High Prevalence of *Leucocytozoon* Parasites in Nestling Northern Goshawks (*Accipiter gentilis*) in the Northern Great Basin, U.S.A.

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HIGH PREVALENCE OF \textit{LEUCOCYTOZOON} PARASITES IN NESTLING NORTHERN GOSHAWKS (\textit{ACCIPITER GENTILIS}) IN THE NORTHERN GREAT BASIN, U.S.A.

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\textbf{ABSTRACT.}—The Northern Goshawk (\textit{Accipiter gentilis}) is currently listed as a sensitive species by the U.S.D.A. Forest Service. Previous research in our study area, the South Hills of the Minidoka Ranger District of the Sawtooth National Forest, Idaho, identified possible signs of parasite infections among the banded adult and nestling goshawks, which could influence their survival and breeding success. Therefore, we sought to quantify the prevalence and intensity of \textit{Leucocytozoon} parasites among a sample of nestling goshawks in the South Hills during the 2012 breeding season. We sampled 27 nestlings from 12 nests for \textit{Leucocytozoon} parasites by examining blood smears. All sampled nestlings were infected with \textit{Leucocytozoon} parasites. The infection intensity ranged from 0.82–10.05 \textit{Leucocytozoon} parasites per 1000 erythrocytes (mean $\pm$ SE = 4.35 $\pm$ 0.54). Using site elevation, distance-to-water, nestling age, nestling sex and nest tree species as predictor variables for infection intensity by \textit{Leucocytozoon} parasites, we employed an information theoretic approach to select a top model to determine the presence of an effect. The top model included nest tree species as the sole predictor for infection intensity. Specifically, higher \textit{Leucocytozoon} parasite intensity was associated with quaking aspen (\textit{Populus tremuloides}) nest trees, as compared to lodgepole pine (\textit{Pinus contorta}). Further research will help identify management implications for this species of concern in this high altitude forest surrounded by a shrub-steppe ecosystem.

\textbf{KEY WORDS:} Northern Goshawk; Accipiter gentilis; blood parasite; Idaho; Leucocytozoon; parasite.

PREVALENCIA ELEVADA DE PARÁSITOS DE \textit{LEUCOCYTOZOON} EN POLLOS DE \textit{ACCIPITER GENTILIS} EN LA GRAN CUENCA DEL NORTE, EEUU

\textbf{RESUMEN.}—Actualmente \textit{Accipiter gentilis} está catalogada como una especie sensible por el Servicio de Bosques del Departamento de Agricultura de los Estados Unidos. Investigaciones previas en nuestro área de estudio, las South Hills del Distrito de Minidoka perteneciente al Bosque Nacional Sawtooth, Idaho, identificaron posibles signos de infecciones parasitarias entre los individuos anillados adultos y pollos de \textit{Accipiter gentilis}, que pueden influir en su supervivencia y en el éxito reproductor. Por ello, buscamos cuantificar la prevalencia y la intensidad de la presencia de parásitos del género \textit{Leucocytozoon} en una muestra de pollos de \textit{A. gentilis} en las South Hills durante la época reproductiva del 2012. Analizamos muestras de sangre de 27 pollos provenientes de 12 nidos en busca de parásitos del género \textit{Leucocytozoon}. Todos los pollos estudiados estaban infectados con parásitos \textit{Leucocytozoon}. La intensidad de infección osciló entre 0.82–10.05 parásitos de \textit{Leucocytozoon} por cada 1000 eritrocitos (medía $\pm$ DE = 4.35 $\pm$ 0.54). Utilizando parámetros descriptores del lugar de cría tales como altitud, distancia al agua, edad del pollo, sexo del pollo y especie del árbol donde se ubicaba el nido como variables predictivas de la intensidad de

