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Article

Haemosporidian prevalence in northern saw-whet owls *Aegolius acadicus* is predicted by host age and average annual temperature at breeding grounds

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Understanding factors that determine haemosporidian prevalence is critical in predicting how parasite and avian host populations will respond to environmental change. Here, we used molecular screening of blood samples from migrating northern saw-whet owls *Aegolius acadicus* in eastern North America to characterize haemosporidian infections and examine parasite prevalence with respect to abundance of migrants, timing of migration, climatic conditions at the breeding grounds, and avian host age. We identified three haemosporidian genera (*Leucocytozoon*, *Plasmodium*, and *Haemoproteus*) and discovered a new lineage of *Leucocytozoon* that is thus far specific to the northern saw-whet owl. We found no significant relationship between parasite prevalence and northern saw-whet owl abundance or timing of migration. After-hatch-year birds were significantly more likely to be parasitized by *Leucocytozoon* than hatch-year birds, whereas prevalence of *Plasmodium* was higher in hatch-year birds. Of the three climatic variables analyzed at owl breeding grounds (temperature, precipitation, and snowpack days), lower average annual temperatures significantly increased the chance of a bird being parasitized by *Leucocytozoon*; no significant temperature-dependent relationship was found for *Plasmodium.* This study contributes to our general understanding of the relationship between parasite prevalence and host density, host age, resource abundance, and abiotic factors such as temperature and precipitation.

Keywords: *Aegolius acadicus*, haemosporidia, climate change

Introduction

Haemosporidians are obligate protozoan parasites that infect vertebrate hosts (Valkiūnas 2005). Three genera of haemosporidians, *Haemoproteus*, *Leucocytozoon*, and *Plasmodium,* are known to infect avian hosts. These parasites are transmitted to the blood of intermediate hosts by a variety of biting flies (Insecta: Diptera): *Plasmodium* is vectored by mosquitoes (Culicidae), *Haemoproteus* by biting midges (Ceratopogonidae) and hippoboscid flies (Hippoboscidae), and *Leococytozoon* by black

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flies (Simuliidae) (Valkiūnas 2005). Haemosporidians infect all 23 bird orders (Valkiūnas 2005), and studies of haemosporidian infections in birds have demonstrated a variety of effects. For example, several studies have found no deleterious effects of haemosporidian infection on reproductive fitness (Dufva 1996, Bensch et al. 2007) or body condition (Ashford 1971, Dufva 1996, Young and Proudfoot 2014), and Zylberberg et al. (2015) report a positive correlation of haemosporidian infection on fitness. In contrast, numerous studies have demonstrated that haemosporidian infection can have a negative effect on reproduction (Allander and Bennett 1995, Merino et al. 2000, Knowles et al. 2010, Asghar et al. 2011), feather growth rate (Marzal et al. 2013, Coon et al. 2016), body condition (Dawson and Bortolotti 2000, Marzal et al. 2013), telomere-length (Asghar et al. 2015), and can cause anemia and death (Valkiūnas 2005, Atkinson and Samuel 2010, Santiago-Alarcon et al. 2013). Because of their effects on red blood cells, haemosporidians may have more subtle impacts on fitness via mechanisms such as inhibiting or delaying migration (Valkiūnas 1989, Asghar et al. 2011). Late arrival on the breeding grounds has subsequently been shown to negatively affect reproductive success (Alatalo et al. 1986, Smith and Moore 2005).

Most studies of haemosporidian parasites found in birds during migration are conducted on passerine species (Passeriformes) (Manwell and Herman 1935, Rintamäki et al. 1998, Garvin et al. 2006, Krone et al. 2008) and waterfowl (Anseriformes) (Figuerola and Green 2000, Reeves et al. 2015), with much less attention given to raptors (Accipitriformes, Falconiformes, and Strigiformes) (Krone et al. 2008, Clark et al. 2014, Ciloglu et al. 2016). Perhaps fewer studies are conducted on raptors because they are at the top of the food chain and, thus, there are fewer species that provide opportunities for research. However, their position in the trophic pyramid may justify studying raptor haemosporidian infections because of the role that raptors play in maintaining balance within an ecosystem and because of the diversity of haemosporidians previously identified infecting raptors (Sehgal et al. 2006, Krone et al. 2008, Jasper et al. 2014, Ciloglu et al. 2016).

