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Avian malaria in a boreal resident species: long-term temporal variability, and increased prevalence in birds with avian keratin disorder

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ABSTRACT

The prevalence of vector-borne parasitic diseases is widely influenced by biological and ecological factors. Environmental conditions such as temperature and precipitation can have a marked effect on haemosporidian parasites (*Plasmodium* spp.) that cause malaria and those that cause other malaria-like diseases in birds. However, there have been few long-term studies monitoring haemosporidian infections in birds in northern latitudes, where weather conditions can be highly variable and the effects of climate change are becoming more pronounced. We used molecular methods to screen more than 2,000 blood samples collected from black-capped chickadees (*Poecile atricapillus*), a resident passerine bird. Samples were collected over a 10 year period, mostly during the non-breeding season, at seven sites in Alaska, USA. We tested for associations between *Plasmodium* prevalence and local environmental conditions including temperature, precipitation, site, year and season. We also evaluated the relationship between parasite prevalence and individual host factors of age, sex and presence or absence of avian keratin disorder. This disease, which causes accelerated keratin growth in the beak, provided a natural study system in which to test the interaction between disease state and malaria prevalence. Prevalence of *Plasmodium* infection varied by year, site, age and individual disease status but there was no support for an effect of sex or seasonal period. Significantly, birds with avian keratin disorder were 2.6 times more likely to be infected by *Plasmodium* than birds without the disorder. Interannual variation in the prevalence of *Plasmodium* infection at different sites was positively correlated with summer temperatures at the local but not statewide scale. Sequence analysis of the parasite cytochrome *b* gene revealed a single *Plasmodium* spp. lineage, P43. Our results demonstrate associations between prevalence of avian malaria and a variety of biological and ecological factors. These results also provide important baseline data that will be informative for predicting future changes in *Plasmodium* prevalence in the subarctic.

1. Introduction

Ecological factors can affect the diversity and distribution of vector-borne pathogens. In northern climates, where the transmission season may be limited, local environmental effects may significantly influence disease dynamics. Study of a resident northern passerine species provides an ideal system in which to examine the influence of environmental factors and host traits on parasite transmission and prevalence of infection.

Blood parasites of the order Haemosporidia, which infect amphibians, reptiles, birds and mammals and are transmitted by blood-sucking insect vectors, are one of the best-studied groups of parasites (Valkiūnas, 2005). Three genera of haemosporidians cause malaria and malaria-like diseases: *Plasmodium* (transmitted by mosquitoes, Culicidae), *Haemoproteus* (transmitted by biting midges, Ceratopogonidae), and *Leucocytozoon* (transmitted by black flies, Simuliidae). Asexual reproduction occurs in the vertebrate (intermediate host), while sexual reproduction occurs in the vector (definitive host). Avian malaria, caused by parasites of the genus *Plasmodium*, is responsible for the extinction and endangerment of numerous bird species (Beier and Stoskopf, 1980; van Riper et al., 1986; LaPointe et al., 2012). Morbidity is more likely in birds that have not evolved with *Plasmodium*; sublethal effects on host fitness such as mate selection, reproductive success and immune response are seen in wild bird populations that have long-standing associations with the parasite (LaPointe et al., 2012). Wild birds may retain mild chronic infections, potentially

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accumulating adverse side effects that impair genetic fitness or reduce their life span (Asghar et al., 2015).

Individual host characteristics may play an important role in exposure to vector-borne parasites. Adults commonly have higher prevalence than juveniles (Cosgrove et al., 2008; Atkinson and Samuel, 2010), a pattern which may be attributed to a longer period of exposure to vectors (Valkiūnas, 2005). The influence of sex on parasite prevalence is less clear, but some studies have reported a difference between males and females (Schall and Marghoob, 1995; Wood et al., 2007). In addition, individual health or disease status can also affect susceptibility to blood parasite infection (Knowles et al., 2011).

The occurrence of avian blood parasites can also be influenced by site characteristics, interannual or seasonal variation, temperature and precipitation (Lachish et al., 2011; Sehgal et al., 2011; Ramey et al., 2012; Gonzalez-Quevedo et al., 2014; Oakgrove et al., 2014; Krama et al., 2015). The relationship between climate and the prevalence of haemosporidians has been documented, with warmer temperatures facilitating the development of parasites (Freed et al., 2005; Garamszegi, 2011; Loiseau et al., 2012a, 2013; Zamora-Vilchis et al., 2012) as well as the abundance and development of vectors (LaPointe et al., 2012; Zamora-Vilchis et al., 2012). Multi-year studies including the effect of temporal, biotic and abiotic factors on haemosporidian infections have shown uneven prevalence across years (Fallon et al., 2004; Bensch et al., 2007; Clark and Clegg, 2015), highlighting the likely interactions of abiotic, vector and host-related factors on parasite prevalence.

Previous research has demonstrated that haemosporidians not only occur but are locally transmitted in Alaska, USA (Ramey et al., 2014, 2015), with Loiseau et al. (2012a) providing the first evidence of *Plasmodium* transmission in the state. Locally acquired infections of *Haemoproteus* and *Leucocytozoon* have been documented in the white-winged crossbill (*Loxia leucoptera*), a species that is a year-round resident of the boreal forest zone (Deviche et al., 2010). *Leucocytozoon* was found in both current and historic samples of goslings from tundra-nesting geese, indicating local transmission of the parasite for at least 20 years (Ramey et al., 2014). In the migratory Swainson's thrush (*Catharus ustulatus*), juveniles in Alaska were infected with *Leucocytozoon*, indicating that they had obtained the parasite locally (Dodge et al., 2012). A recent study across a broad latitudinal gradient in Alaska showed that the prevalence of four genera of haematozoa in 47 avian species varied with location, age, avian species, migratory status and, for *Leucocytoozoon*, co-infection by other parasites (Oakgrove et al., 2014). However, with the exception of Ramey et al. (2014), these studies were based on data collected during only 1 or 2 years, highlighting the need for analysis of a broad, long-term data set from a northern system to understand spatiotemporal patterns of infection at northern latitudes.

We capitalised on an extensive collection of blood samples gathered in Alaska during the course of an ongoing, long-term study of the black-capped chickadee (*Poecile atricapillus*), hereafter "chickadee." Chickadees form a monogamous pair bond and are strongly territorial during the breeding season before joining with neighbouring birds to forage in small flocks over their combined territories in the autumn and winter (Smith, 1991). Flocks have a dominance hierarchy, with an individual's rank affecting its behaviour and chances for survival (Smith, 1991). Age, sex, size and social dominance all play a role in this hierarchy and females may avoid foraging in microhabitats used by dominant males (Desrochers, 1989). The dynamics between the age and sex groups make for possible differences in the microhabitats to which each is exposed, potentially allowing for a variance in parasite prevalence within the species.

