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Research Paper

Golden-winged Warbler body fat and blood parasites are associated with anthropogenic and environmental habitat metrics

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ABSTRACT. The extent of early successional vegetation communities is declining worldwide. Sometimes species use early successional vegetation communities associated with anthropogenic development (e.g., rangeland, cropland, transportation corridors, aggregate mines), given that, at face value, these ecosystem types can share many of the same characteristics (e.g., lack of mature trees). This may have a negative impact on species' health by lowering access to resources or by increasing exposure or susceptibility to vectors carrying disease agents leading to infections. We investigated whether proximity to anthropogenic development had a negative impact on several health metrics of a threatened, early successional species, the Golden-winged Warbler (*Vermivora chrysoptera*). Golden-winged Warblers with more rangeland/grassland within 200 m of their capture location were more likely to be infected with *Haemoproteus* parasites, whereas those with greater marsh cover within 200 m of their capture location were more likely to be infected with *Leucocytozoon* parasites, and those near rivers had higher body-fat scores. As infection prevalence and body-fat scores may have an impact on avian fitness, the variable response of these land-cover types indicates an additional conservation concern for this threatened species.

La graisse corporelle et les parasites sanguins de la Paruline à ailes dorées sont associés à des paramètres anthropiques et environnementaux de l'habitat

RÉSUMÉ. L'étendue des communautés végétales de début de succession décroît dans le monde entier. Les espèces utilisent parfois des communautés végétales de début de succession associées aux milieux d'origine anthropique (p. ex. pâturages, terres cultivées, couloirs de transport, mines), étant donné qu'à première vue, ces types d'écosystèmes partagent de nombreuses caractéristiques (p. ex. l'absence d'arbres matures). Cette réalité peut avoir un effet négatif sur la santé des espèces en réduisant l'accès aux ressources ou augmentant l'exposition ou la sensibilité aux vecteurs porteurs d'agents pathogènes conduisant à des infections. Nous avons examiné si la proximité avec un milieu d'origine anthropique avait un effet négatif sur plusieurs paramètres de santé d'une espèce menacée spécialiste de débuts de succession, la Paruline à ailes dorées (*Vermivora chrysoptera*). Les Parulines à ailes dorées fréquentant des lieux où il se trouvait plus de pâturages ou de prairies dans un rayon de 200 m de leur lieu de capture étaient plus susceptibles d'être infectées par des parasites *Haemoproteus*, tandis que celles ayant une plus grande superficie de marais dans un rayon de 200 m de leur lieu de capture étaient plus sujettes à être infectées par des parasites *Leucocytozoon*, et celles ayant été capturées près de rivières avaient des scores de graisse corporelle plus élevés que les autres. Comme la prévalence des infections et le taux de graisse corporelle peuvent avoir un impact sur la condition physique des oiseaux, la réponse variable de ces types d'occupation du sol révèle une nouvelle préoccupation de conservation pour cette espèce menacée.

Key Words: *anthropogenic disturbance; body condition; early successional forests; haemosporidia; host-parasite interactions; Vermivora*

INTRODUCTION

Early successional vegetation communities are tracts of land that are in the initial stages of regeneration after a disturbance, such as a clear cut or a fire. Diverse early successional vegetation communities are decreasing in abundance around the world (Litvaitis 1993, Hunter et al. 2001, Swanson et al. 2011). In North America, the amount of early successional vegetation communities increased rapidly following farm abandonment in the early 1900s (Gill 1980), but has decreased in recent decades (Litvaitis 1993) and is now at a historic low (Hunter et al. 2001, King and Schlossberg 2014). Moreover, early successional vegetation communities continue to be lost because of land conversion, development, and disturbance suppression (Askins 2001). Recent research in eastern North American forests suggests that early successional vegetation communities are declining at approximately 3% per year, although this is highly variable by

specific area (King and Schlossberg 2014). On account of this loss, many early successional species have become threatened, extirpated, or extinct; for example, loss of early successional vegetation communities likely caused the extinction of the Bachman's Warbler (*Vermivora bachmanii*; Litvaitis 1993, Hunter et al. 2001), a close relative of the Golden-winged Warbler (*V. chrysoptera*; Lovette et al. 2010). Even in the Pacific Northwest, where old growth forest loss is of widespread concern, early successional obligates make up a similar proportion of species at risk to mature forest obligates, and more than 50% of at-risk species used early successional ecosystems in some way (Swanson et al. 2011).