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The Northern Goshawk (Accipiter gentilis; hereafter “goshawk”) is a generalist predator occupying boreal and temperate forests of the Holarctic (Squires and Reynolds 1997). The species is currently listed as a sensitive species by the U.S.D.A. Forest Service and has been identified as a local management indicator species for the Sawtooth National Forest (D. Santini pers. comm.; U.S. Forest Service 2008, 2011). Previous research within the Sawtooth National Forest has suggested that this goshawk population may be suffering from low survival rates (Kaltenecker et al. 2004). In a 7-yr study, Kaltenecker et al. (2004) found no occurrences of young nestlings banded in the area returning to breed as adults, despite banding 74 nestlings between 1997 and 2003. Skin lesions consistent with symptoms of some parasite and fungal infections were regularly observed on both nestlings and adult females of this population during the banding process (Fig. 1; K. Hasselblad pers. comm.). Moreover, black flies (Family Simuliidae), a known vector for Leucocytozoon parasites, are commonly present in southern Idaho (Twin Falls County Pest Abatement District 2012) and have been frequently observed within goshawk nesting stands. Given these indicators and conditions, we determined to evaluate the prevalence of blood parasites in raptors and other avian species. For example, Quillfeldt et al. (2014) suggested that nesting substrate contributed to infection status in two seabird genera. Habitat structure characteristics, such as entire tree stands, have also been related to varying parasite prevalence (Arriero et al. 2008). Zamora-Vilchis et al. (2012) found that both age and sex of Blue Tits (Cyanistes caeruleus) influenced the infection intensity of Plasmodium spp. Environmental factors also play a role in determining prevalence and intensity of blood parasites in raptors and other avian species. For example, Quillfeldt et al. (2014) suggested that nesting substrate contributed to infection status in two seabird genera. Habitat structure characteristics, such as entire tree stands, have also been related to varying parasite prevalence (Arriero et al. 2008). Zamora-Vilchis et al. (2012) found elevation to be negatively correlated with blood parasite prevalence. The distance between a nest and a water source may also be an important factor for infections, because the black fly vector requires a water source to complete its life cycle (McCreadie and...
Adler 1998). Given the potential burden that Leucocytozoon parasites impose on raptors, and that Leucocytozoon parasite prevalence varies as a function of biotic and abiotic factors, it is important to document the prevalence and intensity of these parasites when assessing the health of a raptor population.

Previous studies have documented Leucocytozoon parasites in goshawks in other regions and countries (Toyne and Ashford 1997, Muñoz et al. 1999, Wieliczko et al. 2003), and in one case it is thought that these parasites may have been responsible for the death of a juvenile in Maryland, U.S.A. (Ward 1975). Leucocytozoon parasites were previously found in a population of Sharp-shinned Hawks (Accipiter striatus) originating from the vicinity of the Sawtooth National Forest (Smith et al. 2004). Given this information in addition to previous reports of skin lesions, we speculated that goshawks in the area might also be at risk of infection.

Our objectives in the present study were to (1) measure Leucocytozoon prevalence and intensity among nestling Northern Goshawks from the population in the Sawtooth National Forest in south-central Idaho, and (2) identify potential factors that may influence the prevalence and intensity of parasitism. We hypothesized that the prevalence of Leucocytozoon parasites in this population would be high. We further predicted that the infection intensity among nestlings may be influenced by the nest tree species, distance to water, nestling age, nestling sex, and site elevation.

METHODS

We conducted this study in the South Hills of the Sawtooth National Forest in south-central Idaho, an area that encompasses the Cassia division of the Minidoka Ranger District (41° 58.8’–42° 20’N, 113° 58.8’–114° 28.8’W, elevation: 1468–2456 masl). The Cassia division contains approximately 125 000 ha and is bordered primarily by Bureau of Land Management lands (U.S. Forest Service 2003). The naturally fragmented landscape is dominated by grasslands and mountain big sagebrush (Artemisia tridentata vaseyana; approximately 80%; U.S. Forest Service 1980). The remaining forested landscape consists predominantly of quaking aspen (Populus tremuloides), lodgepole pine (Pinus contorta), and subalpine fir (Abies lasiocarpa; U.S. Forest Service 1980).

We located goshawk nests by searching historical nesting territories and additional areas prioritized through Geographic Information System (GIS).
analysis (Miller et al. 2013). We acquired Digital Elevation Model (DEM) data (resolution = 30 m) and stream location data from the Inside Idaho database (U.S. Geological Survey 1999, Idaho Department of Environmental Quality 2006). We used the stream data to create a 30-m-resolution raster file representing the distance of each pixel from the nearest stream location. We identified nest tree species as either lodgepole pine or aspen.