Recently, chickadees and other species in Alaska and the Pacific Northwest have been affected by a condition called avian keratin disorder (Handel et al., 2010). This disease, which was the primary focus of the original study and whose aetiology is still unknown, causes rapid growth of the outer keratinized layer of the bill and may also affect the skin, legs, feet, claws and feathers (Handel et al., 2010; Van Hemert and Handel, 2010; D'Alba et al., 2011; Van Hemert et al., 2013). The resulting beak deformity reduces the bird's ability to preen and some affected individuals have been found heavily infested with parasitic feather mites (Handel et al., 2010). Birds with this disorder alter their dietary habits and behaviour, and their reproductive fitness, health and survival are also negatively affected (Handel et al., 2010; Van Hemert et al., 2012).

Here we investigate environmental and host factors associated with the prevalence of infection in a resident subarctic passerine bird by the haemosporidian parasites *Plasmodium* and *Haemoproteus*. We describe the dynamics of haemosporidian prevalence in populations of wild-caught chickadees over a 10 year period, primarily during winter, at several sites in Alaska. We aimed to identify possible relationships between parasite prevalence and variables associated with the local environment including summer climate, season, location and year. We also sought to evaluate the relationship between parasite infection and characteristics of the individual host, including age, sex and disease status. We predicted that the prevalence of haemosporidian infection in resident birds at different sites during winter would be positively correlated with climatic variables (temperature and precipitation) at those sites during the previous summer, when vectors are active and parasite transmission may occur. We also predicted a seasonal decline each winter as individuals possibly cleared infections acquired during the previous summer. We predicted that the prevalence of parasite infection would be higher among adult than juvenile birds due to differences in exposure to vectors during summer and that the prevalence might differ between sexes if birds used different microhabitats due to the birds' dominance hierarchy (Smith, 1991). Finally, we hypothesised that birds affected by avian keratin disorder would have a higher prevalence of haemosporidian parasites than unaffected birds, due to possible health effects that the disorder has on the individual.

2. Materials and methods

2.1. Sample collection

We collected 2070 blood samples from chickadees at seven sites in southern central and interior Alaska between the years 2000 and 2011 (Fig. 1), primarily during the non-breeding season, as part of a long-term capture–recapture study of avian keratin disorder in this species (Handel et al., 2010). Non-breeding chickadees were captured systematically every 2 months between September and April (hereafter 'winter') using modified funnel traps and mist nets at three 10 ha sites in wooded parklands surrounded by residential areas in the greater Anchorage, USA, area (Campbell Creek Science Center, Eagle River Nature Center, Mirror Lake Middle School in Chugiak). Additional non-breeding birds were captured during various years at two rural residences in southern and western Anchorage, on the wooded campus of Matanuska-Susitna College, on the wooded campus of the University of Alaska Fairbanks, and on the Kenai National Wildlife Refuge (Table 1, Fig. 1). Study areas ranged in elevation from 56 to 155 m above sea level and all were in mature mixed forests dominated by white spruce (*Picea glauca*) and paper birch (*Betula papyrifera*) interspersed with lowland black spruce (*Picea mariana*) bogs. A few blood samples collected during a nest-box study in the Anchorage area during the summers of

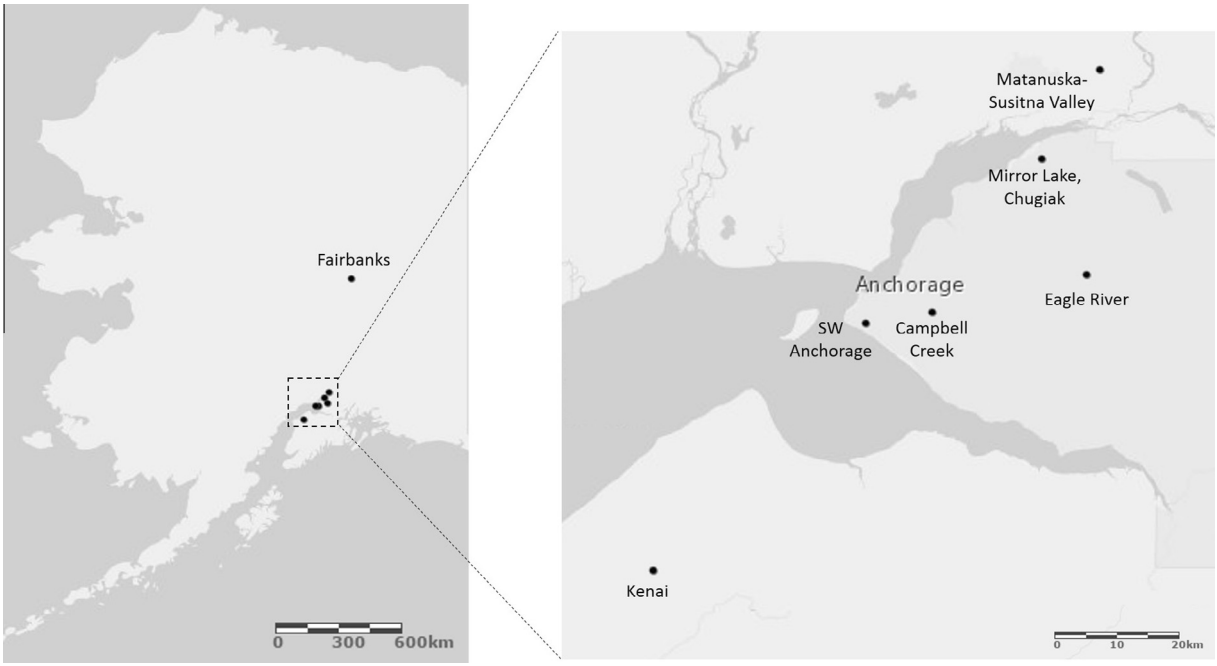

Fig. 1. Sampling sites for a long-term study of *Plasmodium* infection in black-capped chickadees (*Poecile atricapillus*) in subarctic Alaska, USA, 2001–2011. SW, southwestern.

Table 1
Location and elevation of study areas in Alaska, USA, where black-capped chickadees (*Poecile atricapillus*) were captured and nearest weather stations where summer climatic data were collected.