Although some anthropogenic development activities destroy early successional vegetation communities, others create them. For the purposes of this paper, we define anthropogenic

development as human alteration of landscapes for economic or social-development purposes (i.e., not habitat restoration). Anthropogenic development that includes tree removal or livestock grazing, followed by natural succession, can create shrubby edges that are used by some early successional forest species (Bulluck and Buehler 2006, Moulton 2017). However, proximity to anthropogenic development might negatively affect bird health if these areas have lower invertebrate prey availability, increased infection risk due to higher vector presence, or coincide with the occurrence of other stressors such as noise (Ewers and Didham 2006, O'Brien and Dawson 2016). It is important to identify the relative quality of different ecosystems to ensure that land managers can prioritize areas most likely to support wildlife populations.

Avian health metrics that may be affected by anthropogenic development include body condition and parasite prevalence. Anthropogenic development of landscapes may affect the body condition of birds by reducing the availability of important resources, such as food, or by causing birds to re-allocate resources to fight-or-flight responses in the presence of an increased number of predators or disturbance than in non-developed landscapes (Frid and Dill 2002, Quinn et al. 2006, Liker et al. 2008). Blood parasites are a potentially informative health metric because they tend to negatively affect birds and their presence may indicate a compromised immune system and related inability to respond to stressors (Lee et al. 2006, LaPointe et al. 2012).

Other environmental characteristics, particularly water sources, are also likely to have an impact on the likelihood of infection by blood parasites, especially by affecting habitat availability of parasite vectors. In particular, *Leucocytozoon* parasites are associated with black fly (*Simuliidae*) vectors, which breed near flowing water (Rozendaal and WHO 1997, Valkiūnas 2005). Similarly, wetlands can provide habitat for numerous insects including *Culicoides* midges (some of which are vectors for *Haemoproteus*; Rozendaal and WHO 1997, Valkiūnas 2005). Mosquitoes (*Culicidae*) have diverse habitat requirements depending on the species (Rozendaal and WHO 1997) but are often associated with wetlands and temporary pool habitat (Dixon and Brust 1972, Dale and Knight 2008).

Plasmodium, *Leucocytozoon*, and *Haemoproteus* are widespread and abundant avian blood parasites (LaPointe et al. 2012). These parasite species have been shown to decrease host fitness by decreasing reproductive success or survival (Figuerola et al. 1999, Freeman-Gallant et al. 2001, Marzal et al. 2005, Asghar et al. 2011, Lachish et al. 2011, LaPointe et al. 2012). Birds with higher mass or body-fat scores, or those without infections, are often more capable of surviving migration and successfully producing young (Møller and Saino 2004, Smith and Moore 2005, Martinez-de la Puente et al. 2010, Labocha and Hayes 2012). Monitoring individual health, such as the absence of infection, body fat, and seasonally corrected body mass normalized to body size, i.e., body condition, can help us understand whether vegetation communities with both plentiful resources and low levels of vectors help to support healthier birds. Therefore, there is value in monitoring avian health across a spectrum of environments, from anthropogenically developed to natural, to examine the impact of anthropogenic development on individuals and populations.

To date, research examining the consequences of anthropogenic development on avian blood parasitism has largely been conducted in tropical or Neotropical regions (see Laurance et al. 2013, Mendenhall et al. 2013, Okanga et al. 2013), leaving a knowledge gap regarding its evolutionary and ecological importance in temperate systems. This is especially important because studies have indicated that avian haemosporidian parasites can be transmitted on both the wintering and the breeding grounds (e.g., Soares et al. 2020). Parasite prevalence does not respond uniformly to anthropogenic development: parasitism often increases in areas surrounded by anthropogenic development (e.g., Vaz et al. 2007, Cottontail et al. 2009, Okanga et al. 2013), but the opposite can also be true (e.g., Bonneaud et al. 2009). Variable responses to anthropogenic development are unsurprising because there are many different types of anthropogenic development, and each may have a different effect on the vector communities that transmit blood parasites (LaPointe et al. 2012, Brearley et al. 2013, Sehgal 2015). Therefore, further research is needed to understand the relationship between parasites and specific types of anthropogenic developments (Brearley et al. 2013). This is especially important in non-tropical environments where the few studies that have examined the link between anthropogenic development and blood parasites have generally restricted their analysis to a single variable such as urbanization or agriculture (e.g., Evans et al. 2009, Fourcade et al. 2014, Bailly et al. 2016).

