Human disturbance, host dispersal, and hybridization influence blood parasite communities in a threatened songbird species: the golden-winged warbler (*Vermivora chrysoptera*).

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ABSTRACT

Vector-transmitted blood parasites cause infections of birds that often negatively affect the fitness of their host. The golden-winged warbler is a threatened species in Canada, and the impact of blood parasites to the conservation of this species is poorly understood. Land cover, long-range, historical host dispersal patterns, and host genetics can influence the prevalence of blood parasites. I investigated whether landscape cover of agriculture, local human disturbance, historical dispersal of golden-winged warblers, and hybridization with a closely related species affected the prevalence of parasites in this species. Golden-winged warbler populations that had more recently colonized an area, and had higher landscape cover of agriculture, were more likely to be infected with *Leucocytozoon* and *Haemoproteus* parasites. Golden-winged warblers with local home ranges containing more rangeland were more likely to be infected with *Haemoproteus*, and hybrid golden-winged x blue-winged warblers were more likely to be infected with *Leucocytozoon* than pure golden-winged warblers. Because blood parasite communities in golden-winged warblers are shaped by factors that are important to conservation, their role is worth further exploration for this and other species.

ACKNOWLEDGEMENTS

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Chapter 1. INTRODUCTION

1.1 Definitions

Dispersal - The movement of an organism from the place that it was born (hatched) to the place that it will attempt to breed (Howard 1960), or the movement of an organism between year to year breeding locations. For the purpose of this thesis dispersal of birds typically refers only to large-scale dispersal, which spans at least the radius of a study site in this study (at least 50km).

Early Successional Habitat – Any habitat that is regenerated after a disturbance. This type of disturbance can be anthropogenic (eg. aggregate mine) or natural (eg. fire). These habitats consist of vegetation that regrows quickly after a disturbance, such as grasses, herbaceous plants, saplings, and scrubby bushes. This type of habitat is ephemeral and will only last until the successional stage progresses into a late successional stage (often, but not always, full forest cover) once again. In terms of forest habitat, early successional habitat generally exists from 0 – 20 years following a disturbance, if another disturbance does not occur.

Haplotype – A unique genetic variant of a species or population, wherein the DNA sequence differs from every other genetic variant by at least one base pair. For the purpose of this thesis haplotypes refer only to genetic variants of Haemosporidian parasites.

Lineage – A monophyletic group of any kind, ie. a group that is descended from a single ancestor. For the purpose of this thesis, a lineage refers to a closely related group of parasite haplotypes, none of which are more than two base pairs different from a central haplotype. A unique haplotype can be its own lineage, and therefore a lineage can refer to a single haplotype. However, lineage can also refer to several, closely related haplotypes.

Local Scale – For the purpose of this thesis, local scale refers to anything that occurs within a bird's home-range (or estimated home range). For example, local scale land cover is the type of land that is found within an individual bird's home range.

Landscape Scale – For the purpose of this thesis, landscape scale refers to anything that occurs within a 50km radius of the centre point of a study site.

1.2 Background

The Golden-winged Warbler (*Vermivora chrysoptera*) has been designated as threatened by the Species at Risk Act in Canada since 2007 (Species At Risk Act 2002, 2007). The North American range of the golden-winged warbler is restricted to the Northeast, and it occurs only in Quebec, Ontario, Manitoba and Saskatchewan in Canada (Confer et al. 2011). An immediate threat to this species is hybridization with the blue-winged warbler (*Vermivora cyanoptera*); historically, golden-winged warblers have been extirpated within 50 years of secondary contact (defined as contact following geographic isolation and speciation) with blue-winged warblers (Gill 1980, Confer and Knapp 1981, Buehler et al. 2007). The golden-winged warbler population in Manitoba contains over 4, 600 pairs, is large and potentially increasing, and remains allopatric to blue-winged warblers (Environment and Climate Change Canada 2016). While there is evidence of blue-winged warbler genes in this population (Moulton et al. unpublished data), it has the lowest levels of genetic introgression in the world; Vallender (2009) reported that zero out of 95 golden-winged warblers sampled from Manitoba had blue-winged warbler mitochondrial DNA, while other Canadian populations had levels of introgression that were as high as 30% (Vallender et al. 2009). Thus, the conservation of this population is of international significance. However, the role of hybridization in the conservation of the species is under debate; genomic research revealed that golden-winged and blue-winged warblers are nearly