Study area	Location	Elevation (m)	Weather station[a]	Elevation (m)	Distance (km)	Year[b]
Campbell Creek, Anchorage	61.164 N, 149.777 W	81	GHCND:USC00501220	79	0	2001–2010
Eagle River	61.234 N, 149.271 W	155	GHCND:USC00503163	143	20	2001
			GHCND:USC00502656	152	11	2002–2010
Mirror Lake, Chugiak	61.428 N, 149.425 W	113	GHCND:USC00505733	52	18	2001
			GHCND:USC00502737	195	6	2002–2010
SW Anchorage	61.150 N, 150.004 W	56	GHCND:USW00026541	37	2	2001–2010
Fairbanks	64.867 N, 147.850 W	111	GHCND:USW00026411	132	5	2008
Matanuska-Susitna Valley	61.579 N, 149.241 W	97	GHCND:USC00505733	52	2	2001–2003, 2008
Kenai	60.728 N, 150.723 W	74	GHCND:USW00026523	28	33	2002, 2003, 2007

[a] Station code from National Climatic Data Center, USA (www.ncdc.noaa.gov).
[b] Climatic data for the summer in each year, preceding the autumn/winter during which birds were captured. SW, southwestern.

2000–2006 were also screened for parasites, including 16 breeding adults and five nestlings, all from different nest boxes.

Birds captured during the study were banded with a uniquely numbered U.S. Geological Survey aluminium leg band, measured and released after capture. Blood samples were collected from the brachial vein and stored in 400 μL of Longmire buffer solution (Longmire et al., 1988). Sex was determined genetically from blood or buccal samples (Handel et al., 2006). We used beak morphometrics to classify birds as normal or diseased (affected by avian keratin disorder) according to criteria established by Handel et al. (2010). Birds were aged by degree of skull pneumatization and by narrowness of the outermost rectrices and absence of white on the tips (Meigs et al., 1983; Pyle, 1997). During autumn and early winter, most birds could be aged reliably as hatch year (HY; hatched that summer) or after hatch year (AHY; adult at least 1 year old) but the age of some individuals with completely pneumatized skulls and worn rectrices could not be determined. Although a few young birds in their second calendar year (second year; SY) and some older birds (after second year; ASY) could be distinguished by tail characteristics after 1 January, most could not, so all birds captured between January and June were classified as AHY birds.

2.2. Parasite screening

DNA was extracted from whole blood using the Wizard® SV Genomic DNA Purification System (Promega, Madison, Wisconsin, USA). Extraction success was verified by PCR using primers that amplify the gene encoding the brain-derived neurotrophic factor (Sehgal and Lovette, 2003). We used PCR protocols as described below following Waldenström et al. (2004). *Haemoproteus* and *Plasmodium* spp. were detected by a nested PCR, amplifying sections of the mitochondrial cytochrome *b* (cyt *b*) gene. The primers HaemNF and HaemNR2 were used for the first reaction and HaemF and HaemR2 were used for the second nested reaction. The nested reaction yielded a cyt *b* sequence of up to 524 bp. All reactions were performed in 25 μL volumes and were accompanied by negative (ddH_20) and positive controls (samples from infected birds as confirmed by PCR) to control for any contamination and to confirm success of the PCR. We randomly selected 120 samples for retesting to verify PCR results, confirming 112 initial results and revealing eight false negatives. This concordance of 93% is comparable with other studies (Ramey et al., 2015). Nested reaction products were separated on a 1.8% agarose gel using 1× TBE buffer and visualised by ethidium bromide staining under UV light. PCR products

were purified using ExoSap-IT according to the manufacturer's instructions (U.S. Biochemical Corporation, Cleveland, Ohio, USA) and were sequenced to identify parasite lineages (BigDye® version 1.1 sequencing kit, Applied Biosystems, Foster City, California, USA) on an ABI Prism 3100™ automated sequencer (Applied Biosystems). Sequences (both forward and reverse) were edited and aligned using the program Sequencher 4.8 (Gene Codes, Ann Arbor, Michigan, USA). The BLAST algorithm was used to compare the consensus sequences of new lineages to known *Haemoproteus* and *Plasmodium* spp. lineages deposited in GenBank. Sequences were also compared with the MalAvi database of avian haemosporidian cyt *b* lineages (Bensch et al., 2009). Blood smears were not made from the original blood samples, so unfortunately, we were not able to identify parasites morphologically.

2.3. Climatic data

We obtained data for daily maximum and minimum temperatures and total precipitation from the National Climatic Data Center, USA (www.ncdc.noaa.gov) at weather stations nearest to each of our seven study sites during the avian keratin disorder study. From these data we calculated mean daily temperature (average of maximum and minimum) and mean daily precipitation during the summer months (May–September) preceding each winter season of bird captures at a given site. Weather stations ranged from 2 to 44 km from capture sites but were generally at similar elevations and considered to be representative of conditions where birds were captured (Table 1). For two capture sites, we augmented missing data from the primary weather station with those from the next nearest weather station with similar summer levels of temperature and precipitation.

2.4. Statistical analysis

We analysed data from 1770 of the original 2070 blood samples collected across 10 winters and seven sites to test for associations between *Plasmodium* infection status of individual chickadees and biological, ecological and climatic factors (Table 2). To assure independence, we excluded 134 samples from chickadees bled multiple times; we also excluded 140 samples from birds of unknown age, 21 from adults and nestlings collected during summer, the only four samples collected during winter 2000/2001, and one sample from a captive bird of unknown origin.

We used generalised linear models (Proc GENMOD with binomial distribution of errors and logit link function; SAS Institute, version 9.4) to investigate the relationship between parasite infection status (dependent, binary variable) and the following explanatory variables associated with capture of each bird: (i) capture site, (ii) year (winters of 2001/2002 through 2010/2011), (iii) season (autumn: September–October, just after adults have completed prebasic molt and juveniles have become independent; early winter: November–December, the beginning of adverse weather; mid-winter: January–February, harshest winter weather; and late winter: March–April, immediately before adults begin to disperse to breeding territories), (iv) age (HY versus AHY), (v) sex and (vi) presence or absence of avian keratin disorder. To test effects of climatic factors, we also modelled associations with (vii) mean daily temperature and (viii) mean daily precipitation during summer months (May–September) preceding captures at each site.