The Golden-winged Warbler is an at-risk early successional species. In 2007, it was listed as threatened on Schedule 1 of the Canadian Species at Risk Act (SARA). The primary threats to the species were initially thought to be habitat loss and hybridization with an early successional–breeding congener, the Blue-winged Warbler (*V. cyanoptera*; Gill 1997, Buehler et al. 2007, Vallender et al. 2009). More recently, habitat loss on the wintering range has been identified as a likely important factor (Kramer et al. 2018). Genomic analysis recently revealed that hybridization between the species predates European colonization (Toews et al. 2016), which suggests that hybridization may be a less important threat than originally assumed and reinforces the importance of understanding the impact of habitat loss. Both Golden-winged and Blue-winged Warbler populations are decreasing; population estimates have dropped by 1.85% and 0.67% per year, respectively, since 1966 (Sauer et al. 2020). Thus, identifying and retaining high quality early successional vegetation communities is expected to benefit both *Vermivora* species. The SARA Recovery Strategy for the Golden-winged Warbler indicates the need to identify and maintain high quality habitat, and to identify and mitigate anthropogenically caused ecological traps (Environment and Climate Change Canada 2016). For example, Golden-winged Warblers will nest in utility right-of-ways, but nesting success can be low in this environment because of higher nest predation rates than in other areas (Bulluck and Buehler 2006, Kubel and Yahner 2008). If anthropogenic development has a negative impact on the health of Golden-winged Warblers, this suggests that Golden-winged Warblers may face health consequences if they are selecting for, or are forced to use, these types of ecosystems.

The Golden-winged Warbler population in Manitoba is potentially valuable from a conservation perspective because it has the lowest rate of genetic introgression with the Blue-winged

Warbler across the breeding range (Vallender et al. 2009, Moulton et al. 2018). However, this population also has a high prevalence of blood-parasite infections, relative to other populations (i.e., in Kentucky, Ontario, and Wisconsin), perhaps because of high levels of anthropogenic development in the form of agriculture on the landscape, or because this population occurs near the edge of the species' historical north-west range expansion (Vallender et al. 2012, Enslow et al. 2020). If anthropogenic development leads to decreased body condition, or increased parasite prevalence, in Manitoba's Golden-winged Warblers, this may pose a conservation concern to this important population.

We studied body condition and parasite infection rates of Golden-winged Warblers in Manitoba in relation to anthropogenic development, including cover of rangeland (land grazed by cattle), aggregate (sand and gravel) mines, and transportation features (roads and rail lines). We predicted that rangeland would be positively associated with parasite prevalence, because *Culicoides* vector habitat may exist when water accumulates in cattle footprints and drinking areas (González et al. 2013). We predicted that body-condition indices would have a negative relationship with aggregate mines and transportation features because these developments remove foraging habitat and may increase stress in birds from traffic or loud equipment (Francis et al. 2009, McClure et al. 2013). We also included distance to river and proportion of marshland in models, to control for effects of aquatic features on abundance of parasite vectors.

METHODS

Study areas

We captured Golden-winged Warblers in southeastern Manitoba and Riding Mountain National Park, in western Manitoba. The southeastern Manitoba study area is approximately 60 km southeast of Winnipeg, Manitoba, within 15 km of the town of Richer (49.764° N, 96.528° W). The Riding Mountain National Park study area was near the eastern border of the park and included regions immediately outside of the park, from McCreary to Dauphin, Manitoba (49.764° N, 96.528° W). The Golden-winged Warbler population is somewhat disjunct in Manitoba. Most birds breed either in the center-west near Riding Mountain National Park, in the southeast, or in a small population in the center of the province (the Interlake; Environment and Climate Change Canada 2016; C. Artuso, *personal communication*). Thus, these study areas encompass most of the known distribution of Golden-winged Warblers in the province, and so by sampling these areas, we expected to have sampled much of the geographic variation in parasite prevalence in the species in Manitoba. The study areas are similar; both areas contain large quantities of aspen parkland and boreal transition zone forest, consisting of tracts of undeveloped forest interspersed with anthropogenic development of various sizes (from small country roads to large acreages of farmland). Anthropogenic development types are similar in these two study areas, dominated by rangeland/grassland (defined as open grassland, used for hay or grazed by cattle, generally with less than 10% shrub cover), cropland (defined as land cultivated for row crops such as cereals and seeds), transportation features (defined as paved or gravel roads, cut-survey lines, railway corridors, and transmission lines), or aggregate mines (which we verified using orthographic imagery

