosterone can be beneficial in the short-term by encouraging behaviors such as increasing begging for food to facilitate survival (Kitaysky et al. 2003). However, prolonged exposure to elevated corticosterone levels, in response to ectoparasitism, can severely impede nestling growth and development and may have long-term detrimental effects on survival (Raouf et al. 2006). Because ectoparasites incur negative effects on their hosts, understanding how host-parasite interactions influence host condition,

development, and physiology is important for the conservation and management of threatened avian species.

Hematophagous ectoparasites associated with birds include blood-sucking flies, fleas, ticks, and hemipteran insects in the family Cimicidae (Philips 2007). Commonly referred to as bed bugs, cimicids can have a significant impact on their hosts because both adults and nymphs require blood meals (Usinger 1966). Cimicids are typically associated with colonial-nesting birds like swallows and swifts (Brown and Brown 1986; Loye and Regan 1991), but they have also been observed in raptor nests (Hickman 1968; Platt 1975; Sitter 1983; Grubb et al. 1986). Many ectoparasite species expanding or shifting their spatial distributions in response to climate change (Møller et al. 2013), and introductions of new cimicid species to raptor populations have recently been documented (McFadzen et al. 1996; Santillán et al. 2009). McFadzen et al. (1996) first reported the presence of Mexican chicken bugs (*Haematosiphon inodorus*) in the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA) in southwestern Idaho, which at the time was the northern-most record for this species. *Haematosiphon inodorus* lives in nest material and surrounding substrate (Usinger 1966). Individuals are extremely active, feed diurnally and nocturnally, and both adults and nymphs will emigrate in search of available hosts (Lee 1955).

Repeated biting of ectoparasites can lead to hemorrhages, muscle weakness, and chronic stress of nestlings (Brown et al. 1995; Eggert et al. 2010; Justice-Allen et al. 2016). Sever infestation can adversely affect nestling mass, hematocrit, and survival (Brown and Brown 1986; Chapman and George 1991; Richner et al. 1993; Merino and Potti 1995; Heeb et al. 2000). Although few studies have considered on the ecological

impact of *H. inodorus* on raptors, McFadzen and Marzluff (1996) found young prairie falcons in nests infested by *H. inodorus* had lower mass and hematocrit levels, similar to what has been reported in other avian species.

Chronically elevated stress hormones produce detrimental effects in many bird species. The release of glucocorticoid hormones in response to environmental stressors, characterized as a stress response, has short-term benefits, including increased foraging and energy uptake, which may facilitate short-term survival during adverse conditions (Raouf et al. 2006). However, a stress response may carry significant costs for developing birds, such as reduced growth efficiency and compromised immune defense and cognitive abilities for long-lived species (Kitaysky et al. 2003). Increased corticosterone levels have been linked to locomotor activity (Breuner et al. 1998) and nest departure (Heath 1997; Corbel and Groscolas 2008). Thus, increases in corticosterone in response to ectoparasitism (Kavaliers et al. 2003; Quillfeldt et al. 2004; Raouf et al. 2006) could facilitate early fledging behavior and have negative consequences for nestling survival. Early fledging in response to ectoparasitism, along with a corresponding increase in fledgling mortality, has been reported in barn swallows (*Hirundo rustica*) (Møller 1990), and early fledging may be particularly deleterious for nestlings of cliff-nesting raptor species such as golden eagles (*Aquila chrysaetos*).

Golden eagles are a widespread, but uncommon, raptor currently facing threats across their North American range (Kochert et al. 2002). Golden eagle breeding ecology has been well studied in southwestern Idaho and, in Chapter 2, we previously report the occurrence of *H. inodorus* in golden eagle nests in the NCA. The present study is the first attempt to document physiological costs associated with infestation in eagle nestlings.

Our objectives were to assess the cost associated with *H. inodorus* infestation to determine the impact of ectoparasitism on nestling mass, hematocrit levels, corticosterone levels, and the probability of fledging early.

Methods

Study Area

Our study area was located along the Snake River Canyon in the NCA and the adjacent Upstream Comparison Area (UCA), which extends east from the NCA to Hagerman, Idaho. The steep basalt cliffs of the Snake River Canyon provide nesting locations for golden eagles. The surrounding uplands are a mosaic of land cover types and habitats that include native shrub-steppe and salt-desert communities characterized by big sagebrush (*Artemisia tridentata*), shadscale (*Atriplex confertifolia*), gray rabbitbrush (*Ericameria nauseosa*), and disturbed grasslands and rangeland dominated by exotic annual grasses, native perennial grasses, irrigated agricultural land, and rural and suburban development (U.S. Department of the Interior 1996).