We analysed four different subsets of the data in a series of models for several reasons: not all sites were sampled in all years; we could only reliably age birds during the first two seasonal periods; only birds at least 6 months of age are affected by avian keratin disorder; and sample sizes of birds affected by the disorder were small. In the first analysis ($n = 508$), we restricted the data set to individuals with normal beaks (i.e. unaffected by the disorder) of known age and sex that were captured during autumn or early winter at one of the three standard banding sites in the greater Anchorage area (Campbell Creek, Eagle River, Mirror Lake), where birds were captured systematically every year of the study. Samples from winter 2008/2009 were excluded because no infections were found in birds with normal beaks at these sites that winter and prevalence was therefore inestimable. This restricted subset (birds whose age could be reliably determined) was used to maximise power to test if prevalence differed between adults and juveniles, since young birds would likely have been exposed to vectors for a shorter period of time.

In the second analysis ($n = 1215$), the data set was expanded to include all individuals of known age and sex with normal beaks captured at the same standard sites in Anchorage during September–April (again excluding winter 2008/2009), although most young birds could not be distinguished from older birds after 1 January and all were classified as AHY birds. In this analysis, the variable for age provided a comparison of birds <6 months old versus all older birds. Thus, older birds sampled after 1 January would have included some individuals that had hatched the previous summer. This data set was the most robust for examining seasonal, interannual and spatial variation in parasite prevalence at a local level (within the greater Anchorage area).

In the third analysis ($n = 1649$), the data set was further expanded by adding all individuals with normal beaks (adults and juveniles) captured at other sites throughout Alaska, including samples from all years and seasons. Although capture effort was imbalanced across seasons and years, this data set allowed us to examine spatial and interannual variation in parasite prevalence at a broader geographic scale.

Table 2
Numbers of black-capped chickadees (*Poecile atricapillus*) tested for haemosporidian parasites at seven study sites in Alaska, USA, during autumn and winter (September–April) from 2001/2002 to 2010/2011 and included in statewide analysis.

Autumn–Winter	Site						
	Campbell Creek	Eagle River	Mirror Lake	SW Anchorage	Fairbanks	Mat-Su	Kenai
2001/2002	50	15	73	48		14	
2002/2003	42	36	66	57		27	13
2003/2004	67	27	48	19		27	8
2004/2005	41	30	26	51			
2005/2006	43	91	45	9			
2006/2007	70	49	43	12			
2007/2008	78	21	50	14			21
2008/2009	61	15	42	12	18	2	
2009/2010	38	41	58				
2010/2011	45	45	47	15			
Total	535	370	498	237	18	70	42

SW, southwestern; Mat-Su, Matanuska-Susitna Valley.

In the final analysis (n = 761), the data set was structured to test for an association between *Plasmodium* infection and disease status (presence of avian keratin disorder) in adults in addition to year, site and climatic variables. All AHY birds (with and without avian keratin disorder) captured at any of the Anchorage sites during any season were included. It was possible to include winter 2008/2009 because some individuals with avian keratin disorder within this sample were infected by *Plasmodium*.

In each analysis, an all-subsets modelling approach was used, in which the full model included all additive combinations of the independent variables; all possible subsets of that model were included in the candidate model set. We compared full models with an autoregressive versus independent correlation structure and found no evidence of temporal correlation in prevalence across years based on the quasi-information criterion (QIC) in the generalised estimating equations. Thus we assumed an independent correlation structure for model subsets and evaluated the relative support for models in each candidate set using Akaike's Information Criterion corrected for sample size (AIC_c) (Burnham and Anderson, 2002). Within each candidate model set, we then calculated the Akaike weight (w_i) for each model, which is the relative likelihood of the model, given the data and the models being considered, and calculated the evidence ratio (w_i/w_j) to evaluate the support for one model (i) over another (j) (Burnham and Anderson, 2002).

Credible intervals (95% CI) were calculated for estimates of prevalence in different groups of birds as described by Swei et al. (2011) using the inverse of the cumulative distribution function of the beta distribution; the shape 1 parameter was specified as $\mu + 1$ and the shape 2 parameter was specified as $N - \mu + 1$, where μ was the number of positive samples and N was the total sample size. These analyses were executed in the R framework (R Development Core Team, 2004. A language and environment for statistical computing. R Foundation for Statistical Computing. R Found. Stat. Comput. Vienna, Austria).

3. Results

Among 1770 blood samples collected from chickadees with and without avian keratin disorder during winter of 2001/2002–2010/2011 at seven sites across Alaska, 417 (24%; 95% CI: 3–33%) tested positive for *Plasmodium* infection, all belonging to a single cyt *b* lineage, P43 (GenBank accession number: DQ839065). When compared with the MalAvi database (December 2015), lineage P43 matched perfectly with lineage BT7, which has been reported as infecting 37 avian host species in 11 families and has been found in Europe, North America, Hawaii and Asia. Only three infections with *Haemoproteus* were detected (0.17%; 95% CI: 0.06–0.49%), also of only one lineage, OZ02DR21 (GenBank accession number: GQ395667), and these were not considered further for analysis.

Table 3
Apparent prevalence (%) of *Plasmodium* infection among black-capped chickadees (*Poecile atricapillus*) sampled during autumn and winter from 2001/2002 to 2010/2011 at seven sites across Alaska, USA.

Site	n	Mean	95% CI
Fairbanks	18	11.1	3.4–33.2
Matanuska-Susitna Valley	70	25.7	16.9–37.1
Mirror Lake	498	31.1	27.2–35.3
Eagle River	370	16.5	13.1–20.6
Campbell Creek	535	15.9	13.0–19.2
SW Anchorage	237	35.9	30.0–42.2
Kenai	42	26.2	15.3–41.2
Overall	1770	23.6	21.6–25.6

CI, credible interval; SW, southwestern.

Across all winters combined, the prevalence of *Plasmodium* infection was variable among the seven sites sampled, ranging from 11% (95% CI: 3–33%; n = 18) in Fairbanks, the most northerly site, to 36% (95% CI: 30–42%; n = 237) in southwestern Anchorage (Table 3). Among the few samples collected from birds in nest boxes in Anchorage during the summers of 2000–2006, the prevalence of *Plasmodium* infection was 50% among breeding adults (95% CI: 28–72%; n = 16) and 20% in nestlings (95% CI: 4–64%; n = 5). Four of the breeding adults had avian keratin disorder and three of these were infected with *Plasmodium*.