and defined as areas with less than 10% vegetation cover). We assumed that all areas classified as rangeland/grassland were grazed, which was consistent with our observations for the area. Forests were characterized by aspen (*Populus tremuloides*) and birch trees (*Betula papyrifera*), interspersed with spruce (*Picea*) or pine (*Pinus*), and considerable shrub cover of hazel (*Corylus cornuta*), raspberry (*Rubus occidentalis*), Saskatoon berry (*Amelanchier alnifolia*), and pincherry (*Prunus pensylvanica*; Moulton 2017). We selected sampling locations based on previous survey data that indicated the presence of a breeding population, and by surveying for Golden-winged Warbler habitat, which includes aspen parkland and boreal transition zone ecosystems interspersed with open grassy patches (Confer and Knapp 1981, Moulton and Artuso 2017, Confer et al. 2020).

Field methods

We surveyed potential sampling locations for territorial singing by Golden-winged Warblers. When we identified a singing bird, we immediately attempted to capture the individual using 6 m long mist nets and territorial song playbacks. We captured birds between 20 May and 1 July 2014. When we caught a bird we processed it by applying a Canadian Wildlife Service aluminum leg band; we sexed and aged it by plumage, according to Pyle (1997); and we collected standard morphometrics, e.g., mass, wing-chord length (Ralph et al. 1993). We assigned a furcular fat score based on a 0–7 scale (Ralph et al. 1993) and took ~20 µL of blood via venipuncture of the brachial vein. Banders placed the blood into tissue lysis buffer and stored it at room temperature (White and Densmore 1992).

Infection prevalence

We compared a non-nested PCR protocol and a nested PCR protocol that are both commonly used to detect haemosporidia to ensure the infection detection methods were robust. We extracted DNA from the blood samples using a homemade kit (Ivanova et al. 2006). The non-nested PCR protocol followed the methods of Vallender et al. (2012). Primers L15183 (Szymanski and Lovette 2005) and H15725 (Ricklefs and Fallon 2002) amplified a 550 base pair region of cytochrome b in *Haemoproteus* and *Plasmodium* parasites but did not amplify *Leucocytozoon* parasites. Next, we used the nested PCR protocol outlined in Hellgren et al. (2004), which detects parasite presence in a dilution as low as 1:10,000 parasite cells to host blood cells. The first two primers in the nested PCR (HaemNF1 and HaemNR3) initially amplified a region of cytochrome b from all three parasite genera (*Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) from the raw DNA. A second and third PCR used the product from the first nested PCR step. In the second PCR, nested primers (HaemF and HaemR2) isolated a smaller 480 base pair region of cytochrome b in *Haemoproteus* and *Plasmodium* parasites. In the third PCR, nested primers HaemFL and HaemR3L only isolated *Leucocytozoon* parasites. We used electrophoresis to detect positive infections. We scored visible bands of PCR product as positive infections. We sequenced positive infections twice, once with the forward primer and once with the reverse primer, using a Big Dye sequencing method, with an ABI 310 3XL Automated sequencer (Applied Biosystems Canada, Burlington, Ontario). We cleaned and aligned all successful sequences using Geneious Version 10 (<http://www.geneious.com>, Kearse et al. 2012), and identified parasites to the genus level using BLAST (Altschul et

al. 1990). We compared parasite haplotypes to known haplotypes in the literature using both the MalAVI BLAST and NCBI BLAST (Altschul et al. 1990, Bensch et al. 2009). The haplotypes found in several study sites across the range of Golden-winged Warblers, including this one, are discussed in a companion paper (Enslow et al. 2020).