Field Techniques

In 2015 and 2016, we surveyed 35 eagle territories in the NCA and UCA that have been historically monitored as part of a long term monitoring project (Steenhof et al. 1997). We visited study nests every 8-10 days beginning when nestlings were approximately 21 days old, the age at which young eagles are able to thermoregulate (Kochert et al. 2002), until nestlings were approximately 51 days old. To minimize stress to the nestlings, we did not visit nests during inclement weather. During each nest visit, we examined nestlings for the presence of ectoparasites. We grouped levels of *H. inodorus* infestation into three categories based on visual observations of adult and

nymph bugs in the nest and on nestlings: (1) no infestation: no bugs observed in the nest material or on nestlings; (2) low infestation: 1-10 bugs observed in total; or (3) high infestation: >10 bugs observed. We assessed nestling age in days using the protocols of Hoechlin (1976) and Driscoll (2010), and we recorded nestling mass using a Pesola scale. We defined early fledging events as nestlings that left their nest before they were 51 days old, which is 80% of mean fledging age (Steenhof et al. *in press*).

Twice during the nestling period, when nestlings were 4 and 7 weeks old, we recorded morphometric measurements, including wing chord, culmen length, and footpad length, and we used a 25-gauge needle to withdraw blood from the brachial vein of nestlings into a syringe. During these visits, we transported nestlings out of the nest and recorded the amount the time between our first contact with a nestling until blood sampling was complete to account for handling effects on corticosterone concentrations. Blood for corticosterone samples was stored in a 0.8 mL, LH Lithium Heparin mini centrifuge tube. For nestling hematocrit samples, blood was drawn into two heparinized 75 mm capillary tubes. All blood samples were immediately placed in a cooler until returning to the Boise State University campus on the same day of sampling. Hematocrit samples were centrifuged for 6 minutes at 10,000 rpm. We measured the proportion of the blood volume that was packed blood cells and used the mean of two samples to represent hematocrit for each nestling during each visit. Nestling sex was determined through DNA test results from nestling blood processed at Purdue University (West Lafayette, IN, USA; 2015 samples) and by Avian Biotech International (Tallahassee, FL, USA; 2016 samples). All field methods followed protocols approved by the Boise State University Institutional Animal Care and Use Committee (Protocol #006-AC14-007).

Corticosterone Sampling

We centrifuged corticosterone blood samples at 6,000 rpm for 10 minutes to separate plasma within 8 hours of collection, and then stored samples at -20°C until processing. We used extracted plasma to determine circulating corticosterone concentrations by running enzyme-linked immunosorbent assays (ELISA, Cayman Chemicals). We ran samples in duplicate, and utilized a pooled sampling consisting of select blood samples to determine inter-assay variability. We twice extracted corticosterone from 30 µL of plasma with 5 ml diethyl ether. The lipophilic supernatant was poured off and evaporated under a stream of nitrogen gas in a warm water bath. Extracted samples were reconstituted with 100 µL of EIA buffer, vortexed, and divided into 50 µL aliquots that were added to 96-well plates coated with mouse monoclonal antibody. We added corticosterone-specific acetylcholinesterase tracer and rabbit corticosterone antiserum and then incubated plates on an orbital shaker for two hours. Plates were then rinsed to remove any non-bound corticosterone and developed in a dark chamber with Ellman's reagent for 1 hour. We read plates at 405 nm with a Biotek EL800 plate reader. We validated the corticosterone assay by comparing the slopes of a plasma dilution curve to the assay standard curve slope. Plasma dilution curves consisted of different ratios of pooled eagle plasma to buffer. There was no significant difference between the slopes of the plasma dilution and assay standard curve. We calculated the concentration of corticosterone in samples by comparing results to a standard curve established with known concentrations. We determined extraction efficiency by analyzing a standard corticosterone sample, and calculated inter-assay variation from repeated values of a pooled sample. We corrected all values for assay extraction

efficiency (mean ± SD) 83.0 ± 7.6%. Inter-assay variation averaged 8.63% and average intra-assay variation was 2.02%. Assays were completed in two consecutive days and the same buffer solutions were used to eliminate any variation in absorbance values due to slight differences in concentration.