3.1. Effects of biological, ecological and climatic factors on parasite prevalence

3.1.1. Anchorage autumn and early winter

The first analysis was restricted to chickadees with normal beaks captured at one of the three standard Anchorage sites during autumn and early winter, when juvenile (HY) birds could reliably be discriminated from adult (AHY) birds. Within the candidate set, the model receiving the most support (w = 0.27) for explaining variation in parasite prevalence included categorical effects for year, site and age, and linear effects for mean precipitation and temperature during the summer preceding capture (Table 4). A similar model excluding temperature, however, received almost equal support (ΔAIC_c = 0.21; w = 0.25). In contrast, there was little support for a difference in prevalence between sexes and virtually none for a seasonal difference between autumn and early winter (Table 4). Across all standard Anchorage sites and years (excluding winter 2008/2009 when no infections were detected in birds with normal beaks), the prevalence of *Plasmodium* infection was estimated to be 23% (95% CI: 19–27%) among 402 HY birds and 36% (95% CI: 27–45%) among 106 AHY birds during autumn and early winter (Fig. 2A). Based on the best-supported model, adult birds with normal beaks were 2.0 (95% CI: 1.1–3.4) times more likely than juveniles to be infected by *Plasmodium* during autumn and early winter.

3.1.2. Anchorage All Winter

When the data set was expanded to include chickadees with normal beaks captured at the same standard Anchorage sites during all four winter capture periods, the model receiving the most support (w = 0.21) again included year, site and temperature but not age or precipitation (Fig. 3, Table 4), although a model that also included precipitation had almost equal support (ΔAIC_c = 0.15; w = 0.19). Younger birds could not be discerned from older birds during middle and late winter, so AHY birds captured during those periods included individuals from both age cohorts. Again there was little support for a difference in prevalence between sexes or among the four seasonal periods (Table 4).

3.1.3. Alaska All Winter

In the statewide analysis of birds with normal beaks, the best-supported model (w = 0.17) included only site and year. There was moderate support (w = 0.06–0.14) for models that also included combinations of age, temperature and precipitation but the remaining parameters (sex, season) were less informative (Table 4).

3.2. Effects of avian keratin disorder on parasite prevalence

Among 761 AHY birds across all of the Anchorage sites during winter, 143 showed signs of avian keratin disorder, 62 of which tested positive for *Plasmodium* (prevalence of 43%; 95% CI: 36–52%). *Plasmodium* prevalence for the remaining 618 unaffected AHY birds was 26% (160 infected; 95% CI: 23–30%; Fig. 2B). The best-supported model explaining parasite prevalence in this

Table 4
Results of generalised linear models testing for ecological, biological and climatic factors associated with *Plasmodium* infection among black-capped chickadees (*Poecile atricapillus*) with normal beaks (i.e., not affected by avian keratin disorder) captured in Alaska, USA, during autumn and winter from 2000/2001 to 2010/2011. Within each candidate set, the model with $\Delta AIC_c = 0$ has the best fit to the data; K is the number of parameters in the model; *w* is Akaike model weight. The top 10 models within each set are shown.

Model by Candidate Set	K	ΔAIC_c	*w*
Anchorage Autumn–Early Winter[a] (*n* = 508)			
Year + Site + Age + Temp[d] + Prcp[d]	14	0.00[e]	0.27
Year + Site + Age + Prcp[d]	13	0.21	0.25
Year + Site + Age + Sex + Temp[d] + Prcp[d]	15	1.67	0.12
Year + Site + Age	12	2.36	0.08
Year + Site + Age + Temp[d]	13	2.57	0.08
Year + Site + Prcp[d]	12	3.52	0.05
Year + Site + Age + Sex	13	3.81	0.04
Year + Site + Temp[d] + Prcp[d]	13	3.84	0.04
Year + Site	11	5.33	0.02
Year + Site + Age + Sex + Season	14	5.93	0.01
Anchorage All Winter[b] (*n* = 1215)			
Year + Site + Temp	12	0.00[e]	0.21
Year + Site + Temp[d] + Prcp[d]	13	0.15	0.19
Year + Site	11	0.92	0.13
Year + Site + Prcp	12	1.53	0.10
Year + Site + Age + Temp[d]	13	1.77	0.09
Year + Site + Age + Temp[d] + Prcp[d]	14	1.92	0.08
Year + Site + Age	12	2.82	0.05
Year + Site + Sex	12	2.95	0.05
Year + Site + Age + Prcp[d]	13	3.44	0.04
Year + Site + Age + Sex + Temp[d] + Prcp[d]	15	3.94	0.03
Alaska All Winter[c] (*n* = 1649)			
Year + Site	16	0.00[e]	0.17
Year + Site + Age	17	0.38	0.14
Year + Site + Temp[d]	17	0.69	0.12
Year + Site + Age + Temp[d]	18	0.93	0.11
Year + Site + Prcp[d]	17	1.25	0.09
Year + Site + Age + Prcp[d]	18	1.66	0.07
Year + Site + Sex	17	1.99	0.06
Year + Site + Temp[d] + Prcp[d]	18	2.04	0.06
Year + Site + Age + Temp[d] + Prcp[d]	10	2.32	0.05
Year + Site + Age + Sex	18	2.36	0.05

[a] Captured during September–December (when juvenile birds could be reliably distinguished from older birds) at one of three standardised banding sites operated annually in the Anchorage area; excludes winter 2008/2009 because no infections were found and prevalence was therefore inestimable in models.
[b] Captured during September–April at one of three standardised banding sites in Anchorage; excludes winter 2008/2009.
[c] Captured during September–April at one of seven sites in Alaska.
[d] Temp, daily mean temperature during the previous summer months (May–September); Prcp, mean daily precipitation during the previous summer months.
[e] Values for Akaike's Information Criterion corrected for sample size (AIC_c) for the best-supported models were 521.02 for Anchorage Autumn–Early Winter, 1172.80 for Anchorage All Winter, and 1554.57 for Alaska All Winter.

