ASSESSMENT OF VARIATION IN THE DETECTION AND PREVALENCE OF BLOOD PARASITES AMONG SYMPATRICALLY BREEDING GEESE IN WESTERN ALASKA

by

Raymond Matthew Buchheit

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ABSTRACT

Haemosporidian parasites may impact avian health and are subject to shifts in distribution and abundance with changing ecological conditions. Therefore, understanding variation in parasite prevalence is important for evaluating biologically meaningful changes in infection patterns and associated population level impacts. Previous research in western Alaska indicated a possible increase in *Leucocytozoon* infection between emperor geese (*Anser canagicus*) sampled in 1996 (<1%, *n*=134), and during 2011–2012 (19.9%, 95% CI: 3.0-36.8%, *n*=77); however, different detection methods were used for these estimates. Prior research in this same region identified a lack of *Leucocytozoon* parasites (0%, *n*=117) in sympatrically breeding cackling geese (*Branta hutchinsii minima*) in 2011. In this study, we molecularly screened blood samples collected from sympatrically breeding emperor and cackling geese in western Alaska during additional breeding seasons to better assess temporal and species-specific variation in the prevalence of blood parasites. We found similar prevalence estimates for *Leucocytozoon* parasites in emperor goose blood samples collected in 1998 and 2014, suggesting consistent infection of emperor geese with blood parasites at these time points. Using samples from sympatric geese sampled during 2014, we found evidence for higher incidence of *Leucocytozoon* parasites among emperor geese (20.3%, 95% CI: 11.8–32.7%) as compared to cackling geese (3.6%, 95% CI: 1.1–11.0%) reinforcing the previous finding of species-specific differences in infection. Furthermore, we detected *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* blood parasites in unflighted goslings of both species, supporting the possible transmission of these parasites at western Alaska breeding grounds. Our results help to clarify that prevalence of *Leucocytozoon* parasites have likely remained consistent among emperor geese breeding in western Alaska since the late 1990s and that this species may disproportionally harbor *Leucocytozoon* parasites as compared to sympatrically breeding cackling geese.

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Contribution of Authors and Co-Authors

Author: Raymond M. Buchheit

Contributions: Implemented the study. Collected and analyzed the data. Wrote the manuscript.

Co-Author: Joel A. Schmutz

Contributions: Conceived the initial project idea, assisted with study design and securing project funding.

Co-Author: Brian Uher-Koch

Contributions: Data collection. Assisted with preparation and review of the manuscript.

Co-Author: John A. Reed

Contributions: Data collection and assistance with training on molecular methods.

Co-Author: Andrew M. Ramey

Contributions: Assisted with study design and securing project funding. Assisted with preparation and review of the manuscript.

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ASSESSMENT OF VARIATION IN THE DETECTION AND PREVALENCE OF BLOOD PARASITES AMONG SYMPATRICALLY BREEDING GEESE IN WESTERN ALASKA

Raymond M. Buchheit,[1] Joel A. Schmutz,[2] John A. Reed,[2] Brian Uher-Koch[2], Andrew M. Ramey[2,3]

[1] Department of Ecology, Montana State University, P.O. Box 173460, Bozeman, MT 59717.

[2] U.S. Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, AK 99508.

[3] Corresponding author: Email: aramey@usgs.gov, Phone: 907-786-7174

Introduction

Haemosporidians are a diverse group of single-celled, intracellular parasites that affect a wide range of vertebrates (Atkinson and van Riper 1991). Avian hosts are commonly affected by haemosporidians of the genera *Leucocytozoon*, *Haemoproteus*, and *Plasmodium*. These parasites are transmitted by dipteran insect vectors, with *Leucocytozoon* being primarily transmitted by black flies, *Haemoproteus* by mosquitoes and biting midges, and *Plasmodium* by mosquitoes (Atkinson and van Riper 1991; Valkiunas 2004). Consequences of infection with these parasites reported in birds range from seemingly benign (Kilpatrick et al. 2006; Bensch et al. 2007; LaPointe et al. 2012) to impacts on reproductive fitness (Merino et al. 2000; Marzal et al. 2005; Asghar et al. 2015) and survival (Herman et al. 1975; Atkinson et al. 2000; Asghar et al. 2015).

Although haemosporidian infection in birds is common (Clark et al. 2018; Ellis et al. 2019), novel introductions of such parasites into naive avian populations have been documented and may be associated with population level impacts (Warner 1968; van Riper et al. 1986; Hellgren et al. 2007; Atkinson and LaPointe 2009). Thus, global climate change has the potential to facilitate future biologically consequential introductions of haemosporidian parasites into naive avian populations through shifts in the ranges of parasites and their vectors to higher latitudes and elevations (Sekercioglu et al. 2008; Chen et al. 2011; Fecchio et al. 2019), lengthened breeding seasons of arthropod vectors, and improved conditions for parasite reproduction (LaPointe et al. 2009; Zamora-Vilchis et al. 2012; Loiseau et al. 2013). Additionally, changes in environmental conditions may alter the abundance of parasites at locations where they are currently uncommon (Brooks and Hoberg 2007). Such events may be

more likely to occur at high latitude regions where the effects of climate change have been most pronounced (Kutz et al. 2009).