Statistical Analysis

Golden eagle young regularly had food in their crops that biased our mass measurements high. We scored the size of nestling crops in quartiles and used a sex- and age-specific crop size equation to estimate the mass of crop contents (Collopy 1984). Crop mass was then subtracted from measured body mass. We used a linear mixed model (LMM) to predict crop-adjusted nestling mass based on *H. inodorus* infestation level, age, age squared, sex and the interaction between infestation level and sex. We used a LMM to test whether nestling hematocrit was influenced by infestation level, age, age squared, or sex. We used a LMM to predict the effect of age, sex, time of day, and infestation level on nestling corticosterone. We included handling time in minutes included as a covariate to account for increased corticosterone levels as a result of handling. We created a generalized linear mixed model to predict the probability that nestlings fledged early or died in the nest as a result of *H. inodorus* infestation. We calculated the mean infestation level at a nest based on all visits to predict the probability that nestlings either left the nest early or died in the nest prior to fledging. Nestling identity and territory identity were included as random variables in all models to account for non-independence of samples. All analyses were performed in R (version 3.2.2, R Core Development Team 2016) and linear models were created using functions lmer and

glmer in the package lme4 (Bates et al. 2015) Descriptive statistics are reported as mean ± standard deviation.

Results

We assessed *H. inodorus* infestation levels at 19 and 16 golden eagle territories in 2015 and 2016, respectively. Ten territories were sampled in both years and one nest was sampled in both years. Of the 35 nests sampled, 23% (n = 8) nests experienced no *H. inodorus* infestation, 46% (n = 16) nests experience low levels of infestation 31% (n = 11) experienced high levels of infestation. Of the 57 nestlings associated with those nests, we found three (5% of total) nestlings dead in highly infested nests and we observed another 10 (18% of total) leave the nest before reaching 51 days of age. Of the 10 nestlings that fledged early, six were recovered dead below the nest. In total, the death of 16% (n = 9) of all nestlings in our study occurred in, or below, highly infested nests and can likely be attributed to *H. inodorus* infestation.

We measured nestling mass from 31 eagle nestlings in 2015 and 26 nestlings in 2016. Increasing *H. inodorus* infestation had a negative effect on nestling mass. Nestlings in nests with high infestations had significantly lower body mass than nestlings in non-infested nests ($\chi^2 = 23.9$, $p < 0.01$). Nestlings in nests with no or low infestation levels added mass at a faster rate than nestlings in highly infested nests (Figure 3.1). The fixed effects of both age ($\chi^2 = 450.7$, $p < 0.01$) and sex ($\chi^2 = 100.5$, $p < 0.01$) had a positive effect on nestling mass. We found no evidence of an interaction between nestling sex and infestation level ($\chi^2 = 0.4$, $p = 0.81$), indicating neither sex was parasitized disproportionately.

We measured hematocrit from 24 nestlings in 2015 and 26 nestlings in 2016. Mean nestling hematocrit over both seasons was 0.29 (range 0.12 - 0.54) and did not vary by nestling age ($\chi^2 = 0.7$, $p = 0.42$) or sex ($\chi^2 = 0.002$, $p = 0.96$). High levels of *H. inodorus* infestation had a negative effect on nestling hematocrit ($\chi^2 = 27.9$, $p < 0.01$, Figure 3.2). Nestlings from highly infested nests had significantly lower hematocrit than nestlings from non-infested nests or nests with low levels of infestation.

Circulating corticosterone was measured from 26 nestlings during 2015. Nestling corticosterone concentrations averaged 22.29 ± 15.17 ng/mL (range 3.70 - 80.65 ng/mL). We found no effect of nestling age ($\chi^2 = 0.4$, $p = 0.51$), sex ($\chi^2 < 0.1$, $p = 0.90$), or time of day ($\chi^2 = 0.3$, $p = 0.60$) on corticosterone levels, and thus combined all nestlings for the subsequent analysis. Circulating corticosterone was significantly higher for nestlings that experienced heavy *H. inodorus* infestation compared to nestlings from nests with either no infestation or low infestation ($\chi^2 = 21.1$, $p < 0.01$, Figure 3.3). We observed an increase in nestling corticosterone caused by handling time ($\beta = 2.53$, CI = 1.23, 3.78), demonstrating that golden eagle nestlings elicit a stress response during handling. We did not find a significant interaction between handling time and infestation level ($\chi^2 = 2.6$, $p = 0.28$), which indicates there were no differences in the rate of nestling stress response based on infestation level. Mean infestation level at a nest predicted the probability that nestlings left the nest before 51 days of age or died in the nest ($\chi^2 = 10.6$, $p < 0.01$, Figure 3.4). Nestlings that experienced high infestation levels throughout their time in the nest were more likely to leave early or die before leaving the nest.