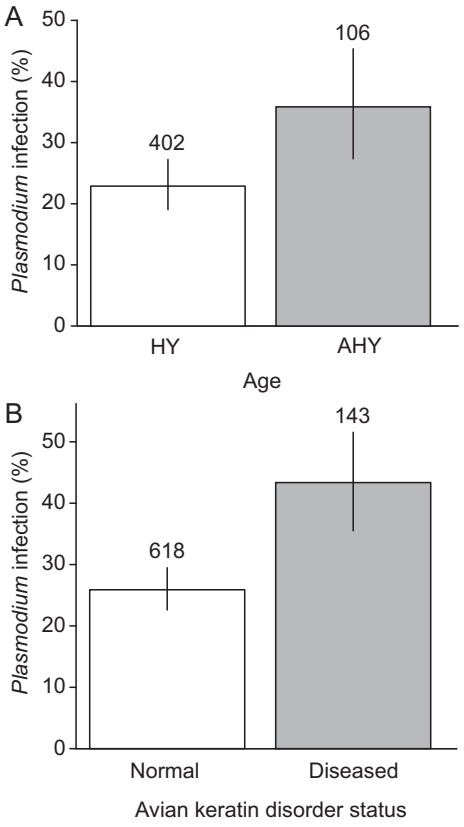

Fig. 2. Prevalence of *Plasmodium* infection (A) in juvenile (HY) versus adult (AHY) black-capped chickadees (*Poecile atricapillus*) with normal beaks and (B) relative to the disease state in adults (unaffected or affected by avian keratin disorder) at three standard sampling sites in the greater Anchorage area, Alaska, USA, during autumn and winter 2001/2002–2010/2011. Samples for comparison of young and adult birds were restricted to autumn and early winter (September–December), when age could be reliably determined; samples for comparison of disease states included all four autumn and winter periods (September–April).

sample included site, year and disorder status ($AIC_c = 851.61$, degrees of freedom = 14). The evidence ratio for this model compared with the model that included only site and year ($\Delta AIC_c = 17.00$) was ~3700, indicating a strong association between avian keratin disorder and parasite infection. Based on this model, adults with avian keratin disorder were 2.6 (95% CI: 1.7–4.1) times more likely than unaffected individuals to be infected by *Plasmodium*. A model that included mean summer temperature had almost equal support ($\Delta AIC_c = 0.15$), whereas models with precipitation ($\Delta AIC_c = 2.07$) or both climate variables combined ($\Delta AIC_c = 2.23$) had less support (Table 5).

4. Discussion

Our analysis of a large number of blood samples over a 10 year period and at seven sites allowed for a broad analysis of factors that could affect parasite prevalence in black-capped chickadees in Alaska. Our study revealed that *Plasmodium* prevalence varied across space and time, and that the strength of association with environmental conditions (e.g., summer temperature) varied at different geographic scales. One of our most striking findings was the strong association between *Plasmodium* infection and the prevalence of avian keratin disorder in adult chickadees, providing evidence that, in this case, individuals affected by an existing disease condition are more likely to harbor parasite infections. Understanding the dynamics of vector-borne parasites and their hosts requires knowledge of the factors affecting their interactions, including conditions of both the environment and the host that facilitate parasite transmission. Previous studies at more southern latitudes have found that the prevalence of avian malaria infection often exhibits substantial variation across years (Bensch et al., 2007; Atkinson and Samuel, 2010) or locations (Bensch and Åkesson, 2003). Rarely, however, have they been of sufficient scope to document variation in both time and space simultaneously or the factors underlying those patterns (but see Wood et al., 2007; Knowles et al., 2011).

Plasmodium prevalence in chickadees differed by site and year, suggesting that microhabitats and microclimates likely affect vector distribution, parasite transmissibility or both. Other studies have documented spatial variation in parasite prevalence and transmission, providing evidence of both broad geographic (Sehgal et al., 2011; Loiseau et al., 2012b) and habitat-level differences (Wood et al., 2007; Lachish et al., 2011; Ramey et al., 2012;

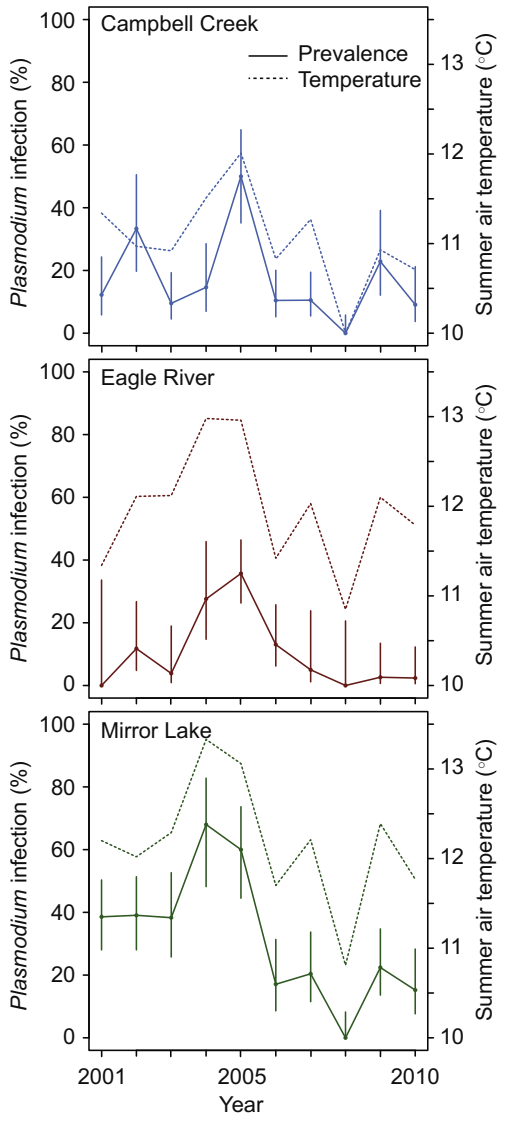

Fig. 3. Annual prevalence of *Plasmodium* infection in black-capped chickadees (*Poecile atricapillus*; solid line) with no signs of avian keratin disorder during autumn and winter from 2001/2002 to 2010/2011, relative to the mean daily air temperature (dashed line) during the preceding summer at three standard sampling sites in the greater Anchorage area, Alaska, USA.

Gonzalez-Quevedo et al., 2014; Krama et al., 2015). Abiotic factors such as temperature, precipitation and landscape characteristics may help to explain such patterns (Sehgal, 2015).