**Captures and Blood Samples.** To gain access to nestlings, we used climbing spurs or a climbing rope to ascend nest trees. We used a photographic key to determine nestling age (Boal 1994). We banded and collected blood samples from nestlings when they were 23–34 d old, between 28 June and 11 July of 2012. All nestlings that could be safely captured and removed from the nest were placed in a sleeve to protect their wings and lowered individually to the ground. We determined the sex of nestlings based on their tarsus size while banding (Pyle 2008) or via genetic analysis. Using a needle and syringe, we collected approximately 1 ml of blood from the jugular vein. One drop of the blood was used to prepare a thin blood smear on individual labeled microscope slides. Two slides were created for each individual nestling to better ensure the availability of a quality blood smear sample. The rest of the blood sample was stored in Queen’s Lysis buffer for genetic analysis (Hobson et al. 1997, Brumfield and Dittmann 2012). The slides were fixed in absolute methanol and stained using Wright–Giemsa stain (Hauska et al. 1999).

**Parasite Identification and Quantification.** *Leucocytozoon* parasites were identified on each slide using the morphological traits described by Valkiūnas et al. (2010) with the 100× oil immersion microscope objective (Fig. 2). Once all slides were viewed and prevalence (i.e., the proportion of infected individuals to non-infected individuals) known, a Ken-A-Vision PupilCAM camera was used to record 30–35 unique photos of each nestling blood smear. We took photos until the viewer estimated a minimum count of 3500 erythrocytes. This is well...
above previously reported erythrocyte counts of 2000 (King et al. 2010, Ortego and Cordero 2010). ImageJ software was then utilized as described by Gering and Atkinson (2004) to count the total number of erythrocytes. Leucocytozoon parasites in these photos were counted by consensus of two consistent viewers. We defined intensity of infection as the quantity of erythrocytes infected with Leucocytozoon parasites per 1000 noninfected erythrocytes. Normality was achieved by log transforming the infection intensities.

**Statistical Analysis.** We modeled infection intensities using a Gaussian distribution with Generalized Linear Mixed Models (GLMM; Zuur et al. 2009). We created models using various ecologically relevant combinations of fixed effects (nestling sex, nestling age, site elevation, distance-to-water, and nest tree species) and a random effect of nest ID to predict the log-transformed intensity of parasite infection. We ranked models using the Akaike Information Criterion adjusted for small sample size (AICc; Burnham and Anderson 2002). We measured the presence of an effect if the coefficient for a given predictor was represented in the top model and the top model was ranked above the null model (Burnham and Anderson 2002).

We conducted all statistical analyses in R (R Development Core Team 2012). We fit GLMM models using the R package “lme4” (Bolker et al. 2013). We calculated AICc and related values using the R package “AICcmodavg” (Mazerolle 2012). Standard error was calculated and reported as ± from the mean.

This study was conducted with all necessary permits and authorizations including a sub-permit (RAM) under master Federal Bird Banding Permit #21633, a State of Idaho Wildlife Collection/Banding/Possession permit #870115, with authorization from Idaho Fish and Game Jerome Field Office and under Boise State University Institutional Animal Care and Use Committee permit #006-AC11-004.

**RESULTS**

We sampled 27 nestlings from 12 separate nests. Only one of these nests was occupied in the previous year. We measured the continuous variables site elevation, distance-to-water, and nestling age for each nest (Table 1). We categorized samples by sex and nest tree species (Table 2). All 27 sampled nestlings were positive for Leucocytozoon parasites. The intensity varied from 0.82 to 10.05 Leucocytozoon parasites per 1000 erythrocytes, with a mean of 4.35 ± 0.54. We also observed unconfirmed infections of Haemoproteus spp. or Plasmodium spp. in the blood of three nestlings.

Our top model included nest tree species as the sole predictor for infection intensity (Table 3). Specifically, we found that increased Leucocytozoon parasite intensity was associated with aspen nesting substrate (Fig. 3).

**Table 1.** Nestling age, nest elevation, and distance to water for nestling Northern Goshawks during the summer of 2012 in the South Hills of the Sawtooth National Forest, Idaho. Elevation and distance to water were calculated to the nearest 30 m. Nestling age was estimated using a photographic key.