Discussion

Ectoparasitism by *H. inodorus* has a detrimental effect on the physiological condition of golden eagle nestlings in southwestern Idaho. Higher levels of *H. inodorus* infestation were associated with lower nestling mass and hematocrit, both of which suggest that *H. inodorus* ectoparasitism created an energetically expensive cost to nestling development. We also found that nestling circulating corticosterone levels in nestlings increased in direct relation to *H. inodorus* infestation. Although increased corticosterone levels may facilitate nestling survival in the short-term by increasing food begging, chronic exposure to elevated levels of corticosterone can result in a range of deleterious effects, including reduced immune function (Wingfield et al. 1997) and reductions in cognitive capabilities (Kitaysky et al. 2003), both of which are likely to affect the lifespan of individual birds that survive to independence. Finally, *H. inodorus* infestations were positively associated with early fledging, which can lead to the death for young birds leaving nests on high cliffs before they are capable of flight.

High ectoparasite infestations can negatively affect fitness-related traits in birds (Møller 1990; Hurtrez-Boussès et al. 1997; Heeb et al. 2000). Magrath (1991), Arizaga et al. (2015), and Jones et al. (2017) all report a positive relationship between nestling body mass and condition and juvenile survival, thus reductions in nestling mass not only reduces condition at fledging, but could also reduce post-fledging survival as well (Tinbergen and Boerlijst 1990). Similar to other studies (e.g., Chapman and George 1991; Whitworth and Bennett 1992; Potti et al. 1999), we found that nestling hematocrit decreased as infestation increased, suggesting a direct effect of ectoparasitism on nestling condition. The reported hematocrit range for healthy raptors is 0.35 – 0.55 (Monks and

Forbes 2007); however, lower values have been obtained from apparently healthy birds (Rehder et al. 1982). A study evaluating the range in hematocrit values of five adult golden eagles found a range of 0.38 – 0.46 (Polo et al. 1992). In our study, nestling hematocrit ranged from 0.12 – 0.54 (mean = 0.29), and high levels of *H. inodorus* ectoparasitism were associated with the lowest hematocrit values. Although we do not know the survival fate of all nestlings from nests with high levels of infestation, we observed that some of those nestlings in highly infested nests survived to fledge and disperse from their natal nest. The long-term impact of low mass and hematocrit on the survival of fledgling eagles is unknown and warrants further study.

Chronically elevated corticosterone due to ectoparasitism could be deleterious to the survival of eagle nestlings by reducing cognitive function as well as facilitating movements that could cause early fledging. Corticosterone has been found to stimulate activity in numerous avian species including dispersal in juvenile western screech-owls (*Megascops kennicottii*; Belthoff and Dufty 1998), pre-migratory restlessness in red knots (*Calidris canutus*; Piersma et al. 2000), and migratory flights of bar-tailed godwits (*Limosa lapponica*; Landys-Ciannelli et al. 2002). Heath (1997) demonstrated corticosterone increases in American kestrel nestlings just prior to fledging. In our study, we recorded 10 instances of early fledging from highly infested nests, most of which resulted in mortality. High *H. inodorus* infestation has been documented nestlings fledging early from heavily parasitized nests in several raptor species (McFadzen and Marzluff 1996; D. Driscoll, pers. comm.) increased the likelihood of nestlings dying in their nests or jumping early to their death. Given the positive effect that corticosterone has on nestling movement (Breuner et al. 1998), it is possible that increased

corticosterone levels resulting from high infestation levels facilitated fledging movements before eagle nestlings were able to fly. Furthermore, decreased cognitive ability as a result of ectoparasitism can reduce foraging ability of recently fledged birds, which could increase the likelihood of post-fledging mortality due to starvation (Kitaysky et al. 2003).