genetically identical, leading some to speculate that the two species may actually be two phenotypes of a single species (Toews et al. 2016). Additionally, hybridization between these species predates European colonization, which directly contrasts the long-standing hypothesis that hybridization was caused by humans clearing the late successional forest that separated the species and allowing the two early successional species to come together (Gill 1980, Toews et al. 2016). Some conservationists may remain wary of combining the two species because their mitochondrial divergence is high at 3%, which is on par with other species of wood warbler (Gill 1997), and it is likely that anthropogenic disturbance has increased the rate of hybridization (Toews et al. 2016). However, even if they are considered a single species, the complex still warrants attention from conservationists; both species/morphs are early successional habitat specialists, and both are declining at least in part because of loss of this early successional habitat (Gill et al. 2001, Confer et al. 2011). According to breeding bird survey data, from 1966 to 2015 blue-winged warblers declined by an average of 1.24% per year from 1966 to 2015, while golden-winged warblers declined by an average of 2.95% per year, across both species' ranges (Sauer et al. 2017). Because hybridization is likely not as great a threat as initially suspected, it is important to address the multiple landscape factors that may affect the long-term survival and productivity of the *Vermivora* complex, especially the large and distinct Manitoba population.

Landscape structure is important to golden-winged warblers, as illustrated by previous research (Moulton and Artuso unpublished data) that demonstrates that pure golden-winged warblers select territories in less anthropogenically-disturbed sites than golden-winged warbler x blue-winged warbler hybrids. The golden-winged warbler is a habitat specialist that requires early successional scrub; their natural habitat contains early successional vegetation surrounded by mature forest (Confer et al. 2011). This habitat was historically preserved and regenerated by

natural disturbances such as fire (Askins 2001), but it can also be created by anthropogenic

disturbances (Hunter et al. 2001, Bulluck and Buehler 2006). Anthropogenically disturbed

habitat contains early successional habitat surrounded by human-influenced landscapes such as

mining operations or agricultural land. Species may respond either positively or negatively to

anthropogenic disturbance; it can reduce population numbers of forest dwelling species, while

increasing the numbers of early successional or 'edge species' (Ethier and Fahrig 2011). A

species' use of anthropogenically disturbed habitat does not necessarily mean that it is quality

habitat for the species, and if golden-winged warblers are attracted to this habitat but have low

survival or reproductive capacity in it, it may be an ecological trap (Battin 2004). Current

research on golden-winged warblers in southeastern Manitoba is being undertaken to investigate

the effects of fragmentation on nesting success and habitat selection (L. Moulton, personal

communication). The exact mechanisms driving different fitness or population changes of a

species near anthropogenic disturbance are often unclear, but may involve habitat

selection/specialization, predation, or change in exposure to vectors of infectious agents (Burger

et al. 1994, Ewers and Didham 2005, Ethier and Fahrig 2011).

The role of disease is a potentially important, but little explored threat to golden-winged

warblers. Avian blood parasites are common causes of infectious diseases of birds that have an

impact on the fitness of species, globally (LaPointe et al. 2012). There are many different blood

parasites, with different effects on their hosts, and this study focuses on three common and

widespread genera: the mosquito transmitted malarial genus *Plasmodium* and closely related

genera *Leucocytozoon* and *Haemoproteus* (Greiner et al. 1975). Impacts of parasites on their

hosts vary from reduced mating success (Figuerola and Munoz 1999, Freeman-Gallant et al.