<table>
<thead>
<tr>
<th>CONTINUOUS VARIABLE</th>
<th>MEAN ± SE</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nestling age</td>
<td>28.4 ± 0.6 d</td>
<td>23 – 34 d</td>
</tr>
<tr>
<td>Site elevation</td>
<td>2071 ± 17 m</td>
<td>1858 – 2255 m</td>
</tr>
<tr>
<td>Distance to water</td>
<td>372.0 ± 28.2 m</td>
<td>184.39 – 644.75 m</td>
</tr>
</tbody>
</table>

**Table 2.** Nestling sex and nest tree species for nestling Northern Goshawks during the summer of 2012 in the South Hills of the Sawtooth National Forest, Idaho. Sex was determined through band size and genetic analysis.

<table>
<thead>
<tr>
<th>NEST TREE SPECIES</th>
<th>SEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspen</td>
<td>MALE</td>
</tr>
<tr>
<td>Lodgepole pine</td>
<td>FEMALE</td>
</tr>
</tbody>
</table>

**Table 3.** Generalized Linear Mixed Models used to predict Leucocytozoon parasite infection with a suite of environmental parameters. These parameters included fixed effects of nestling age, nestling sex, nest tree species, site elevation, and distance to water. We also included a random effect of nest ID. We ranked models using the Akaike Information Criterion adjusted for small sample size (AICc). The top model included nest tree species as the sole predictor for infection intensity.

<table>
<thead>
<tr>
<th>MODEL</th>
<th>K</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>w_0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nest tree species</td>
<td>4</td>
<td>57.35</td>
<td>0.00</td>
<td>0.44</td>
</tr>
<tr>
<td>Null</td>
<td>3</td>
<td>58.55</td>
<td>1.20</td>
<td>0.24</td>
</tr>
<tr>
<td>Distance-to-water</td>
<td>4</td>
<td>60.31</td>
<td>2.96</td>
<td>0.10</td>
</tr>
<tr>
<td>Nestling age</td>
<td>4</td>
<td>60.88</td>
<td>3.52</td>
<td>0.08</td>
</tr>
<tr>
<td>Nestling sex</td>
<td>4</td>
<td>61.03</td>
<td>3.68</td>
<td>0.07</td>
</tr>
<tr>
<td>Site elevation</td>
<td>4</td>
<td>61.26</td>
<td>3.91</td>
<td>0.06</td>
</tr>
</tbody>
</table>
DISCUSSION

All nestling goshawks sampled in our study were infected with *Leucocytozoon* parasites. Although similarly high prevalence values for *Leucocytozoon* parasites have been reported in nestlings of other raptor species (90%, 100%; Forrester et al. 2001, King et al. 2010), prior studies on nestling goshawks have reported much lower values (18.8%, 53.6%; Toyne and Ashford 1997, Wieliczko et al. 2003). Due to the dearth of research showing a high prevalence of *Leucocytozoon* parasites in goshawks, this population is of interest.

Although the goshawks in our study showed a high prevalence of *Leucocytozoon* parasites among the population of nestlings, it was unclear to what degree these parasites may have negatively affected the population. In 2011 and 2012, occupancy rate and reproductive rate in the study area were equal to or above average (Bechard et al. 2006, Kenward 2006, Miller et al. 2014), and we now have evidence that nestlings fledged in the South Hills have survived to reproduce in the area. These cases include three adult female breeders that hatched and later bred in this area. The observed subadult breeding rate in the area was <10%, which further suggested the population was stable (R. Miller unpubl. data, Kenward 2006).