The Yukon-Kuskokwim Delta (YKD), a globally important wetland complex where more than a million geese congregate annually to breed, lies in one such high latitude region within western Alaska. Three species of geese sympatrically breeding on the YKD have previously been sampled for haemosporidian parasites including emperor geese (*Anser canagicus*), cackling geese *(Branta hutchinsii minima*), and black brant (*Branta bernicla nigricans*). A single *Leucocytozoon* parasite infection was identified from 134 emperor goose (<1%) blood smears collected on the YKD in 1996 (Hollmén et al. 1998), in contrast to a higher prevalence estimate (19.9%, 95% CI: 3.0–36.8%, *n*=77) for parasite infections in this same species and location as inferred from molecular screening of whole blood samples collected during 2011–2012 (Ramey et al. 2014). In the same investigation using molecular techniques, *Leucocytozoon* parasite infections were also detected in black brant (11.1%, 95% CI: 0–29.5%, *n*=49) sampled on the YKD during 2011–2012 but not in cackling geese sampled at this same locale in 2011 (*n*=117). Thus, previous sampling of geese on the YKD for haemosporidian parasites suggests that there may be considerable variation in prevalence among sympatrically nesting geese.

In this study, we further assessed the detection and prevalence of avian haemosporidian parasites in emperor and cackling geese breeding on the YKD. Specifically, we used consistent methods to examine whether: (1) emperor geese sampled in 1998 had a different incidence of parasitic infections as compared to those sampled in 2014, (2) emperor geese harbor a higher prevalence of parasites than cackling geese raising broods in the same geographic area, and (3) transmission is potentially occurring within the YKD. Results of this study help to elucidate

variation in haemosporidian parasite prevalence among sympatrically breeding geese on the YKD which may inform future assessments of change in parasite distribution and abundance.

Materials and Methods

We obtained blood samples from emperor geese during incubation and brood rearing in 1998 and 2014 and from cackling geese during brood rearing in 2014 at locations adjacent to the Manokinak River (approx. 61°N, 165°W) on the YKD. Bow nets or mist nets were used to capture incubating adult female emperor geese. Flightless hatch year and adult geese were captured during brood rearing by herding birds into corrals. Capture and handling protocols were reviewed by the Animal Care and Use Committee at the U.S. Geological Survey (USGS) Alaska Science Center and authorized under USGS federal bird banding permit #20022. Sex and age class (hatch year vs. adult) for both species were determined by plumage and cloacal examination. Whole blood was collected via jugular venipuncture and stored in Longmire buffer. Samples were kept at ambient temperature for up to ~12h before freezing and remained frozen until molecular analysis.

DNA was extracted from whole blood using a DNeasy Blood and Tissue kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol. To confirm successful DNA extraction, a 481 base pair fragment of the mitochondrial DNA (mtDNA) cytochrome oxidase I (COI) gene of the avian host was amplified using previously published primers in Ramey et al. (2014), and thermal-cycling conditions reported by Kerr et al. (2007). Each DNA extraction was screened three times for the presence of haematozoa using a nested polymerase chain reaction (PCR) protocol described by Hellgren (2004). A minimum of one negative control for every 24 wells was incorporated into each set of PCRs and reactions were conducted in eight well strip

tubes with individual caps that remained closed, except while loading template and reagents to prevent cross contamination. Primer sequences and amplification protocols are briefly summarized in Supplemental File S1. Amplicons were visualized on 0.8% agarose gels stained with Gel Red Nucleic Acid Gel Stain (Biotium, Hayward, California, USA).

PCR products appearing to represent the 479 base pair (bp) cytochrome b (cyt b) mitochondrial DNA (mtDNA) target were treated with ExoSap-IT (USB Inc., Cleveland, Ohio, USA) and sequenced with identical primers used for PCR and with BigDye Terminator version 3.1 mix (Applied Biosystems, Foster City, California, USA) on an Applied Biosystems 3730xl automated DNA sequencer (Applied Biosystems, Foster City, California, USA). Sequence data were cleaned and edited using DNA Dynamo (Blue Tractor Software Ltd., North Wales, U.K.). Sequences were then assigned to haemosporidian parasites of three genera (*Leucocytozoon*, *Haemoproteus*, or *Plasmodium*) using the nucleotide BLAST function available through the National Center for Biotechnology Information (NCBI). Assignment was based on the top NCBI BLAST result with a minimum max identity score of 90%. A sample was identified as positive for *Leucocytozoon*, *Haemoproteus*, or *Plasmodium* parasites if any of the three replicate PCR's resulted in a bidirectional double-stranded target product that was verified through genetic sequencing. Samples from which single-stranded or otherwise ambiguous products resulted were considered negative. Information on cyt b mtDNA haplotypes are available in Reed et al. (2021) and NCBI GenBank (accession numbers MW574916 – MW574929).