Here, we used DNA from blood samples collected from migratory northern saw-whet owls *Aegolius acadicus* (hereafter saw-whet owls) between 2006 and 2016 to investigate haemosporidian prevalence in a natural raptor population with extreme resource-driven fluctuations over time. We identified lineages of haemosporidians and investigated the relationship between infection prevalence, host age, host density, and timing of migration. Northern saw-whet owls prey mostly on small mammals, nest in cavities, and are among the most common owls in the boreal forests of Canada and the northern United States (Cannings 1993). North American populations of northern saw-whet owl are migratory (Mueller and Burger 1967, Holroyd and Woods 1975, Weir et al. 1980, Duffy and Kerlinger 1992); however, the number of birds migrating annually fluctuates in relation to annual breeding success. Thus, during years when food is abundant and nests produce large numbers of fledglings, the number of migrating hatching year birds, in part, increases the total number of migrants moving south during the winter (Cannings 1993). The northern saw-whet owl is therefore an ideal candidate for investigating the relationship between irruptive migration patterns and haemosporidian prevalence, a relationship which heretofore has not been investigated using PCR-based haemosporidian detection. Furthermore, past research on saw-whet owls revealed a geographic discrepancy in haemosporidian diversity and prevalence (Taft et al. 1997, Leppert et al. 2008, Young and Proudfoot 2014). Based on previous studies of avian haemosporidians, we predicted that hatch-year birds would have higher infection prevalence (Murdock et al. 2013, Ramey et al. 2014, Lutz et al. 2015, Hammers et al. 2016). Thus, we expected an increase in parasite prevalence in the population during irruption years, when the number of hatch-year birds is $\geq$ 15% greater than the 11 yr mean estimated from 54 348 encounter records kept at the US Geological Survey Bird Banding Laboratory (Confer et al. 2014). We also predicted that infected saw-whet owls would migrate later than those that revealed no sign of infection.

In addition to host-related factors, we also analyzed infection prevalence with respect to three climatic variables at the inferred saw-whet owl breeding grounds: average annual temperature, total annual precipitation, and number of days with $\geq$ 2.54 cm of snow on the ground. We chose these climatic variables because they are likely abiotic factors associated with the life history of both the haemosporidians and their vectors. Abiotic conditions impact avian haemosporidian prevalence in large part because dipteran vectors of haemosporidians thrive under particular temperature and precipitation conditions (LaPointe et al. 2010, Loiseau et al. 2013, Okanga et al. 2013, Pérez-Rodríguez et al. 2013). Most studies of *Plasmodium* found a positive relationship between temperature and prevalence because the mosquito vectors and the malarial parasites require warm conditions to survive and breed (LaPointe et al. 2010, Sehgal et al. 2011, Loiseau et al. 2013, Okanga et al. 2013). In Hawaii, for example, birds living at altitudes greater than 1500 m a.s.l. were spared from introduced malarial parasite infection because of the inability of *Plasmodium* to complete their life cycle at the colder temperatures of the higher elevations (LaPointe et al. 2010). Ross and Merritt (1978) also demonstrated that warmer temperatures accelerate simuliid black fly larval development and thus may increase *Leucocytozoon* prevalence. Unseasonably warm temperatures may also increase *Leucocytozoon* prevalence by melting snow and icepack on bodies of water earlier or quicker than usual, thereby allowing for earlier emergence of simuliid haemosporidian vectors (Ross and Merritt 1978, Bernotiene and Bartkeviciene 2011, Murdock et al. 2013). We therefore predicted that average annual temperature would be positively correlated with prevalence of both *Plasmodium* and *Leucocytozoon* infection, and negatively correlated with total annual snowpack days. We also predicted that infection prevalence would positively

correlate with total annual precipitation because both mosquitoes and black flies breed in water, and therefore higher total annual precipitation would benefit breeding mosquitos by increasing the surface area of standing water and would benefit breeding black flies by increasing water flow in rivers and streams.

Material and methods

Sample collection and age classification

We collected whole blood from 1253 saw-whet owls between 2006 and 2016. All saw-whet owls were captured at the Mohonk Preserve (latitude 41°46′49.07″N, longitude 74°7′26.87″W) near New Paltz, NY, USA using mist nets and acoustic lures (Erdman and Brinker 1997). We mist netted northern saw-whet owls from 1 October to the first week of December, when the number of owls is usually reduced to one or two per night. The rate of capture followed the saw-whet owl migration pattern reported by Beckett and Proudfoot (2011), a gradual increase in the number of owls netted per night with a peak about half-way through the season and a gradual decline after the peak, producing a gaussian curve. The peak wave moves south at about 1° latitude every 3.8 d (Beckett and Proudfoot 2011). Therefore, we can reasonably assume that our study focused on migrating saw-whet owls. Individuals were aged from molt patterns of primary and secondary flight feathers (Evans and Rosenfield 1987, Pyle 1997, Weidensaul et al. 2011), tagged with USGS aluminum leg bands, and released at the capture location. Two age classes were assigned, hatch-year (HY) and after hatch-year (AHY), via visual inspection of primary and secondary feathers. The flight feathers of HY birds lacked contrasts in color and were determined to have an equal amount of wear. Feathers of AHY individuals showed obvious variation in color tones: older feathers were dull and faint in comparison to newer feathers. Occasionally an ultraviolet light was used to validate age assignments, because the ultraviolet light allows visualization of fluorescing porphyrin pigments in the heme of new feathers, whereas older generation feathers lack these pigments because porphyrins break down over time when exposed to sunlight (Weidensaul et al. 2011). Insulin syringes (0.5 cc, 29 gauge) were used to collect ca 200 µl of whole blood from each owl. Whole blood was stored in 2 ml cryotubes with 1 ml of preservation buffer (Longmire et al. 1988).