Anemia and metabolic stress can cause immunosuppression and increase the susceptibility of birds to pathogenic agents (Folstad and Karter 1992). During development, nestling birds make trade-offs between growth, survival, and immune functions (Brommer 2004). Although *H. inodorus* is not a known vector of any pathogens, the closely related swallow bug (*Oeciacus vicarius*) is a vector for the Buggy Creek virus (Togaviridae: Alphavirus) and transmits the pathogen to cliff swallows (Fassbinder-Orth et al. 2013). Future studies might investigate the potential role of *H. inodorus* as a vector of arboviruses and other diseases. Further, Justice-Allen et al. (2016) reported that parasitism by argasid ticks caused paresis and ataxia in bald eagle nestlings. Avian tick paralysis is caused by neurotoxins associated with many tick species (Luttrell et al. 1996). Although ectoparasitism decreased the physiological condition of nestlings, we noted no other abnormal effects of *H. inodorus* ectoparasitism on eagle nestlings and repeated biting did not appear to cause additional symptoms.

Our study is the first to report circulating corticosterone concentrations for golden eagles and we validated the use of ELISA assays on eagle plasma. Our results show that the presence and intensity of *H. inodorus* infestation influences circulating corticosterone levels in eagle nestlings, and suggest that glucocorticoids are a mechanism to overcome the effects of ectoparasitism. Ectoparasitism has been linked to elevated corticosterone levels in some bird species (Kavaliers et al. 2003; Quillfeldt et al. 2004; Raouf et al.

2006), but not others (Lobato et al. 2008; Eggert et al. 2010; Pryor and Casto 2015), leading to the suggestion that an adrenal response to ectoparasitism may depend on the ability of the host species to cope with the parasitism event, and also the pattern of exploitation by the ectoparasite and the history of association between the host and ectoparasite (St. Juliana et al. 2014). Interspecific differences in stress response to ectoparasitism demonstrate the importance of investigating corticosterone response in raptor species. Host species may exhibit less of a response from by parasite species with which they have co-evolved. Thus, relatively new host-parasite relationships may allow parasites to exert a strong effect on their hosts. The glucocorticoids response elicited by eagle nestlings in southwestern Idaho to *H. inodorus* infestation may therefore be related to the relatively recent arrival of *H. inodorus* in this area.

Given the costs of ectoparasitism to nestling condition and survival, it is important to understand the physiological response to infestation. When avian populations are stressed by habitat reduction or alteration, the negative response to hematophagous ectoparasites may compound negative population-level effects (Loye and Carroll 1998). North American golden eagle populations face threats from habitat loss and the reduction of their historical prey populations (Kochert and Steenhof 2002), and monitoring and understanding the effects of ectoparasitism on nestling condition and survival may be important for future conservation efforts of the species.

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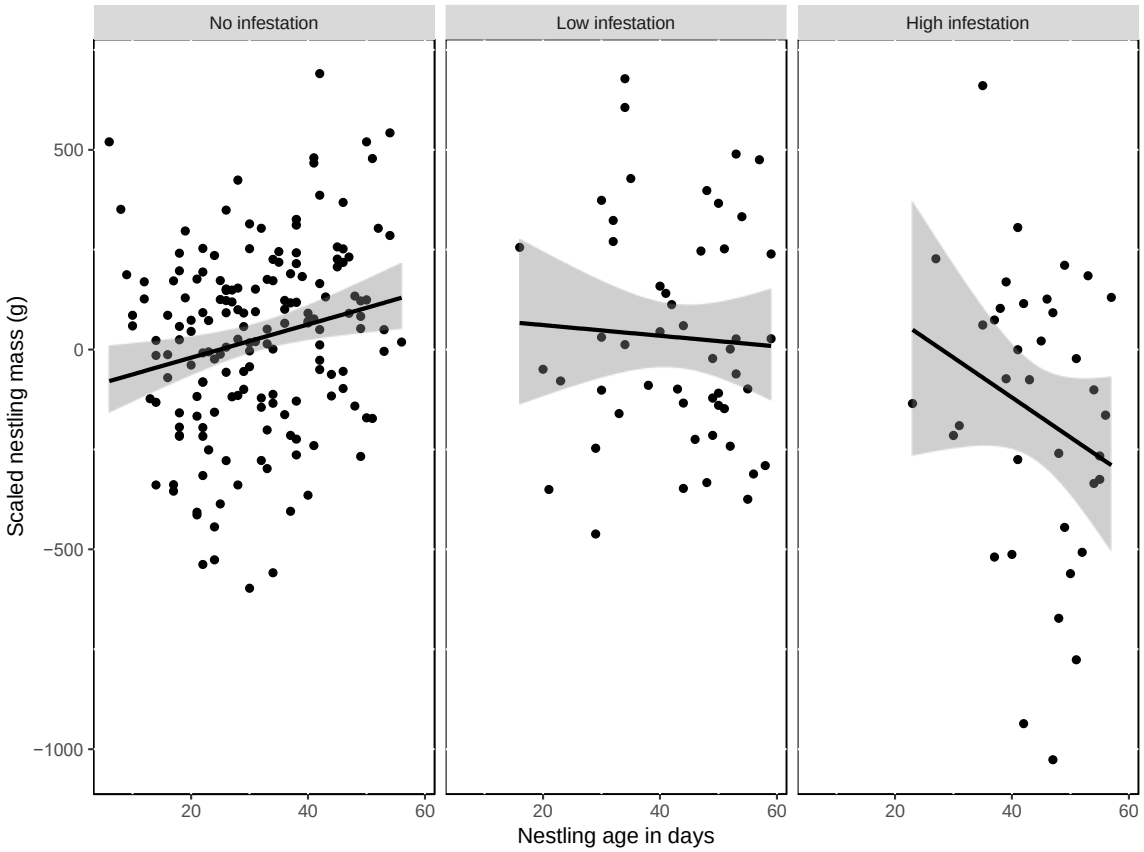