Given imperfect parasite detection (i.e., sensitivity) using our PCR-based approach, we employed occupancy modeling (MacKenzie et al. 2017) to provide estimates of detection and prevalence considering the potential effects of species, age, year, breeding stage, and sex. We

used program MARK (White and Burnham 1999) through the RMark package (Laake 2013) in program R (v. 4.0.2; R Development Core Team 2020) to build models and address the two primary study objectives (i.e., assessing temporal variation and differences between sympatric species in detection and prevalence of parasites) with separate analyses. First, we directly compared emperor geese sampled in both nesting and brood drive periods in 1998 and 2014. Second, since cackling geese were only sampled in 2014 during brood rearing, we directly compared these birds to emperor geese sampled only in 2014 during the same period. Due to small sample size, we limited our model sets to all additive combinations of variables for prevalence (Ψ) while holding detection (ρ) to a single variable or constant. We did not include interactions between variables influencing prevalence to be conservative in our inference (Supplementary Tables S1 & S2).

First, we focused on comparing results for emperor geese sampled in 1998 vs 2014 to examine temporal variation in parasite detection and prevalence. We assessed variation in parasite detection and prevalence in emperor geese through a series of models incorporating covariates for age class (hatch year vs adult), breeding stage (nesting vs brood rearing), and year (1998 and 2014). Next, we compared results between emperor geese and cackling geese sampled in 2014 to examine species-specific variation in detection and prevalence. We assessed species-specific variation through a series of models incorporating covariates for species, age class, and sex. In both analyses, model fit was assessed to inform which model structures best fit the observed data. We used the Fletcher's *c*-hat values for the most general models in each analysis to evaluate goodness of fit and quantify appropriate variance inflation factors. These inflation values were used to create quasi-likelihood Akaike's information criterion corrected for small

sample size ($QAIC_C$) for model selection. An information theoretic approach was used to determine influences on parasite prevalence and detection. Models were ranked by $\Delta QAIC_C$ scores and quasi-Akaike weights. The $\Delta QAICc$ scores were calculated as the difference between each model and the most parsimonious model. Detection and prevalence estimates were obtained by interpreting the most parsimonious model, unless one did not emerge, then model averaging was used to obtain unbiased point estimates and confidence intervals.

Results

We extracted DNA from 307 blood samples collected from geese at sites on the YKD during 1998 (*n*=72 emperor geese) and 2014 (*n*=125 emperor geese, *n*=110 cackling geese) and molecularly detected *Leucocytozoon, Haemoproteus,* and *Plasmodium* parasites (Table 1). Parasites of all three genera were identified in hatch year birds captured during brood drives (Table 1). The majority (45/57 or 79%) of haemosporidian infections molecularly identified were assigned to the genus *Leucocytozoon* and therefore small numbers of *Haemoproteus* (*n* = 7) and *Plasmodium* (*n* = 6) detections were excluded from modelling efforts.

In our first analysis to assess temporal variation in parasite detection and prevalence among emperor geese sampled in 1998 and 2014, we detected moderate lack of fit in the most general model incorporating covariates for detection by sample year and prevalence by sample year, breeding stage, and age class (Supplemental Table S1). Therefore, a variance inflation factor of 1.67 was applied to the entire set of models and parameter estimation. Given the absence of a clear top model (Table S1), we used model averaging to attain unbiased point estimates and confidence intervals. Using this approach, we found model averaged point estimates of *Leucocytozoon* prevalence ranging from 17.8% (95% CI: 9.4–31.1%) for goslings in

2014, to 26.9% (95% CI: 14.0–45.3%) for incubating females in 1998 (Figure 1). We also found little support for differences in the detection of *Leucocytozoon* parasites in emperor geese sampled in 1998 vs. those sampled in 2014 or for variation in parasite detection and prevalence by breeding stage and age class (Figure 1, Table S1). Estimates of detection probability ranged between 0.72–0.77 (95% CI: 0.53–0.88) per PCR run or 0.90–>0.99 per sample when run in triplicate.