Sample screening and identification

We extracted DNA from 200 µl of the blood-buffer solution from each saw-whet owl using a DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA), following the manufacturer's protocol. We used a nested PCR protocol (Hellgren et al. 2004, Waldenström et al. 2004, Bell et al. 2015) to screen DNA extracts for haemosporidian DNA. We included both a negative (purified distilled water) and positive control (a known positive sample from a previous study) on each PCR plate to insure that the PCR assay was functioning and the reagents were free of contaminants. The PCR protocol uses two sets of primers, one set designed to amplify *Plasmodium* and *Haemoproteus* (H332F, HaemNR2; H350F, HaemR2) and the other set designed to amplify *Leucocytozoon* (HaemNFi, HAEMNR3; L350F, L890R) (Bell et al. 2015). The nested PCR will amplify a fragment of the mitochondrial cytochrome-*b* (cyt-*b*) gene if haemosporidian DNA is present. We ran the PCR products on a 1% agarose gel containing 10 000× SYBR Safe DNA gel stain (Life Technologies, Carlsbad, CA) to visualize whether the samples were positive or negative for haemosporidians. We sent PCR positive products to Functional Biosciences (Madison, WI, USA) for PCR cleanup and Sanger sequencing. We followed protocols of Lutz et al. (2015) and used the same primers for sequencing as were used for the final nested PCR for the first 760 samples. However, we used sequencing primers FIFI and R2 for *Plasmodium*/*Haemoproteus* and L545F and L825R for *Leucocytozoon* for the remaining 493 samples (Bell et al. 2015). We edited the sequences visually in Geneious (ver. 8.1.8, Biomatters) and identified lineages using the BLAST sequence function in the MalAvi database (Bensch et al. 2009). Sequences that were either shorter than the assigned MalAvi gene segments or whose base pairs were less than a 100% match to a published sequence were identified to the genus level. All sequences were deposited in the MalAvi database and in GenBank (MK062185-MK063641).

Determination of variables related to irruptive migration

We used owl capture rate (number of owls captured per hour) as a proxy for population density. We used linear regression to determine whether our capture rate data was correlated with encounter data ('Bandings Count') acquired online from the USGS Bird Banding Laboratory; results revealed a significant ($p < 0.01$) relationship. Since we did not record banding hour data for year 2006, we extrapolated the 2006 capture rate data point from the equation obtained from the linear regression model fit to the dataset from 2007–2016. We defined irruptions as years with 15% more hatch-year birds than the weighted mean, as in Confer et al. (2014). The years 2010, 2012, and 2016 met this criterion when calculated from both the USGS banding data as well as our capture rate data. Although the capture rate data from 2007 met the irruption criteria, no blood samples were collected from owls during 2007 and thus, data from 2007 were excluded from further analyses.

We investigated the relationship between timing of migration and parasite prevalence by including banding date in the analyses. Across all years, banding day number was defined starting with 1 October as banding day 1, continuing sequentially until the latest capture day of 6 December, banding day 67; thus a banding day of 32, for example, would correspond to 1 November in any year.

Determination of environmental variables at owl breeding grounds

Warne et al. (2015) used an integrative approach that included assay of deuterium (δD) isotope in feathers and corticosterone profiles in blood and feathers to test for associations between blood parasite levels, geographic location, and long and short-term stress in saw-whet owls in varied ecological conditions. The isotope map presented in that study was generated from δD values of HY saw-whet owls (n = 58) sampled on the Mohonk Preserve in 2011, samples which were included in our molecular analysis. Capitalizing on this research overlap, we used the δD isotope map from Warne et al. (2015) to estimate the location of breeding grounds of the owls included in this study (Fig. 1a). From NOAA, we obtained data from 19 weather stations in the estimated breeding ground region (Fig. 1b). To obtain the average annual temperature, we calculated the monthly average temperature across all 19 stations and used those averages to estimate the average annual temperature/year. To obtain total annual precipitation, we calculated the average of the total monthly precipitation across all stations, then added these values across 12 months for each year; total annual number of snowpack days (days with ≥ 2.54 cm of snow on the ground) was calculated in the same manner as total annual precipitation (Fig. 2). We calculated correlation coefficients for the three climatic variables and found no relationship between temperature and precipitation ($z = -0.11$, $p = 0.74$) nor between precipitation and snowpack days ($z = 0.41$, $p = 0.21$); the relationship between temperature and snowpack days was borderline significant ($z = -0.61$, $p = 0.05$).

Statistical analysis

We performed mixed effects logistic regressions using stepwise predictor selection with the following dependent variables: infection status (all single infections); infection status per genus (*Plasmodium* or *Leucocytozoon*); and mixed infections. We did not run statistical analyses on *Haemoproteus* infection rates separately because we found this parasite genus in only 2 out of 1253 saw-whet owls screened. Fixed effects included host age, host density (capture rate), banding day, average annual temperature at breeding grounds, total annual precipitation at breeding grounds, and total annual snowpack days at breeding grounds. Year was included as a random effect.