Many studies have examined the link between avian malaria and climate variables (van Riper et al., 2002; Freed et al., 2005; LaPointe et al., 2010, 2012; Garamszegi, 2011; Sehgal et al., 2011; Loiseau et al., 2013), indicating an influence of warmer temperatures and moisture on mosquito abundance and parasite transmissibility. At high latitudes, periods of active transmission of haemosporidians are usually associated with warmer seasons (Valkiūnas, 2005). In our study, variation in the prevalence of *Plasmodium* infection at different sites in the greater Anchorage area during winter was associated with variation in the mean daily temperature during the preceding summer and there was little support for precipitation as an important explanatory factor. A related parasite, *Plasmodium relictum*, requires a minimum of 13 °C for sporogonic development and optimal growth occurs at 21–28 °C (LaPointe et al., 2010). Mean daily summer temperatures at all of our sites ranged from 10 to 13.3 °C; thus our results are consistent

Table 5
Results of generalised linear models testing for effect of disease status (affected or unaffected by avian keratin disorder) in addition to ecological, biological and climatic factors on the probability of *Plasmodium* infection among adult black-capped chickadees (*Poecile atricapillus*) captured in Anchorage, Alaska, USA, during autumn and winter from 2000/2001 to 2010/2011. Within the candidate set, the model with $\Delta AIC_c = 0$ has the best fit to the data; K is the number of parameters in the model; *w* is Akaike model weight. Only models with $\Delta AIC_c < 10$ are shown.

Model	Anchorage All Winter[a] (*n* = 761)		
	K	ΔAIC_c	w
Year + Site + Disease	14	0.00[c]	0.38
Year + Site + Disease + Temp[b]	15	0.15	0.36
Year + Site + Disease + Prcp[b]	15	2.07	0.14
Year + Site + Disease + Temp[b] + Prcp[b]	16	2.23	0.13

[a] Captured during September–April at one of four sites in Anchorage.
[b] Temp, daily mean temperature during the previous summer months (May–September); Prcp, mean daily precipitation during the previous summer months.
[c] Value for Akaike's Information Criterion corrected for sample size (AIC_c) for the best-supported model was 851.61.

with the hypothesis that summer temperature is a key factor influencing *Plasmodium* infection of birds in subarctic regions.

What was surprising was that this relationship with temperature held at the local but not the broader geographic scale. Although summer temperatures were relatively warmer in Fairbanks, prevalence was significantly lower there than at the other sites. Loiseau et al. (2012a) examined birds for *Plasmodium* infection during a single summer across a slightly broader latitudinal gradient, at Anchorage (61°N), Fairbanks (64°N) and farther north in Coldfoot (67°N). At that spatial scale, the probability of infection was best explained by long-term bioclimatic variables that incorporated the minimum temperature of the coldest month, seasonality in temperature and precipitation, and annual precipitation. A subsequent study (Oakgrove et al., 2014) of the prevalence of haematozoan co-infection at more sites across the same gradient confirmed the same latitudinal pattern. Thus, we are not certain why summer temperatures may affect local but not broader distributional patterns of infection in chickadees where the *Plasmodium* parasites occur; identification of the vector and knowledge of its ecology are clearly needed.

We found a strong site effect at both the local and broader geographic scale, even after controlling for summer temperature, suggesting that *Plasmodium* persistence or transmission is affected by site characteristics that we did not measure. Given that P43 has been found in genera across 10 different avian families, it is a generalist (as opposed to specialist) parasite. Generalist parasites are thought to be favored in areas where host diversity is high, as there may be reduced host availability for specialist parasites (Moens and Pérez-Tris, 2015). Avian species diversity was lower at Fairbanks, the most northern site sampled, than at the other six sites, which were all in southern-central Alaska and had similar avian communities (C. Handel, personal observation). Thus, the combination of low host diversity and a seasonally compressed window for transmission might partially explain why chickadees from the most northern site had the lowest estimated prevalence of parasite infection. Although Loiseau et al. (2012b) found that generalist parasites elsewhere occurred in hosts with a limited geographical range, this factor did not apply to our system as chickadees range broadly across northern North America (Smith, 1991). Other studies at temperate latitudes have found spatiotemporal variation in the prevalence of *Plasmodium* infection to be associated with elevation, forest characteristics, distance from poultry farms and the presence of water (Wood et al., 2007; Atkinson and Samuel, 2010; Knowles et al., 2011; Lachish et al., 2011; Gonzalez-Quevedo et al., 2014; Krama et al., 2015). Sampling resident birds from a larger number of sites with varied ecological characteristics

across the arctic and subarctic, coupled with experimental work on vector populations, would help elucidate the limiting factors for *Plasmodium* persistence and transmission in northern regions.

Although we found high interannual variation in *Plasmodium* prevalence, we found no evidence of a long-term trend, nor a consistent cyclical pattern, during the 10 year period. In a 17 year study of great reed warblers (*Acrocephalus arundinaceus*), Bensch et al. (2007) found evidence of a 3–4 year cycle, and Schall and Marghoob (1995) found suggestions of a 10 year cycle in *Plasmodium* infection of western fence lizards (*Sceloporus occidentalis*) in California, USA. Conditions in subarctic regions may not be conducive to cyclical patterns of infection or, alternatively, our study may not have been long enough to detect one.

In contrast to our study, several studies at more southern latitudes have found evidence of strong seasonal variation in *Plasmodium* prevalence (Cosgrove et al., 2008; Atkinson and Samuel, 2010). Beaudoin et al. (1971) proposed a model to explain such seasonal variation whereby a peak in malaria prevalence occurs in late summer and autumn, when populations of vectors and naïve juvenile hosts are both high, followed by a drop in host blood levels during winter as vector activity wanes, and then a relapse of infection during spring. We found no evidence of any seasonal differences. If a seasonal peak does occur in this subarctic region, it likely occurs during July or August, when populations of juvenile birds and potential vectors (mosquitoes) are most abundant. Thus, it is possible that we failed to detect a seasonal pattern either because we did not sample birds during late summer or some infections were latent during winter. More information should be gathered about this parasite (lineage P43), including identification of its vector and monitoring how parasitemia varies with time. Ideally, experimental infections could verify if infection status changes with season.

Our study joins an ever-growing body of literature suggesting that individual host factors, including infection with other diseases, play a significant role in avian malaria prevalence. Surprisingly, chickadees with avian keratin disorder were 2.6 times more likely to be infected with avian malaria than those without the disease. Although the cause of avian keratin disorder is still under investigation, the disease affects birds' preening and foraging abilities and has a negative impact on their health and fitness (Handel et al., 2010). A significantly high proportion of birds with pox-like lesions in Hawaii also had malaria infections, suggesting an interaction between these diseases (Atkinson et al., 2005). On the other hand, avian malaria was inversely correlated with infection by West Nile virus (Medeiros et al., 2014a). It is possible that chickadees affected by avian keratin disorder have depressed immune function that makes them more susceptible to malaria infection. In a study of canaries experimentally infected with *Plasmodium*, Cornet et al. (2014) found that parasitemia was higher in a control group than in a group provided with supplemental food. Although birds with the higher nutritional state were able to maintain body mass during the infection period whereas control birds did not, supplemented birds paid a higher cost of infection than did the control birds in terms of reduction in hematocrit levels. Birds suffering from poor nutrition may have reduced immune function and thus be more susceptible to disease (Cornet et al., 2014). Chickadees affected by avian keratin disorder have compromised foraging ability, resulting in dietary shifts that may further compromise their health (Handel et al., 2010; Van Hemert et al., 2012). The elevated prevalence of malaria in birds with avian keratin disorder highlights the importance of further study of interactions between haemosporidian infection and other disease conditions at both the individual and population level.