In the second analysis to examine species-specific variation in parasite detection and prevalence, we again detected moderate lack of fit in the most general model incorporating covariates for detection by species and prevalence by species, age class, and sex (Table S2). Therefore, a variance inflation factor of 1.58 was applied to the entire set of models and parameter estimation. However, model averaging was not necessary in this analysis to obtain point estimates and confidence intervals as a clear best model was identified, incorporating covariates for detection by age class and prevalence by species (*QAICc weight* = 0.41; Table S2). This top model received approximately three times the weight of the next best ranked model. Furthermore, models including species as a covariate for prevalence received over 80% of total model weight in the candidate set. The prevalence estimates from our top model were 20.3% (95% CI: 11.8–32.7%) for emperor geese, and 3.6% (95% CI: 1.1–11.0%) for cackling geese (Figure 1). We found covariates for age class and sex to be uninformative for prevalence. However, four of the five top models (>75% of model weight in the set) included age class as a detection covariate (Table S2). The detection estimates from our top model were 0.85 (95% CI: 0.67–0.94) for adult geese, and 0.24 (95% CI: 0.10–0.80) for goslings per PCR run (Figure 1).

This equates to estimated detection probabilities of >0.99 and 0.56 for samples from adult and hatch year geese, respectively, when run in triplicate.

Discussion

Results of our investigation provide important insights regarding variation in the detection and prevalence of blood parasites among sympatrically breeding geese on the YKD in western Alaska. Specifically, our results provide evidence that emperor geese have likely consistently harbored *Leucocytozoon* parasite infections through recent time, that sympatrically breeding emperor geese and cackling geese may differentially harbor *Leucocytozoon* infections, and that hatch year geese on the YKD may harbor a diversity of haemosporidian parasite infections that may not be as readily detected using PCR as compared to adult birds. These findings help to establish an important baseline for future assessments of changes in parasite prevalence and potential population level effects in response to predicted ecological change.

Using blood samples obtained from additional breeding seasons, our data are congruent with previous molecular screening efforts that support the consistent infection of emperor geese breeding on the YKD with *Leucocytozoon* parasites at a prevalence of approximately 18-27% (Ramey et al. 2014). Given relatively consistent evidence for the prevalence of *Leucocytozoon* parasites among emperor geese at all time points assessed using molecular methods (this study and Ramey et al. 2014), we infer that the apparent increase in *Leucocytozoon* prevalence since the first record by Hollmén et al. (1998) in 1996 is most likely a function of methodology. In this case, the increased sensitivity of modern molecular methods likely accounted for the higher *Leucocytozoon* prevalence estimates in recent investigations, as opposed to an actual increase in

parasite prevalence since 1996. Conclusions regarding trends of infection through time could be strengthened through testing of emperor goose blood samples from additional years.

Also consistent with a previous report (Ramey et al. 2014), we found evidence that emperor geese may indeed differentially harbor *Leucocytozoon* infections as compared to sympatrically breeding cackling geese. In our analysis, the prevalence of *Leucocytozoon* infections in emperor geese was estimated to be more than five times that of cackling geese sampled on the YKD despite these species nesting and rearing broods in the same geographic area. Differential parasite prevalence among sympatrically breeding species has also been observed in other systems where closely related taxa inhabit different wintering areas (Yohannes et al. 2009; Emmenegger et al. 2018). In the case of geese inhabiting the YKD, cackling geese tend to utilize agricultural fields within temperate lower latitudes during the non-breeding season (Bogiatto et al. 2009), as opposed to emperor geese which are dependent on natural food sources within subarctic high latitude locations throughout the wintering period (Hupp et al. 2008; Uher-Koch et al. 2021). Consequently, cackling geese may more readily procure food resources and attain better physical condition prior to the onset of breeding which may also facilitate a robust immune response sufficient to quickly resolve haemosporidian infections (Arriero et al. 2018). In contrast, emperor geese may not arrive on the YKD with ample energetic reserves to meet the combined physiological demands of breeding (i.e., egg laying and incubation) and in mounting a sufficient immune response to suppress parasitic infections to undetectable levels. Alternatively, geographic variation in environmental conditions (Fecchio et al. 2020), or some degree of host specificity of parasites or vector preferences of biting insects (Hellgren et al. 2008) may play some role in parasite epidemiology on the YKD. Regardless of the underlying mechanism(s), our

results suggest that avian haemosporidian parasites have the potential to disproportionally affect emperor geese breeding on the YKD and therefore, research to assess detrimental effects of *Leucocytozoon* infections in this species is warranted.