Geographic analysis

We obtained land-use data (raster converted to shapefile, 30-m resolution) from the Manitoba Land Initiative and overlaid the GPS position of each Golden-winged Warbler sampled (Manitoba Conservation and Water Stewardship 2005). We assumed birds were captured near their territories and created a 200-m buffer (hereafter referred to as the capture buffer) around each bird point to attempt to capture the potential influence of nearby land-use types, and then intersected the capture buffer with the land-use layer. We recognize that there are limitations of this approach because Golden-winged Warbler song territories are rarely circular and tend to follow forest edges. Additionally, song perches may not be representative of where the birds are spending the majority of their time (Streby et al. 2012). We estimated percent cover of each of the following land-cover types for each capture buffer: transportation features (referred to as Roads/Trails in the dataset, and visually verified as composed of roads and rail lines); and rangeland/grassland, marsh, and aggregate mines (referred to as Bare Rock/Gravel/Sand in the dataset, and visually verified as aggregate mines; ArcMap 10.2, ESRI 2011). We verified land-use classifications around each bird by comparing the land-use layer to field observations and an orthographic base map. Only one small change from agriculture to rangeland/grassland appears to have occurred between 2009 and 2015, and this change affected three buffered points. We also calculated distance from each bird to the nearest river, which included both intermittent and permanently flowing watercourses (Harrison 2005). Most watercourses in the study area are moderate to small.

Statistical analysis

To determine whether there was an association between local land use on prevalence of infection, we used two binomial generalized linear models with binomial link functions. We used *Haemoproteus* occurrence as the response variable in one model and *Leucocytozoon* occurrence as the response in another model (R 3.6.2; R Core Team 2019). Because of low prevalence of *Plasmodium*, we did not create a model for this parasite genus. We evaluated the association between three anthropogenic percent cover predictor variables and parasite occurrence: rangeland/grassland, aggregate mines, and transportation features. We also evaluated the association between two natural landscape characteristics and parasite occurrence: distance to river and percent of buffer covered by marshland. We compared models with and without ordinal date (numerical value for each day of the year: 1–365) and bird age using Akaike's Information Criterion (AIC) to evaluate whether these covariates were associated with the response variables. Because these covariates were not the subjects of our research, we removed them from subsequent analyses if they did not improve model fit more than 2.0 AIC units (Arnold 2010).

We also tested whether parasite occurrence and anthropogenic development were associated with Golden-winged Warbler body

condition and body fat. Mass and fat scores were not available for all birds, and thus their sample sizes were $n = 63$ and $n = 66$, respectively. To test this association, we created a body condition index from the residuals (R1; body condition index) of a regression between body mass and ordinal date to correct for seasonal changes that occur throughout the breeding season ($R^2 = 0.30$, $\beta = 0.031$, $p < 0.0001$). We also tested a regression between R1 and wing length, but the results were not significant and so the residuals were not used ($R^2 = 0.035$, $\beta = 0.058$, $p = 0.14$; Whittingham and Dunn 2000, R Core Team 2019). We then performed a linear regression, with body condition index as the response variable, and as independent variables the presence of *Haemoproteus* and *Leucocytozoon* as well as date, bird age, and the above land-use variables. We tested for an association between presence of parasites and land-use variables on body fat using a Cumulative Link Model from the R package Ordinal (Christensen 2015). A maximum likelihood goodness-of-fit test indicated that the log-log link function best fits this model. Diagnostic graphs and Shapiro-Wilk tests confirmed that model assumptions were met.

We took a null-hypothesis significance testing (frequentist) approach (Mundry 2011), and a significance threshold of $p < 0.1$. This threshold was chosen to reduce risk of type II error, which is of significant concern in conservation biology (Taylor and Gerodette 1993). In particular, because the species is relatively uncommon it is difficult to achieve large sample sizes, and we were concerned about potentially missing issues that may be a significant conservation concern for this threatened species. All statistical tests were two tailed and means and effects sizes in the results section are presented with standard error (SE).