Figure 3.1 Observed golden eagle nestling mass (black circles, scaled to remove the effect of nestling age and sex), predicted mass (dark line), and associated 95% confidence intervals (solid gray area) measured from nestlings experiencing different levels of *H. inodorus* infestation in nests in southwestern Idaho, USA in 2015 and 2016. Nestlings that experienced high levels of infestation had lower mass than nestlings in nests with no or low levels of infestation ($\chi^2 = 23.86$, $p < 0.01$).

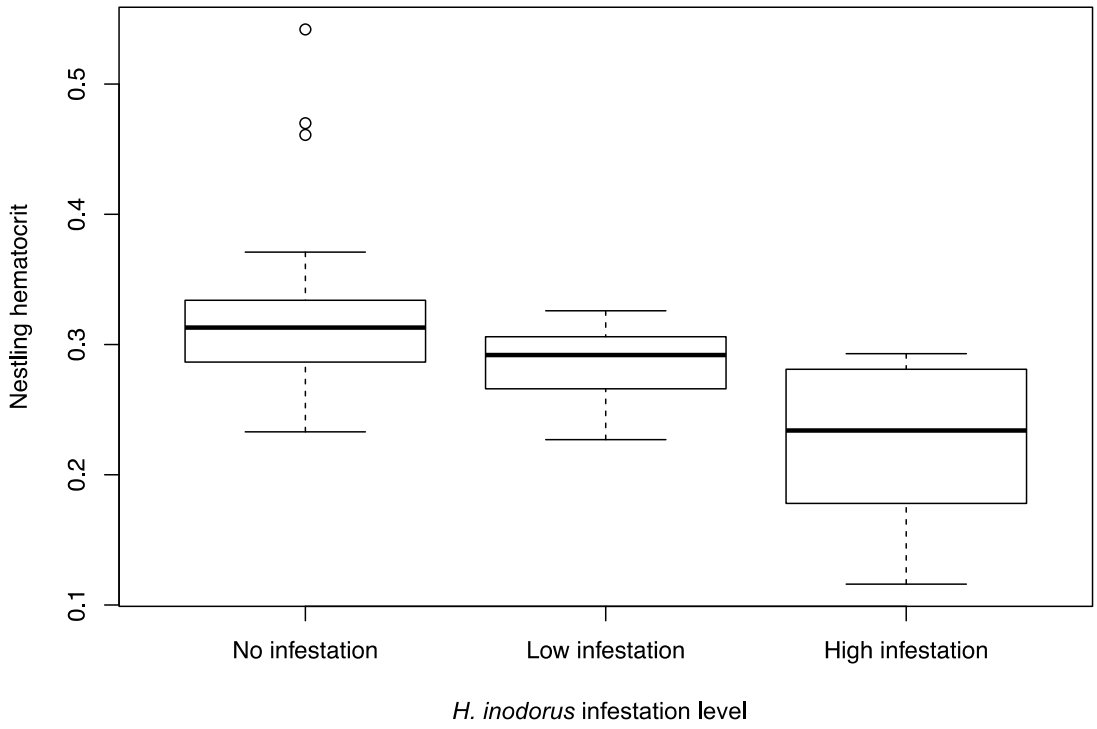

Figure 3.2 Hematocrit measured from golden eagle nestlings experiencing different levels of H. inodorus infestation in nests in southwestern Idaho, USA in 2015 and 2016. Bold lines within boxes represent the median, upper and