Results

Parasite type, prevalence, and species richness

We found an overall haemosporidian infection rate of 73.1% in saw-whet owls (n = 1253) that were tested; 42.9% of the samples were positive for more than one parasite (Fig. 3). Haemosporidian genera included *Leucocytozoon*, *Plasmodium*, and *Haemoproteus*. Mixed infections consisted of *Leucocytozoon* and *Plasmodium* (LP), *Leucocytozoon* and *Haemoproteus* (LH), or two different strains of *Leucocytozoon* (LL); we also detected one individual with a quadruple infection (LLLH) (Fig. 3). This individual was one of 19 birds whose blood sample was inadvertently amplified and analyzed twice; each of these 19 samples was counted as one observation in our analyses.

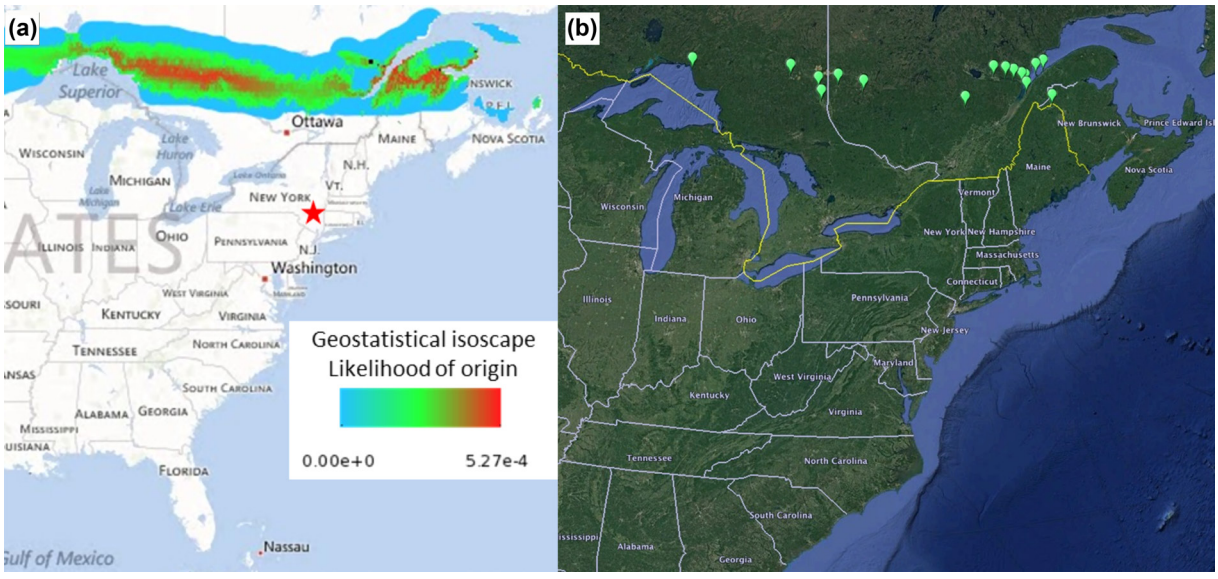

Figure 1. Proposed breeding grounds of northern saw-whet owls captured at the Mohonk Preserve near New Paltz, NY during fall migration (a) and locations of weather stations (b) that provided climatic data used to assess relationships between Haematozoa prevalence in saw-whet owls and annual precipitation, snow cover, and temperature at proposed breeding grounds of saw-whet owls. The proposed breeding grounds are from Warne et al. (2015, Fig. 2); estimates are based upon δ^2Hydrogen analysis of feathers of hatch-year owls; star shows capture site.

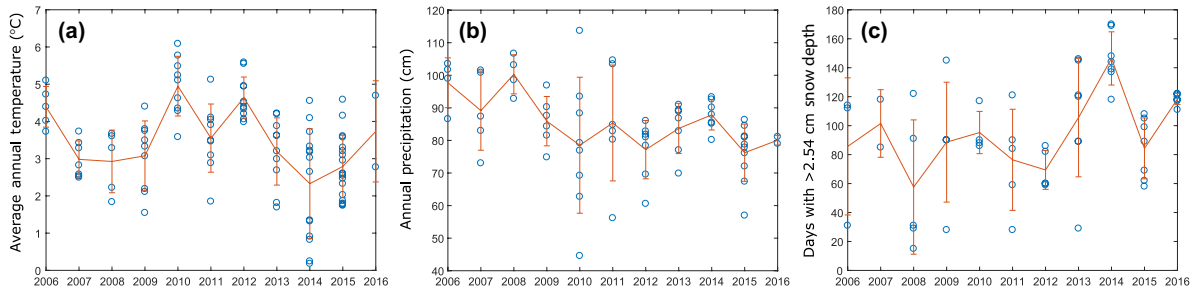

Figure 2. Average annual temperature (a), total annual precipitation (b), and total days with > 2.54 cm of snow depth (c) determined from data provided by NOAA weather stations located within the proposed northern saw-whet owl breeding grounds established from stable isotope analysis (Warne et al. 2015).