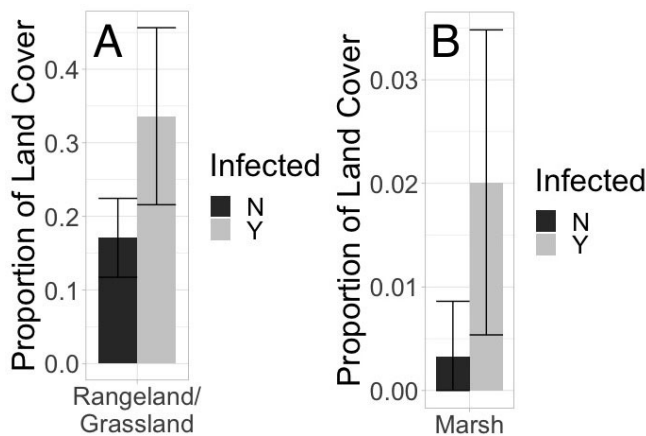
RESULTS

We captured 67 adult male Golden-winged Warblers during the breeding season in 2014: 27 in and near Riding Mountain National Park and 40 in southeastern Manitoba. Because of the low prevalence of *Plasmodium*, we only used *Haemoproteus* and *Leucocytozoon* in the rest of the analyses. Two positive infections produced weak PCR bands that corresponded to either *Haemoproteus* or *Plasmodium*, but could not be sequenced and were removed from further analyses. Total apparent prevalence of infection was 59.7% (40/67): 30.8% were infected with *Haemoproteus* (20/65), 6.2% were infected with *Plasmodium* (4/65), and 37.3% were infected with *Leucocytozoon* (25/67). Overall, 13.3% of Golden-winged Warblers were infected with more than one genus of parasite (10/67).

The nested PCR detected a *Plasmodium* or *Haemoproteus* infection in 37.3% (25/67) of our samples. This was higher than the single-PCR method, which detected an infection of one of these two genera in 26.9% (18/67) of the same samples. All individuals that tested positive for *Haemoproteus* or *Plasmodium* in the non-nested PCR also tested positive in the nested PCR. The quality of sequences obtained with the nested PCR method was higher and resulted in fewer ambiguous base pairs after cleaning. The two methods isolated DNA fragments that overlapped considerably, and we were able to accurately assign individuals to the same parasite haplotype if different primers were used. 85% (17/20) of *Haemoproteus* infections belonged to a single haplotype, which is accessible on genbank as MN114079.

A Golden-winged Warbler's probability of infection with *Haemoproteus* increased by 1.25 times as the proportion of rangeland/grassland in their capture buffer increased by 10% ($\beta = 2.27$, $SE = 1.28$, $p = 0.076$, $df = 60$), but no other anthropogenic or natural habitat variables were associated with *Haemoproteus* prevalence (all p values > 0.235 ; Fig. 1). The proportion of rangeland/grassland within capture buffers varied from 0–94.9%, and in 46.3% of samples cover was below 10% (Fig. 2). For parasite presence, fat score, and body condition, neither age class nor ordinal date improved the fit of the model greater than two AIC units (Arnold 2010), so we removed these variables from all subsequent analyses. These variables were also non-significant, for age class $p > 0.341$, and for ordinal date $p > 0.218$. *Leucocytozoon* occurrence did not vary significantly with any anthropogenic development variables, but it was positively correlated with marsh cover. When marsh cover in a Golden-winged Warbler's capture buffer increased by 2%, the bird's odds of being infected with *Leucocytozoon* increased by 1.48 times ($\beta = 19.0$, $SE = 10.7$, $p = 0.077$, $df = 66$). Marshes were not abundant near capture locations: 90% of sampling locations were not within 200 m of any marsh cover, and when a sample buffer overlapped marsh cover it was by a maximum of 13.78% (Fig. 2).

Fig. 1. Proportion of land use within a 200-m radius significantly associated with (A) *Haemoproteus* and (B) *Leucocytozoon* infection in Golden-winged Warblers (*Vermivora chrysoptera*) sampled in southeastern and southwestern Manitoba in 2015, $n = 67$.



None of the land-use characteristics we studied had a significant association with body condition of Golden-winged Warblers: transportation features ($p = 0.90$), rangeland/grassland ($p = 0.41$), marsh ($p = 0.97$), aggregate mines ($p = 0.60$), and distance to river ($p = 0.98$). The only variable that was associated with body fat was distance to river, with Golden-winged Warblers situated closer to a river having higher body-fat scores ($\beta = -0.354$, $SE = 0.160$, $p = 0.0274$, $df = 57$; Fig. 3).