lower limits of the box are the first and third quartiles, whiskers contain 1.5 times the interquartile range, and open circles are outliers. Nestling hematocrit decreased as cimicid infestation increased ($\chi 2 = 27.85$, $p < 0.01$).

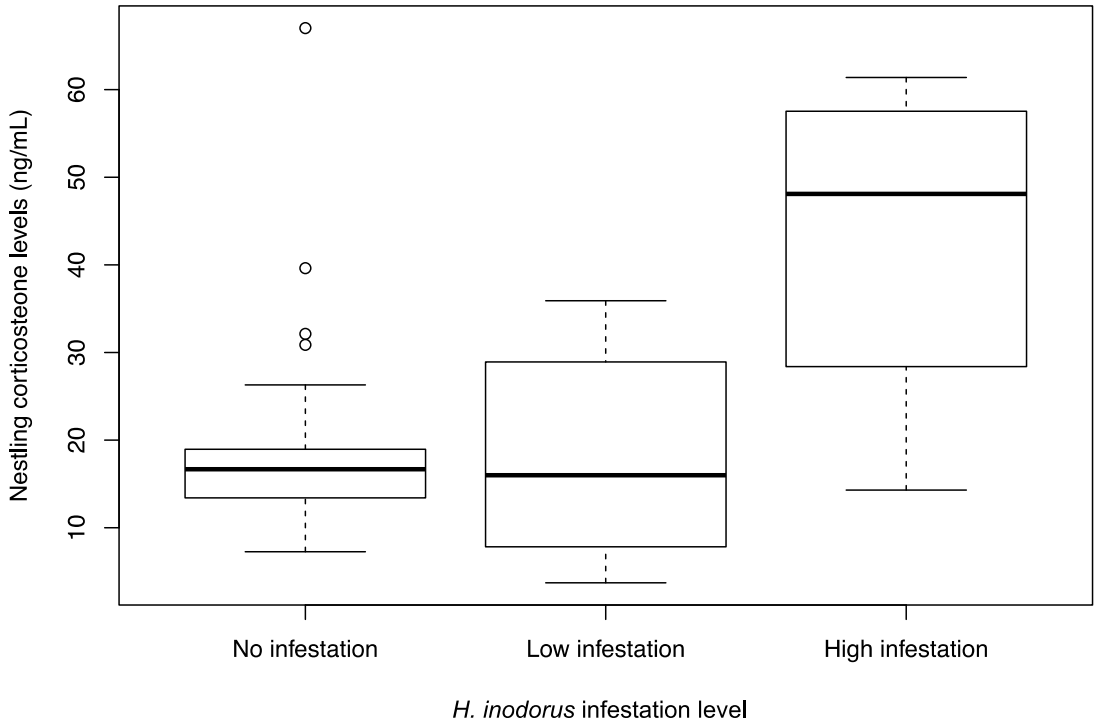

Figure 3.3 Corticosterone levels measured from golden eagle nestlings experiencing different levels of H. inodorus infestation in nests in southwestern Idaho, USA in 2015. Bold lines within boxes represent the median, upper and lower limits of the box are the first and third quartiles, whiskers contain 1.5 times the interquartile range, and open circles are outliers. Nestling corticosterone levels (ng/mL) increased as H. inodorus infestation increased ($\chi 2 = 21.1$, $p < 0.01$).