We identified 13 haemosporidian cyt-*b* lineages that were full-length and matched 100% of the base pairs in corresponding sequences published in the MalAvi database (*Leucocytozoon*: STOCC06, BUVIR04, AEFUN02, BNOW04, STOCC13, STOCC03; and *Plasmodium*: PADOM11, SEIAUR01, BT7, SW5, CATUST05, RWB01, BAEBIC02) (Fig. 4). We also identified an unreported cyt-*b* lineage of *Leucocytozoon*, assigned AEAC01, which is consistently 4 base pairs different from lineage BUVIR04 in MalAvi. The two samples we identified as *Haemoproteus* were both a 99% match to STVAR08.

Differential effects of age, temperature, precipitation, and snowpack on parasite prevalence

We screened blood samples from 624 HY and 629 AHY individuals and found that 66.2% of HY and 80.3% of AHY saw-whet owls were infected with haemosporidians. Stepwise regression analysis revealed that the best model to explain our findings included both age and average annual temperature at the breeding grounds. AHY birds were significantly more likely to be infected by haemosporidians than HY birds ($p < 0.01$) and temperature was negatively correlated with infection by haemosporidians ($p < 0.01$); this relationship was true for both single and mixed infections (Table 1).

We analyzed each haemosporidian genus separately and found that HY birds were significantly more likely to be infected by *Plasmodium* ($p < 0.01$), and AHY birds were significantly more likely to be infected by *Leucocytozoon* ($p < 0.01$). Decreasing average annual temperature was significantly correlated ($p < 0.01$) with *Leucocytozoon* infection, but the correlation between average annual temperature and *Plasmodium* infection was not significant ($p = 0.06$) (Table 1).

We found that total annual precipitation, total annual snowpack days, host density, and banding day were not significant predictors of parasite prevalence either as a whole or separately by haemosporidian genus.

Discussion

We have shown that age is an important factor determining whether a saw-whet owl is infected by a particular haemosporidian, with older birds more likely than younger birds to be infected by *Leucocytozoon*, and younger birds more likely than older birds to be infected by *Plasmodium*. We have also shown that average annual temperature at the breeding grounds may affect haemosporidian infections, with lower temperatures increasing the chance that a saw-whet owl will be infected by *Leucocytozoon*. Thus, our study contributes to the wealth of literature on avian haemosporidians and the factors influencing infection dynamics. Our study also highlights the importance of analyzing parasite infections separately at the genus-level to avoid overlooking correlations of parasite prevalence with potential causal factors.

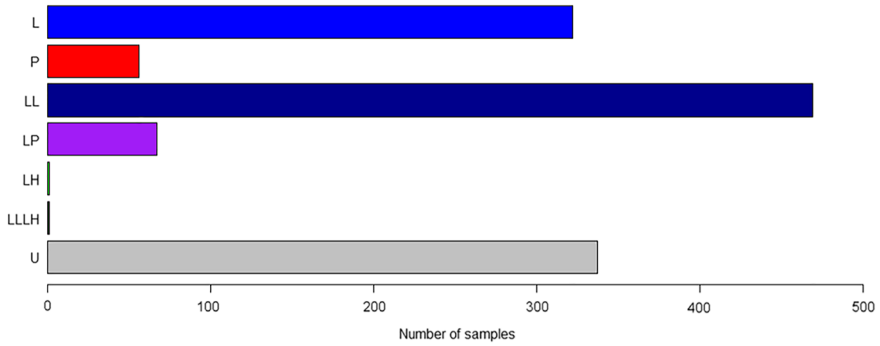

Figure 3. Number and percentage of Haemospordian infections in northern saw-whet owls (n = 1253) captured at the Mohonk Preserve near New Paltz, NY from 2006 to 2016, arranged by infection type. L = *Leucocytozoon*; P = *Plasmodium*; LL = two different *Leucocytozoon* lineages; LP = *Leucocytozoon* and *Plasmodium*; LH = *Leucocytozoon* and *Haemoproteus*; LLLH = mixed *Leucocytozoon* and *Haemoproteus* infection (quadruple infection); U = uninfected.