The infection intensity varied from 0.82 to 10.05 *Leucocytozoon* parasites per 1000 erythrocytes, with a mean of 4.35 ± 0.54. This intensity range and average was lower than that in other studies of nestling raptors (1–87.5, 0–75; King et al. 2010, Ortego and Cordero 2010). This may indicate that this population managed infections to lower levels. There are a number of possible explanations for how this population of goshawks may manage the effects of infection by *Leucocytozoon* parasites. First, food availability may be sufficiently high that nestlings are able to overcome the debilitating effects of parasites. In a study of Great Horned Owls (*Bubo virginianus*), Hunter et al. (1997) found that prey shortages greatly increased the effect of infection by *Leucocytozoon* parasites on fledgling survival. In years of lower than average food supply, birds may allocate more energy to maintaining body condition, rather than to immune defenses, leaving them more susceptible to parasites. Although Miller et al. (2014) found that prey consumption per nestling goshawk in our study area in 2011 and 2012 was similar to or greater than that in other studies (Younk and Bechard 1994, Smithers et al. 2005), the limited duration of our study precluded a test of the effects of different levels of prey abundance.

The stability of a goshawk population in the face of high *Leucocytozoon* parasite prevalence may also be a consequence of nest preference and supplementation behaviors by breeding individuals. Kenward (2006) suggested that intermittent use of nesting structures decreases the buildup of parasites. Having fewer parasitic stressors indirectly allows nestlings to better respond to *Leucocytozoon* parasite infections. Of the 12 nests we sampled in 2012, only one was known to be occupied in 2011. This nest also fell within the upper 74th percentile of infection intensity, consistent with Kenward’s hypothesis. We also observed that adult goshawks delivered fresh aspen and lodgepole pine branches to add to their nests multiple times each day. These branches buried uneaten prey items and unhatched goshawk eggs (R. Miller unpub. data), which reduced exposure of decaying waste and perhaps attraction of black flies. Resin from the broken sprigs may have repelled the black flies due to the presence of volatile chemicals (Lafuma et al. 2001, Keeling and Bohlmann 2006, Lindroth and Clair 2013). Although all nestlings in our study were infected by *Leucocytozoon* parasites, any reduction in fly activity and harassment may lessen the negative effects of these flies (Smith et al. 1998).

Our top model included nest tree species as the sole predictor for infection intensity. Specifically, higher *Leucocytozoon* parasite intensity was associated

![Figure 3. Model results indicating that increased *Leucocytozoon* parasite intensity was associated with aspen nest tree species as compared with lodgepole pine substrate. The point indicates the mean. The bold line indicates the median. The box shows the quartiles. The whiskers designate the range.](image-url)
with aspen nesting substrate rather than with lodgepole pine substrate. Both conifers and aspen contain volatile compounds, and perhaps conifers are more efficient at deterring black flies (Lafuma et al. 2001, Keeling and Bohlmann 2006, Lindroth and Clair 2013). Lodgepole pine branches were the species most frequently deposited in the nests, even for nests located in aspen trees (R. Miller unpubl. data), which supported this idea. Aspen trees are suitable nesting substrate for these goshawks and may provide other advantages, but may be associated with a cost: higher Leucocytozoon parasite infection intensities.

A number of factors may have contributed to our inability to detect strong relationships between Leucocytozoon parasite infection intensity and the various other variables we measured. First, there may have been insufficient variation in infection intensities to detect effects across the range of variables we measured using our available sample size. For example, the nearly ubiquitous access to water, brief appropriate sampling age of nestlings, and the relatively narrow elevation band suitable for nesting at our study site may not have generated enough variation in these parameters to manifest substantial changes in infection intensity. Selecting nests that fall within a greater range of these parameters, assuming goshawks nest in these areas, may improve the resolution of the model. Increasing sample size may also help to address problems associated with low power. Lastly, examining how vector (i.e., Family Simuliidae) distribution, rather than surrogate measures such as elevation and distance-to-water, relates to infection intensity might provide greater insight into variation in infection intensity.

Our results demonstrate that there is a high prevalence of Leucocytozoon parasites in this population of goshawks, and that the birds seem to be enduring nonetheless. The low infection intensity range and rate may indicate that Leucocytozoon parasites are not a limitation to this population. However, further study is needed to more thoroughly understand the consequences of parasitism in this host-parasite relationship. For example, are the effects of parasitism more prominent in years of food and climate stress? What environmental variables explain variation in vector abundance? Unraveling the details of the relationship between Leucocytozoon parasites and goshawks will help address management concerns for this species, as well as other avian species living in this high altitude forest surrounded by a shrub-steppe ecosystem.

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Literature Cited


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