Body fat and body condition varied with *Leucocytozoon* infections, but not with *Haemoproteus* infections. *Leucocytozoon*-infected Golden-winged Warblers were in better body condition ($\beta = 0.350$, $SE = 0.160$, $p = 0.034$) than uninfected birds. *Haemoproteus*-infected birds had similar body condition to uninfected birds ($p = 0.43$). *Haemoproteus*-infected versus

uninfected birds also had similar body-fat scores ($p = 0.80$), with infected birds having an average body-fat score of 1.11, and uninfected birds having an average score of 1.15. *Leucocytozoon*-infected birds had an average body-fat score of 1.38, which was significantly higher than the average of the non-infected birds (1.0; $\beta = 1.05$, $SE = 0.427$, $p = 0.014$). None of the 10 Golden-winged Warblers with a body-fat score of zero were infected with *Leucocytozoon*. We note that the small numbers of zero body-fat score birds is highly unusual for breeding birds.

Fig. 2. Proportion of land use within a 200-m radius of Golden-winged Warblers (*Vermivora chrysoptera*) sampled in southeastern and southwestern Manitoba in 2015, $n = 67$.

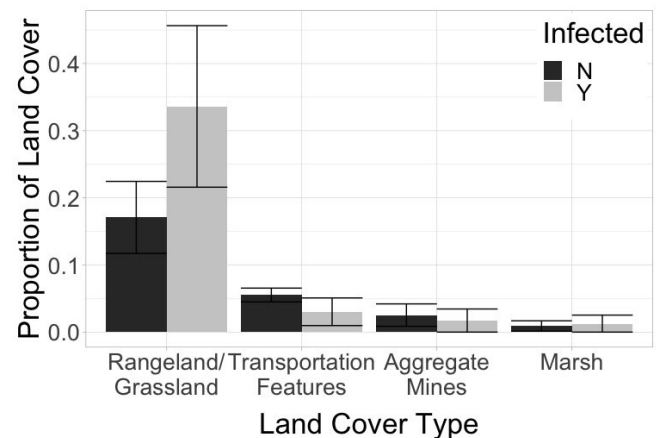
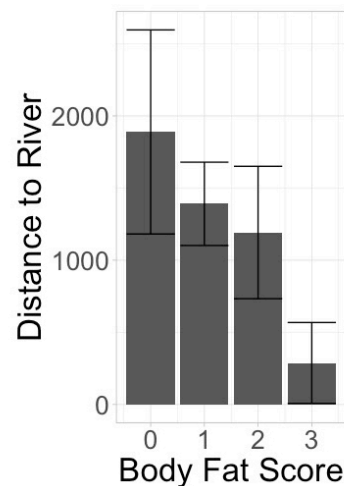


Fig. 3. Mean body-fat scores of Golden-winged Warblers (*Vermivora chrysoptera*) relative to capture distance from a river.



DISCUSSION

Our results suggest that landscape structure may be associated with health and parasite prevalence in early successional avian species such as Golden-winged Warblers. The results were counter

to some of our predictions because transportation features and aggregate mines had no relationships to the metrics we examined. However, the results for rangeland/grassland were consistent with our prediction because it was associated with higher parasite prevalence. Additionally, two natural landscape features were associated with avian health metrics: marshes were associated with increased *Leucocytozoon* prevalence, whereas closer proximity to a river was associated with increased body fat. Anthropogenically developed landscapes are often associated with lower resource availability (Reinoso-Pérez et al. 2016) or increased actual or perceived threats (Frey and Conover 2006, McClure et al. 2013), both of which can lead to lower immune competency (Brearley et al. 2013) and could lead to the observed patterns. Stress and food provisioning have been found to be linked to immune function in some avian species (Yadav et al. 2014, Wilcoxon et al. 2015, Gao et al. 2017). However, immune response has a strong genetic component (Piertney and Oliver 2006), and more research is needed to elucidate the trends and mechanisms associated with resource availability and immunity in wildlife (Strandin et al. 2018).