Although we postulated that prevalence of haemosporidian infection could differ between male and female chickadees due to the dominance hierarchy (Smith, 1991) and potentially different foraging niches, we found no evidence for effects of sex during any period of the non-breeding season. Foraging areas used by the more dominant males may be safer from predators than areas used by lower-ranking females (Smith, 1991) but these microhabitats may not provide safer havens from vectors of parasites. The relatively short time spent by females in the nesting cavity incubating eggs (12–13 days; Smith, 1991) likely provides inconsequential protection from infection. Results of other studies have been mixed, with some studies finding no sex effect (Ricklefs et al., 2005; Cosgrove et al., 2008) and others finding higher prevalence among males than females (Schall and Marghoob, 1995; Wood et al., 2007).

In contrast, we found that adults were twice as likely as juveniles to be infected by *Plasmodium* during autumn and early winter, when we could reliably determine the age of chickadees. Similar age-related differences have been found in the closely related blue tits (Wood et al., 2007; Cosgrove et al., 2008; Knowles et al., 2011; Podmokła et al., 2014), several species of passerines in Hawaii (Atkinson et al., 2005; LaPointe et al., 2005; Atkinson and Samuel, 2010), shorebirds (Mendes et al., 2005), and western fence lizards (Schall and Marghoob, 1995), with some concluding that infection probability increases with cumulative exposure (Mendes et al., 2005). Other studies, however, found no difference in parasite prevalence between age groups (Ricklefs et al., 2005). Adult birds with a longer period of contact with vectors than young birds have a greater chance of being infected and may maintain these infections even during times when factors are not favourable for parasite transmission (Valkiūnas, 2005). In a study of the strategies of specialist versus generalist parasites, Medeiros et al. (2014b) found that specialist parasites were more likely to infect adult hosts, whereas generalists were more likely to infect juvenile hosts. They hypothesised that specialists may have developed a better ability to persist in their host following infection than generalists, resulting in generalists being cleared in the host while specialists evade host immune defenses. Our findings appear to contradict this idea, however, because P43, a generalist parasite, is able to persist in adult birds throughout winter. Experimental infections are necessary to further explore the dynamics of P43 in northern birds.

Dynamics underlying age-related patterns can be difficult to unravel because they may be complicated by characteristics of the host pool available to be infected, characteristics of the vector population and infectivity of the parasite (Cosgrove et al., 2008; Drovetski et al., 2014). Although average life-span of chickadees is about 2.5 years, the longevity record is more than 12 years (Foote et al., 2010); thus, adults can be repeatedly exposed to malaria parasites during multiple years, increasing the probability of their infection. In a study of great tits (*Parus major*), both lineage and parasitemia of *Plasmodium* infection were retained within individual birds across breeding seasons, suggesting either persistent infection or repeated exposure (van Rooyen et al., 2013). The lack of a seasonal decline in prevalence in chickadees during the winter season suggests that chronic infection may persist for at least some individuals. This can be tested in the future by examining repeated samples of individuals within a given year.

When measuring apparent prevalence of disease in wild birds, it is important to consider the possibility that such estimates may be biased. In a capture-recapture study of the blue tit, the probability of capture was affected by infection status, being higher for birds infected by *Plasmodium circumflexum* and lower for those infected by *P. relictum* (Lachish et al., 2011). The costs in terms of survival also varied between the two parasite species and their effects were further influenced by overall prevalence of the parasite in the tit population. Biased estimates of prevalence could also result if mobility of individuals, and therefore capture probability, vary with infection status. Analysis of blood samples from recaptured

chickadees may help us understand whether such complex dynamics might be at play in this subarctic population.

The avian malaria system is complex and by testing only for prevalence as opposed to determining actual parasitemia, we cannot validate the interactions between hosts and parasites after initial infection (Knowles et al., 2011). Using blood smears and quantitative real-time PCR would help determine the levels of infection occurring in individuals throughout the year. Studying parasitemia in chickadees could provide insights about how infections are controlled in the host and how infections vary in response to the environment (Reece et al., 2009; Knowles et al., 2011). More detailed information on parasitemia in individuals affected and unaffected by avian keratin disorder would also enable us to understand potential interactions between diseases. Experimental work on immune response in captive birds would be needed to disentangle these relationships.

It would also be informative to test a larger sample size of chickadees from the late spring and summer months to determine whether parasite prevalence varies according to the model proposed by Beaudoin et al. (1971) with a peak in malaria prevalence in late summer/autumn and a relapse of infection in spring. Retesting individuals across seasons and multiple years would allow us to better understand patterns in prevalence as well as to determine whether malaria infection influences an individual's probability of either capture or survival. Lastly, we know that *Leucocytozoon* is prevalent and diverse in Alaska (Hollmén et al., 1998; Deviche et al., 2001, 2010; Ramey et al., 2012; Oakgrove et al., 2014; Reeves et al., 2015). It would be worthwhile to study the prevalence, diversity and fitness effects of *Leucocytozoon* parasites in chickadees.

The potential impacts of climate change should not be overlooked, especially in sensitive northern climates. Temperature influences both parasite development and vector competency (Paaijmans et al., 2012) and a fluctuation in temperature, not solely an increase, can cause increases or decreases in the rate of vector development (Paaijmans et al., 2009, 2010). We do not have confirmation of the vector mosquito species that transmits lineage P43 in Alaska and identifying this vector will allow for more fine-scale research. The 10 year sample span that we have used here is not long enough to determine long-term effects of climate change but can help elucidate potential changes in parasite transmission.

We have shown evidence that avian malaria prevalence is influenced by a variety of ecological and host factors in Alaska including year, site and temperature, as well as age and disease state in individuals. The importance of understanding the effects that avian keratin disorder is having on bird fitness is evident, as birds with the disease have a higher prevalence of *Plasmodium* infection. This baseline of knowledge will allow for further research on the fine-scale components of malaria transmission as well as potential interactions with other diseases.

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