The molecular identification of *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* parasites in unflighted goslings on the YKD provides important baseline information for future assessments of ecological change in that this finding supports contemporary transmission of all three parasite genera at this locale. The finding of few *Haemoproteus* and *Plasmodium* detections in waterfowl inhabiting the YKD is consistent with prior reports (Ramey et al. 2014; Ramey et al. 2015) and the premise that parasites of these genera may be temperature limited in high latitude regions (Clark et al. 2020). Support for a difference in the estimated molecular detection of *Leucocytozoon* parasites in adult versus gosling emperor geese in our second analysis was unexpected, particularly given that this detection difference was not clearly implicated in our first analysis focused on emperor geese nor in previous models assessing haemosporidian parasite detection in samples collected from geese (Ramey et al. 2014), ducks (Ramey et al. 2015), and crows (Van Hemert et al. 2019) inhabiting Alaska. It is possible that the difference in detection we found in our analysis may have been due to unidentified sampling artifacts (e.g., disproportional sampling of birds exhibiting low parasitemia) among the relatively small sample size of *Leucocytozoon* positive hatch year birds assessed in our analytical framework (n = 7). We encourage future investigations to incorporate age as a covariate when assessing variance in parasite detection until biological significance is resolved.

In summary, our investigation establishes important baseline information regarding variation in the detection and prevalence of blood parasites that will be useful for informing

future assessments of parasite prevalence in geese breeding in western Alaska and for evaluating potential ecological effects in response to predicted environmental change. For example, our investigation provides additional support that *Leucocytozoon* parasites have clearly been established in geese breeding in western Alaska for at least two decades and therefore do not represent an emergent parasitic threat in the region. However, it is still unclear if haemosporidians are causing biologically significant effects in geese breeding on the YKD at the population level which is worthy of future assessment. Furthermore, our study supports the previous finding that *Haemoproteus* and *Plasmodium* parasites are uncommon among breeding waterfowl on the YKD but are transmitted within this region. Therefore, these presumably temperature limited parasites may represent valuable targets for assessing future ecological change within the YKD.

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TABLES

Table 1. Summary of molecular screening of whole blood samples collected from emperor and cackling geese breeding on the Yukon-Kuskokwim Delta of Alaska for haemosproridian parasites of the genera *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* by year, breeding stage, age class, and sex class.

Species	Year	Breeding stage	Age class	Sex	n	Leucocytozoon positive (%)	Haemoproteus positive (%)	Plasmodium positive (%)
EMGO	1998	Nesting	Adult	F	20	5 (25%)	0	0
EMGO	1998	Brood Drives	Adult	F	18	4 (22%)	1 (6%)	0
EMGO	1998	Brood Drives	Adult	M	13	6 (46%)	0	0
EMGO	1998	Brood Drives	Hatch year	F	11	4 (36%)	0	0
EMGO	1998	Brood Drives	Hatch year	M	10	2 (20%)	0	0
EMGO	2014	Nesting	Adult	F	28	6 (21%)	0	0
EMGO	2014	Brood Drives	Adult	F	36	9 (25%)	1 (3%)	0
EMGO	2014	Brood Drives	Adult	M	9	0	0	0
EMGO	2014	Brood Drives	Hatch year	F	24	1 (4%)	2 (8%)	0
EMGO	2014	Brood Drives	Hatch year	M	28	5 (18%)	0	1 (4%)
CACG	2014	Brood Drives	Adult	F	27	0	1 (4%)	1 (4%)
CACG	2014	Brood Drives	Adult	M	22	2 (9%)	0	2 (9%)
CACG	2014	Brood Drives	Hatch year	F	29	1 (3%)	1 (3%)	2 (7%)
CACG	2014	Brood Drives	Hatch year	M	32	0	1 (3%)	0

Supplemental Table S1. Model selection results to assess temporal variation in *Leucocytozoon* detection (ρ) and prevalence (ψ) among breeding emperor geese sampled during the incubation and brood-rearing periods on the Yukon Kuskokwim Delta in 1998 and 2014. Constant models are denoted by (.).

Model	No. parameters	QAICc	ΔQAICc	weight	Deviance
ρ(.)Ψ(*SampleYear*)	3	208.034	0.000	0.110	35.229
ρ(.)Ψ(.)	2	208.401	0.367	0.092	37.659
ρ(*AgeClass*)Ψ(*SampleYear*)	4	209.183	1.149	0.062	34.295
ρ(.)Ψ(*SampleYear* + *AgeClass*)	4	209.432	1.398	0.055	34.543
ρ(.)Ψ(*AgeClass*)	3	209.448	1.415	0.054	36.644
ρ(*AgeClass*)Ψ(.)	3	209.491	1.457	0.053	36.686
ρ(*BreedingStage*)Ψ(*SampleYear*)	4	209.577	1.544	0.051	34.689
ρ(*BreedingStage*)Ψ(.)	3	209.913	1.879	0.043	37.109
ρ(.)Ψ(*SampleYear* + *BreedingStage*)	4	210.096	2.063	0.039	35.208
ρ(*SampleYear*)Ψ(*SampleYear*)	4	210.118	2.084	0.039	35.229
ρ(.)Ψ(*BreedingStage*)	3	210.406	2.372	0.034	37.602
ρ(*SampleYear*)Ψ(.)	3	210.456	2.422	0.033	37.651
ρ(*AgeClass*)Ψ(*AgeClass*)	4	210.707	2.674	0.029	35.819
ρ(*AgeClass*)Ψ(*SampleYear* + *AgeClass*)	5	210.718	2.684	0.029	33.724
ρ(*BreedingStage*)Ψ(*AgeClass*)	4	211.012	2.978	0.025	36.123
ρ(*BreedingStage*)Ψ(*SampleYear* + *AgeClass*)	5	211.021	2.987	0.025	34.027