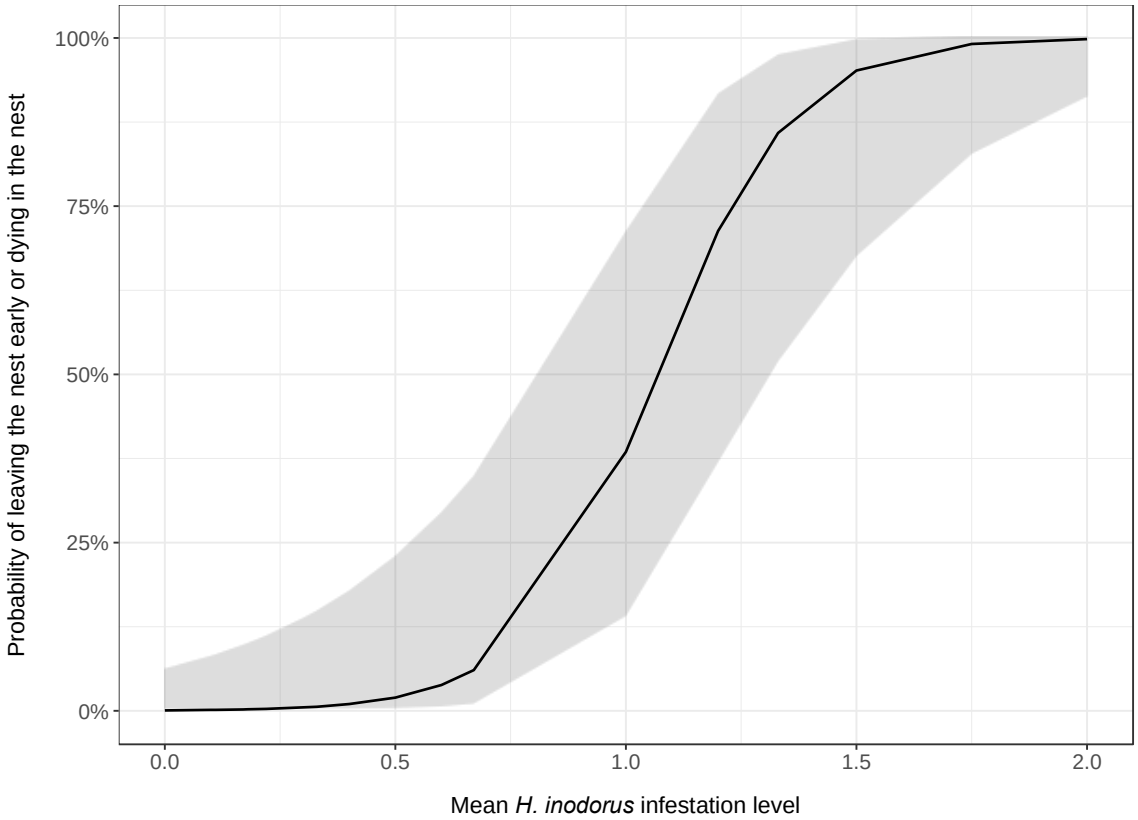

Figure 3.4 Predicted probability (solid dark line) and associated 95% confidence intervals (solid gray area) of golden eagle nestlings leaving the nest early, or dying in the nest, based on the mean infestation level at nests throughout the breeding season in southwestern Idaho, USA in 2015 and 2016. The probability of leaving the nest early or dying in the nest increased as infestation increased ($\chi 2 = 10.58$, $p < 0.01$).

CONCLUSION

Parasites, by definition, are costly to their hosts. However, they become ecologically important when they begin to influence host populations (Newton 1998). Given changes in climate and the expanding human ecological footprint, effects of emerging diseases and parasites are increasingly a concern for threatened or endangered wildlife populations. Introduced diseases and parasite species can drive changes in community structure and facilitate loss of biodiversity (Telfer and Bown 2012). The effects of exposure to new or increased rates of disease and parasite infestations could be compounded by climate-induced habitat degradation or loss of historical prey resources (Staley and Bonneaud 2015). Despite these concerns, community interactions and structure may be resilient enough to react to and moderate parasite dynamics. It has become increasingly important to study and understand host-parasite interactions, especially for the conservation of threatened or endangered species already facing threats from a changing climate or encroaching human impacts. This thesis documents two parasite species, the protozoan *Trichomonas gallinae* and insect ectoparasite *Haematosiphon inodorus,* associated with golden eagle populations in North America. Currently, both parasites negatively affect golden eagle fitness, and projected future climate and anthropogenic land use change may exacerbate these negative effects. Future management of ecosystems that promotes system resiliency will be essential for the conservation of golden eagle populations.

References

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