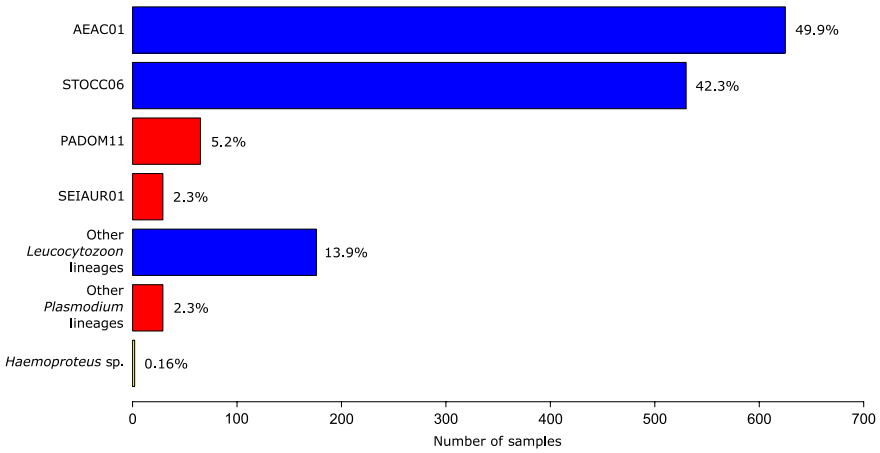

Figure 4. Number and percentage of Haemospordian infections in northern saw-whet owls (n = 1253) captured at the Mohonk Preserve near New Paltz, NY from 2006 to 2016, arranged by haemosporidian lineage. Blue = *Leucocytozoon*; red = *Plasmodium*; yellow = *Haemoproteus*. Other *Leucocytozoon* lineages include BUVIR04, AEFUN02, BNOW04, STOCC13, STOCC03, and sequences that were either < 525 bp and/or contained < 100% matched bp to a sequence in the MalAvi database. Other *Plasmodium* lineages include BT7, SW5, CATUST05, RWB01, BAEBIC02, and sequences that were < 477 bp and/or contained < 100% matched bp to a sequence in the MalAvi database.

Parasite type, prevalence, and species richness

We found higher overall prevalence of haemosporidian infections than reported by Young and Proudfoot (2014) for saw-whet owls in eastern North America. This discrepancy in infection levels in our samples is either due to the influence of sample size, with our study sampling nearly 10-fold as many individuals as those screened by Young and Proudfoot (2014), the sensitivity of screening methods, or a combination of both. Young and Proudfoot (2014) used microscopy of stained blood films for detection, which is generally less sensitive at parasite detection than molecular methods (Ishtiaq et al. 2017). We also found different infection levels for each haemosporidian genus when compared to microscopy results of Young and Proudfoot (2014): *Leucocytozoon* prevalence was higher, *Plasmodium* prevalence was similar, and *Haemoproteus* prevalence was lower. This suggests that some proportion of the *Leucocytozoon* infections that we detected were likely low-intensity infections that were too low to detect with microscopy. If *Plasmodium* is less likely than *Leucocytozoon* to persist in the low-intensity chronic phase in this host species, then this might explain why our PCR screening detected similar *Plasmodium* infection prevalence to Young and Proudfoot's (2014) microscopy screening. Our low detection rate of *Haemoproteus* (0.16% as compared to 5% found by Young and Proudfoot [2014]) may be because we used the same primer to screen for *Haemoproteus* and *Plasmodium.* It is possible that these primers are a better match to *Plasmodium* cyt-*b*, thereby causing us to underestimate *Haemoproteus* infections as has been suggested by other researchers (Ciloglu et al. 2016, Hanel et al. 2016, Meixell et al. 2016).

With regard to species richness, we cannot compare our results to that of Young and Proudfoot (2014) because they did not identify haemosporidians beyond the genus level. However, compared to the number of haemosporidian lineages that we found (n = 14), studies of closely related host species have found far fewer. In Idaho, for example, Leppert et al. (2008) only identified three haemosporidian species in saw-whet owls using blood film microscopy, and Synek et al. (2016) reported only five haemosporidian lineages in Tengmalm's owls *Aegolius funereus* in the Ore Mountains of the Czech Republic using nested PCR. Although this could be due to geographical differences in the ranges of these host species, it may also be attributed to the fact that, unlike northern saw-whet owls, Tengmalm's owl is relatively sedentary and thus may be exposed to a smaller pool of parasite lineages than migratory species

Table 1. Results of mixed effects stepwise logistic regression analysis of host age and average annual temperature at northern saw-whet owl breeding grounds and the likelihood of single or mixed genera infections and parasitism by *Plasmodium* or *Leucocytozoon,* with year as a random effect.

	Single				Mixed				*Plasmodium*				*Leucocytozoon*			
Fixed effect	Est.	SE	Z	p	Est.	SE	Z	p	Est.	SE	Z	p	Est.	SE	Z	p
Intercept	12.70	4.25	2.98	*	14.02	3.33	4.21	*	4.72	3.64	1.30	0.19	11.53	4.10	2.81	*
Host age	0.71	0.14	5.05	*	0.52	0.13	4.11	*	–0.63	0.21	–3.07	*	0.86	0.14	6.31	*
Temp.	–0.32	0.11	–2.85	*	–0.39	0.09	–4.38	*	–0.18	0.10	–1.85	0.06	–0.30	0.11	–2.74	*

*= < 0.01.