ρ(*AgeClass*)Ψ(*SampleYear* + *BreedingStage*)	5	211.277	3.243	0.022	34.282
ρ(.)Ψ(*SampleYear* + *BreedingStage* + *AgeClass*)	5	211.490	3.457	0.020	34.496
ρ(.)Ψ(*BreedingStage* + *AgeClass*)	4	211.490	3.457	0.020	36.602
ρ(*SampleYear*)Ψ(*AgeClass*)	4	211.526	3.492	0.019	36.638
ρ(*AgeClass*)Ψ(*BreedingStage*)	4	211.534	3.501	0.019	36.646
ρ(*SampleYear*)Ψ(*SampleYear* + *AgeClass*)	5	211.537	3.504	0.019	34.543
ρ(*BreedingStage*)Ψ(*SampleYear* + *BreedingStage*)	5	211.671	3.637	0.018	34.677
ρ(*BreedingStage*)Ψ(*BreedingStage*)	4	211.955	3.921	0.015	37.067
ρ(*SampleYear*)Ψ(*SampleYear* + *BreedingStage*)	5	212.202	4.168	0.014	35.208
ρ(*SampleYear*)Ψ(*BreedingStage*)	4	212.483	4.449	0.012	37.594
ρ(*AgeClass*)Ψ(*BreedingStage* + *AgeClass*)	5	212.771	4.737	0.010	35.777
ρ(*AgeClass*)Ψ(*SampleYear* + *BreedingStage* + *AgeClass*)	6	212.799	4.765	0.010	33.677
ρ(*BreedingStage*)Ψ(*BreedingStage* + *AgeClass*)	5	213.061	5.027	0.009	36.067
ρ(*BreedingStage*)Ψ(*SampleYear* + *BreedingStage* + *AgeClass*)	6	213.086	5.053	0.009	33.964
ρ(*SampleYear*)Ψ(*BreedingStage* + *AgeClass*)	5	213.590	5.556	0.007	36.596
ρ(*SampleYear*)Ψ(*SampleYear* + *BreedingStage* + *AgeClass*)	6	213.618	5.585	0.007	34.496

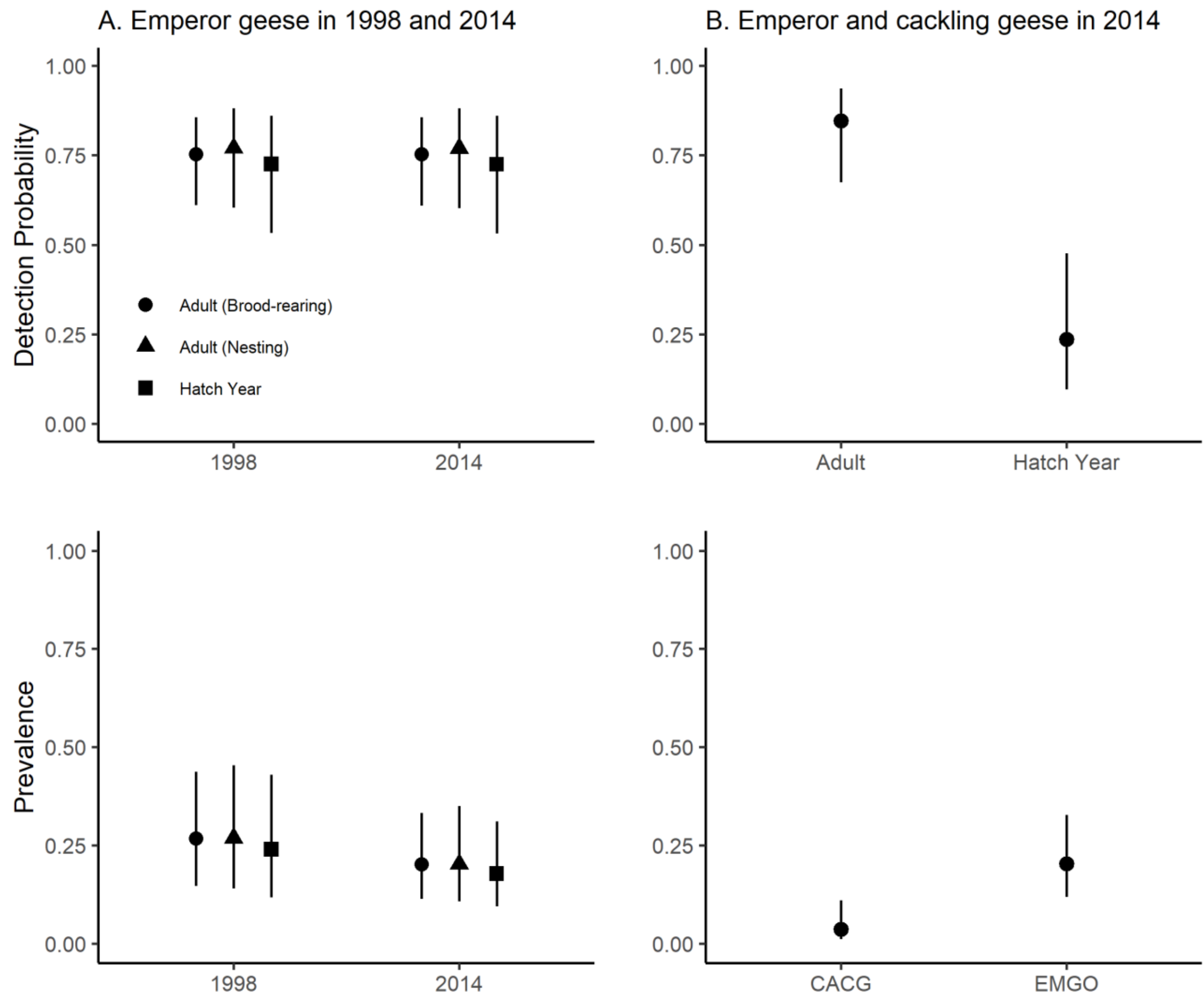
Supplemental Table S2. – Model selection results to examine species-specific variation in *Leucocytozoon* detection (ρ) and prevalence (Ψ) among breeding emperor and cackling geese sampled during the brood-rearing period on the Yukon Kuskokwim Delta in 2014. Constant models are denoted by (.).