Of the anthropogenic development types that were examined, only rangeland/grassland was associated with parasite prevalence, and it was positively associated with the presence of *Haemoproteus*. This could result from increased vector presence in grazing operations. Cattle manure, drinking water, and water accumulation in hoof prints can increase habitat availability for *Culicoides* midges, which are the vectors for *Haemoproteus* (Lysyk 2006, Martinsen et al. 2008, González et al. 2013). Similarly, goat abundance in Mexican highlands was positively correlated with parasitemia in Canyon Towhees (*Melospiza fusca*; Reinoso-Pérez et al. 2016). However, because we were not able to definitively distinguish between grazed and ungrazed grassland, it is also possible that the mechanisms driving this trend are related to grassland ecosystems in general rather than grazing mechanisms specifically.

Surprisingly, *Leucocytozoon* parasite prevalence was not associated with distance to rivers, known breeding habitats for their black fly vector (Valkiūnas 2005), although parasite prevalence was positively associated with marshes. Black flies may be selecting for small rivulets of water from these marshes that could not be mapped at the scale at which our GIS data were compiled. Golden-winged Warblers also had higher body-fat scores near rivers; we hypothesize that proximity to rivers increases availability of food resources for birds, which may improve their condition and their ability to fight off infections (Lochmiller et al. 1993, Isaksson et al. 2013, Hernández-Lara et al. 2017). Because of sample sizes, we were only able to estimate general relationships with the stream network. Because streams of different orders may have divergent influences on the relationships we observed, we recommend that further research investigate these relationships in greater detail. Additionally, we have limited knowledge about how *Leucocytozoon* biology applies in this system, but this could provide a confounding factor to detecting habitat-related trends. *Leucocytozoon* has been associated with higher latitudes (Cuevas et al. 2020), and strains have been found to vary in prevalence seasonally and by elevation (Lynton-Jenkins et al. 2020). Although our study areas were in fairly uniform, low elevation areas, the season varied from spring

to mid-summer, and one of our study areas was at a higher latitude than the other. However, there was no significant difference in *Leucocytozoon* prevalence between these two study areas (Enslow et al. 2020).

Given that *Leucocytozoon* parasites can be virulent (Santiago-Alarcon 2012), it was unexpected that *Leucocytozoon* infections were more common in Golden-winged Warblers that had higher fat scores and were in better body condition. We suggest three possible explanations for this observation. One hypothesis is that individuals in poor body condition may not be able to tolerate infection and do not survive (see Lyimo and Koella 1992, Anderson et al. 2000, Asghar et al. 2011). Alternatively, foraging habitat for Golden-winged Warblers may also be habitat for black flies (the *Leucocytozoon* vector), so Warblers may be attracted to foraging sites that also increase their risk of infection. Further research would be necessary to identify the mechanisms that could explain this pattern. We note that it is also possible that high fat scores and body condition index used in this paper may not be indicators of high quality individuals.

One challenge with our study design is that we cannot determine whether infection status and body condition are a result of a bird's immediate habitat or of poor quality individuals being forced into suboptimal habitats. This is particularly challenging with migratory bird research, because parasites might either be acquired locally or prior to arriving on the breeding range. We thus consider our results to provide a warning that landscape characteristics might alter avian health but note that more research is needed to understand the mechanisms that explain these patterns.

This research suggests that rangeland/grassland may be associated with increased parasite infection in the Golden-winged Warbler and highlights further complex interactions among landscape structure and avian health. Although anthropogenic development activities sometimes create early successional vegetation communities, these developments may lead to suboptimal landscapes that pose risks to wildlife that live there. Because naturally occurring early successional communities continue to decline in their global extent, many species at risk are likely to increasingly depend on habitats that arise because of anthropogenic development. We recommend further research that would help us understand the challenges faced by wildlife that live in anthropogenically developed early successional landscapes, in the hopes that we could mitigate risks faced by wildlife.

Author Contributions:

Chelsea Enslow formulated the hypothesis and study/methods design, conducted the field and lab research and data analysis, and wrote the paper. Rachel Vallender provided lab training and supervision, provided substantial guidance and feedback on project development, analysis, and study design, and substantially edited the paper. Nicola Koper contributed substantial materials and resources for field and lab activities, supervised project development, analysis, and study design, and substantially edited the paper.

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