(Waldenström et al. 2002). Furthermore, the 14 lineages that we report here are most likely an underestimation because we excluded those sequences that were not 100% matches or were shorter than the gene segment used for lineage identification in MalAvi.

Age as a predictive factor of parasite infection rate

Our results indicate that host age is an important factor involved in saw-whet owl haemosporidian infection dynamics. This is a new finding for this particular host species since previous studies of haemosporidian infections in saw-whet owls did not find that age was a significant predictor of infection, although this may have been due to smaller sample size or less sensitive haemosporidian detection methods (Young and Proudfoot 2014). Our age-related findings are similar to Synek et al.'s (2016) study of Tengmalm's owl, which also used nested PCR for detection and attributed this age difference in infection to the fact that adults, being older, have had a longer period of exposure to haemosporidian vectors, and thus were more likely to have become infected.

This explanation does not fully explain our findings, however, because unlike Synek et al. (2016), we analyzed each genus of haemosporidian independently and found different patterns with respect to age: older birds were more likely to be infected by *Leucocytozoon* than were younger birds, but younger birds were more likely to be infected by *Plasmodium* than older birds. Thus, the 'more time for exposure' hypothesis can only explain our findings with respect to *Leucocytozoon*. Three non-mutually exclusive hypotheses have been developed to explain age-related differences in haemosporidian infections (Sol et al. 2003).

The selection hypothesis suggests that young birds that are heavily parasitized have high mortality and therefore do not become part of the adult population (Sol et al. 2003). This could potentially explain our finding that *Plasmodium* prevalence was higher in hatch-year birds than in adults. Infection by *Plasmodium* may be more deleterious than *Leucocytozoon*, thus hatch-years who acquire *Plasmodium* may be less likely to survive their first year and become adults. This hypothesis has been supported in other studies such as Atkinson and Samuel (2010), who found that young Hawaiian apapane *Himatione sanguinea* infected with *Plasmodium* had higher mortality than adult birds. The same pattern was found for *Haemoproteus* infecting Seychelles warblers *Acrocephalus sechellensis* (van Oers et al. 2010) and feral pigeons *Columba livia* (Sol et al. 2003). However, the hatch-year birds from our study were all captured during fall migration. Presumably infected hatch-year birds would have succumbed to a severe infection prior to migrating or failed to migrate. Given the time of capture and the timing of the infectious cycle of both *Plasmodium* and *Leucocytozoon* (Valkiūnas 2005), it is more likely that the infections that we detected were chronic rather than acute, although we cannot be certain given that we did not measure intensity of parasitemia (Young and Proudfoot 2014).

The immunity hypothesis suggests that differences in the immune system of avian hosts in different age classes determine the extent to which they can resist, sustain, or clear infections (Sol et al. 2003). Because *Plasmodium* and *Leucocytozoon* have different life cycles within avian hosts (Valkiūnas 2005), it is not surprising that their effects on older versus younger birds would differ. For example, due to a more developed immune system, adult birds may be better able to resist or clear *Plasmodium* infections than hatch-year birds, but the same may not be true for *Leucocytozoon*. Longitudinal studies of *Plasmodium* and *Haemoproteus* in a variety of species have demonstrated that individuals were able to clear infections (Bensch et al. 2007, Knowles et al. 2011, Wood et al. 2013, Hammers et al. 2016, but see van Rooyen et al. 2013). In contrast, *Leucocytozoon* infections have been reported to persist in avian hosts over time (Appleby et al. 1999, Leppert et al. 2008, Hanel et al. 2016, but see van Rooyen et al. 2013). Thus, it is possible that adult saw-whet owls in our study had higher levels of *Leucocytozoon* than hatch-years because their immune systems enabled them to both clear *Plasmodium* infections more effectively and maintain *Leucocytozoon* infections at low, chronic, sublethal levels. Hatch-year birds' immune systems might not have been sufficiently developed to effectively clear *Plasmodium* infections.

The vector hypothesis suggests that morphological and behavioral differences among birds of different age classes cause them to be differentially exposed to vectors and thus become infected by haemosporidians at different rates. For example, because saw-whet owls are cavity-nesters, black flies may have a more difficult time locating owl nestlings, since the flies rely on a combination of visual and chemical cues to find prey (Weinandt et al. 2012). Thus, *Leucocytozoon* infections of saw-whets may occur more often in older birds that have already left the nest (Dunn et al. 2017). In contrast, mosquitoes rely less on visual cues and instead depend heavily on carbon dioxide, heat, and chemical cues to find a blood meal (McMeniman et al. 2014). Therefore, mosquitoes may be more attracted to the warmth of a saw-whet owl's nest, which likely contains multiple nestlings and is further warmed by being located inside a cavity (Dubiec et al. 2016), thus increasing the chance that younger birds will be infected by *Plasmodium*. Furthermore, plumage may interact with the locomotion or biting abilities of insect vectors (Ramey et al. 2014). For example, mosquitoes may be more capable of penetrating the downy feathers of a young bird than the mature feathers of an adult bird.