Model	No. parameters	QAICc	ΔQAICc	weight	Deviance
ρ(*AgeClass*)Ψ(*Species*)	4	110.223	0.000	0.410	28.206
ρ(*AgeClass*)Ψ(*Species* + *Sex*)	5	112.292	2.069	0.146	28.174
ρ(*AgeClass*)Ψ(*Species* + *AgeClass*)	5	112.320	2.096	0.144	28.201
ρ(*AgeClass*)Ψ(*Species* + *AgeClass* + *Sex*)	6	114.400	4.176	0.051	28.160
ρ(*Sex*)Ψ(*Species*)	4	114.686	4.462	0.044	32.668
ρ(*AgeClass*)Ψ(.)	3	115.184	4.961	0.034	35.246
ρ(*Sex*)Ψ(*Species* + *AgeClass*)	5	115.811	5.587	0.025	31.692
ρ(*Sex*)Ψ(*Species* + *Sex*)	5	116.573	6.350	0.017	32.455
ρ(*Species*)Ψ(.)	3	116.611	6.388	0.017	36.673
ρ(*AgeClass*)Ψ(*Sex*)	4	117.230	7.007	0.012	35.212
ρ(*AgeClass*)Ψ(*AgeClass*)	4	117.257	7.034	0.012	35.239
ρ(*Sex*)Ψ(*Species* + *AgeClass* + *Sex*)	6	117.288	7.065	0.012	31.048
ρ(*Species*)Ψ(*AgeClass*)	4	117.505	7.282	0.011	35.488
ρ(*Species*)Ψ(*Species*)	4	117.748	7.525	0.010	35.731
ρ(.)Ψ(*Species*)	3	118.041	7.818	0.008	38.103
ρ(*Species*)Ψ(*Species* + *AgeClass*)	5	118.658	8.434	0.006	34.539

ρ(*Species*)Ψ(*Sex*)	4	118.671	8.448	0.006	36.653
ρ(.)Ψ(*Species* + *AgeClass*)	4	118.927	8.704	0.005	36.909
ρ(*AgeClass*)Ψ(*AgeClass* + *Sex*)	5	119.328	9.105	0.004	35.210
ρ(*Sex*)Ψ(.)	3	119.448	9.225	0.004	39.510
ρ(*Species*)Ψ(*AgeClass* + *Sex*)	5	119.579	9.356	0.004	35.461
ρ(*Species*)Ψ(*Species* + *Sex*)	5	119.842	9.619	0.003	35.724
ρ(.)Ψ(*Species* + *Sex*)	4	120.115	9.892	0.003	38.097
ρ(*Sex*)Ψ(*AgeClass*)	4	120.477	10.254	0.002	38.460
ρ(*Species*)Ψ(*Species* + *AgeClass* + *Sex*)	6	120.714	10.490	0.002	34.474
ρ(.)Ψ(*Species* + *AgeClass* + *Sex*)	5	120.957	10.734	0.002	36.839
ρ(*Sex*)Ψ(*Sex*)	4	121.492	11.268	0.001	39.474
ρ(*Sex*)Ψ(*AgeClass* + *Sex*)	5	122.421	12.198	0.001	38.303
ρ(.)Ψ(.)	2	123.082	12.859	0.001	45.204
ρ(.)Ψ(*AgeClass*)	3	123.905	13.682	0.000	43.967
ρ(.)Ψ(*Sex*)	3	125.010	14.787	0.000	45.072
ρ(.)Ψ(*AgeClass* + *Sex*)	4	125.966	15.742	0.000	43.948