Our study cannot distinguish between the explanations suggested above. However, given that these hypotheses are non-mutually exclusive, it is likely that some combination of the hypotheses above accounts for our results. Ours is not the first study to find different age-related patterns among different types of haemosporidians. Meixell et al. (2016) presented a similar finding in three species of waterfowl in Alaska: mallard *Anas platyrhynchos*, northern pintail *Anas acuta*, and American green-winged teal *Anas crecca*, although they did not discuss possible explanations for this pattern.

Climatic variables as predictors of parasitization

Based on what is known about the ecology of dipteran vectors, we predicted that all haemosporidian types would be more prevalent during warmer years. Studies of black fly ecology report only positive correlations with speed of pupation and temperature, except at extremely high temperatures (Ross and Merritt 1978); furthermore, warmer temperatures should allow for earlier vector emergence as ice and snow melt, uncovering streams so that emergence of black flies may occur (Murdock et al. 2013). Thus, our finding that *Leucocytozoon* infections are less prevalent at warmer temperatures was surprising. However, our findings corroborate those of a study of blackcaps *Sylvia atricapilla* on the Iberian Peninsula, which found that although *Plasmodium* infections were more prevalent in warmer regions, both *Leucocytozoon* and *Haemoproteus* infections were more prevalent in cooler regions (Pérez-Rodríguez et al. 2013). Pérez-Rodríguez et al. (2014) subsequently predicted that global warming would increase *Plasmodium* prevalence while decreasing prevalence of *Leucocytozoon* and *Haemoproteus*.

It is possible that temperature did not affect infection prevalence via dipteran vectors but rather affected haemosporidians themselves directly. Sporogeny in *Leucocytozoon* occurs at relatively lower temperatures than *Plasmodium* (Valkiūnas 2005). Thus, lower average annual temperature may favor infection by *Leucocytozoon*. A lack of a relationship between *Plasmodium* infections and average annual temperature at the breeding grounds may have been due to lack of power, since the number of *Plasmodium* infections per year in our study was rather low.

Finally, it is possible that abiotic factors such as temperature have effects on the avian hosts themselves that drive the patterns we observed. In warmer years, for example, birds may expend fewer calories on feeding and thermoregulation, leaving more resources for the immune system to successfully fend off infection by haemosporidians. Notably, a connection between energy resources and haemosporidian infection rates has been established in other studies (Norris et al. 1994, Appleby et al. 1999).

While the connection between infection prevalence and temperature was robust in our study, we were surprised that we did not find an effect of precipitation. This could be because studies that have found a correlation between precipitation and haemosporidian infection were comparing moisture levels between different geographical regions (Kulkarni et al. 2010, Sehgal et al. 2011, Cornuault et al. 2013, Pérez-Rodríguez et al. 2013, Coon and Martin 2014, Oakgrove et al. 2014). As our study was looking at differences in precipitation in the same geographical area between years, it is possible that the variance of precipitation in our study was too low to find an effect.

Relationship of migratory patterns and parasite prevalence

Despite our predictions, we found no connection between the density of avian hosts and the prevalence of haemosporidian infection either generally or separately for any one parasite genus. There are several possible explanations for this finding. First, if hatch-year birds are less likely than adults to be infected by *Leucocytozoon*, the primary haemosporidian in our study, and irruption years are driven primarily by an increase in hatch-year birds, then it follows that infection rates would stay roughly the same during irruption years. Alternatively, if irruption years are a consequence of more resources at the breeding grounds (Côté et al. 2007), then the increase in the likelihood of disease transmission due to a larger host population (Isaksson et al. 2013) may be outweighed by the benefits to avian hosts that come with increased food resources, such as having more energy resources to better fight off parasitic infections (Appleby et al. 1999). Finally, it is possible that host density in this species never reaches a level high enough to impact disease transmission. Our study species is not colonial, spending most of their time alone or in pairs (Holt et al. 2017) and are generally found at low densities on the breeding grounds: a study of saw-whet owls in forested areas of Alberta, Canada found an average density of 0.33 birds per square km (Grossman et al. 2008). Thus, even a relatively large increase in the owl population may not significantly increase the proximity of owls or disease vectors, and thereby would not have an impact on disease transmission.

Despite our predictions, we found no connection between migration timing and infection prevalence. Jasper et al. (2014) found a similar lack of connection in migrating red-tailed hawk *Buteo jamaicensis* as did Ishak et al. (2010) in Cooper's hawk *Accipiter cooperii*. One possible explanation for this finding is that each of these studies, including our own, screened individuals for infection using PCR which is known to be highly sensitive at detecting haemosporidian infection; thus many of our positive samples were likely chronic, low-intensity infections that were not severe enough to impact migration. This large number of low-intensity infections may have masked our ability to find a correlation between haemosporidian infection and migration timing. Future studies looking at the connection between haemosporidian infection and migration should measure intensity of parasitemia to differentiate between chronic low-level infections and high-intensity infections.

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