FIGURES

Figure 1. – Detection (per replicate PCR run) and prevalence estimates with 95% confidence intervals for *Leucocytozoon* parasites in: A. emperor geese (*Anser canagicus*) breeding on the Yukon-Kuskokwim Delta of Alaska sampled during the incubation and brood-rearing periods in 1998 and 2014, and B. emperor and cackling geese (*Branta hutchinsii minima*) sympatrically breeding on the Yukon-Kuskokwim Delta sampled during the brood-rearing period in 2014 only.



APPENDIX

SUPPLEMENTARY MATERIALS

Supplemental information regarding molecular methods:

Summary of primer sequences and amplification protocols for host DNA verification and parasite screening.

DNA EXTRACTION AND CONFIRMATION

DNA was extracted from whole blood samples stored in Longmire buffer using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol.

To confirm the competency of DNA in extracted samples, a 481 bp fragment of the mtDNA cytochrome oxidase I (COI) gene of waterfowl hosts was amplified and then visualized on 0.8% agarose gels stained with Gel Red Nucleic Acid Gel Stain 10,000x in DMSO (Biotium, Hayward, CA).

Host DNA extraction confirmation primers and settings:
Goose COI F: (5' – CCATCATGATCGGAGGATTC – 3')
Goose COI R: (5' – TGGTACAGGATTGGGTCTCC – 3')

Thermalcycler Parameters	
Denature	94°C, 1 min
PCR (6 cycles)	94°C, 1 min 45°C, 1.5 min 72°C, 1.5 min
PCR (35 cycles)	94°C, 1 min 55°C, 1.5 min 72°C, 1.5 min
Final Ext	72°C, 1.5 min

NESTED PCR

The first PCR in the nested protocol is a general haemosporidian amplification – targeting *Leucocytozoon, Haemoproteus & Plasmodium* DNA. The product of this is then used in the second step, targeting *Leucocytozoon* parasite DNA in one PCR run, and *Haemoproteus*/*Plasmodium* parasite DNA in another.

General haemosporidian amplification primers and settings:
HaemNFI: (5' – CATATATTAAGAGAAITATGGAG – 3')
HaemNR3: (5' – ATAGAAAGATAAGAAATACCATTC – 3')

Thermalcycler Parameters	
Denature	94°C, 3 min
PCR (20 cycles)	94°C, 30 sec 50°C, 30 sec 72°C, 45 sec
Final Ext	72°C, 10 min

Leucocytozoon specific PCR amplification primers and settings:
HaemFL: (5’ – ATGGTGTTTTAGATACTTACATT – 3’)
HaemR2L: (5’ – CATTATCTGGATGAGATAATGGIGC – 3’)

Thermalcycler Parameters	
Denature	94°C, 3 min
PCR (35 cycles)	94°C, 30 sec 50°C, 30 sec 72°C, 45 sec
Final Ext	72°C, 10 min

Haemoproteus/*Plasmodium* specific PCR amplification primers and settings:
HaemF: (5’ – ATGGTGCTTTCGATATATGCATG – 3’);
HaemR2: (5’ – GCATTATCTGGATGTGATAATGGT – 3’).

Thermalcycler Parameters	
Denature	94°C, 3 min
PCR (35 cycles)	94°C, 30 sec 50°C, 30 sec 72°C, 45 sec
Final Ext	72°C, 10 min

EVALUATION

Samples were visualized on 0.8% agarose gels stained with Gel Red Nucleic Acid Gel Stain 10,000x in DMSO (Biotium, Hayward, CA). A sample was considered positive if at least one of the replicate nested PCR’s resulted in a bidirectional double-stranded, target product that was verified through genetic sequencing. Samples from which single-stranded or otherwise ambiguous products resulted were considered negative. Infections were then assigned to three genera (*Leucocytozoon*, *Haemoproteus*, or *Plasmodium*) using the nucleotide BLAST function available through the National Center for Biotechnology Information (NCBI).

For further information on the background of the molecular methods used in this study, please see the following sources:

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