2001); to reduced reproductive success (Marzal et al. 2005, Asghar et al. 2011); to

immunodeficiency, increased predation, and direct mortality (Asghar et al. 2011, Lachish et al. 2011, LaPointe et al. 2012). Because blood parasite infections have the potential to negatively affect avian fitness, they may be especially detrimental to species and populations that are already at risk. There has been little research on blood parasite infection in golden-winged warblers, but blood parasites in this species appear to increase from the east to the west across Canada, ending with the highest prevalence in Western Manitoba (Vallender et al. 2012). The Manitoba population of golden-winged warblers is at a fitness disadvantage, with smaller clutch sizes, lower survival, and later breeding site arrival times than golden-winged warbler populations in Eastern Canada (Vallender 2007a, Bulluck et al. 2013, Moulton, unpublished data); therefore, it is crucial to further investigate the risk that parasites bring to this population (Vallender et al. 2012).

An important step in characterizing the threat of parasitism to a species is to examine how and why the prevalence of different types of parasites varies across the range of the species. Because temperature strongly influences vector development and parasite incubation within a vector species, climate change may render conditions in northern regions suitable for vector reproduction or parasite transmission, which may cause northern communities to be exposed to novel southern parasites (LaPointe et al. 2010, Hongoh et al. 2012, Loiseau et al. 2012). Similarly, if parasites are dispersed through the dispersal of their hosts, a dispersing species could transmit emergent parasites throughout its range (Hoberg and Brooks 2008). Lastly, anthropogenic land conversion and climate change can affect the spread of vector species, and this factor will be increasingly important as global human disturbances continue (Patz et al. 2008, LaPointe et al. 2012).

Research linking anthropogenic disturbance and avian malarial parasites has largely been conducted in tropical regions (see Laurance et al. 2013; Mendenhall et al. 2013; Okanga et al. 2013). Results from these studies have been somewhat mixed: disturbance effects tend to be negative when human activity increases aquatic habitats for mosquito larvae, or add supplementary hosts such as livestock. However, human disturbance may reduce infection rates in areas where humans destroy vector habitat or use vector control measures (LaPointe et al. 2012). Additionally, deforestation and land development has been shown to increase land temperatures, and lead to an increase in vector abundance and human malaria prevalence in the tropics (Lindblade et al. 2000, Patz et al. 2008). Because blood parasite prevalence appears dependent on landscape use, rainfall, and temperature, the effects of anthropogenic disturbance on parasite transmission are likely to be very different in Canada than tropical regions.

Though many studies have explored the role of parasitism in hybridization dynamics (see Fritz et al. 1999 for a review), the role of parasites in the golden-winged x blue-winged warbler hybrid complex has been understudied to date. The mechanisms behind the genetic swamping of golden-winged warblers with blue-winged and hybrid warblers are poorly understood, but take place rapidly (Gill 1980, Confer et al. 2011, Gill 1997, Vallender et al. 2007b). Parasites may promote hybridization when hybrids are more resistant to parasites than their parental species (eg. Heaney and Timm 1985) or resist hybridization when hybrids are more susceptible to parasites than their parents (Mouila et al. 1991, Goldberg et al. 2005). However, there is no universal rule suggesting when hybrids will be more or less resistant to parasites than their parent species. Hybrids may be resistant to parasites if the increased heterozygosity provided through hybridization increases an animals chance of having an allele that renders the animal successful at defeating infections (Doherty and Zinkernagel 1975, Hedgecock 1995). However, hybrids may

be more susceptible to parasites if mixing genotypes negatively disrupts genes that control immune function (Templeton 1986, Moulia 1991, Fritz et al. 1999). Therefore, each hybrid complex must be examined individually to understand how parasites influence that system. One study found no effect of hybridization on overall infection prevalence in golden-winged warblers, but they did not distinguish between two genera of parasites (*Haemoproteus* and *Plasmodium*), and did not examine a third genus, *Leucocytozoon* (Vallender et al. 2012). Resistance to parasites may be specific to parasite genus or haplotype, and so looking at this interaction in greater detail is warranted (Asghar et al. 2011, Santiago-Alarcon et al. 2012).

1.2. Purpose and Objectives

The purpose of this project is to document the species and prevalence of blood parasites infecting golden-winged warblers across their range, and to examine the relationship between parasitism and geographic characteristics on a range-wide scale, and the relationship between anthropogenic disturbance and hybridization on smaller/regional (Manitoba / Ontario) scales. To do this, several key objectives were met.

1. Determined the haplotypes of blood parasites affecting golden-winged warblers throughout a significant portion of their Canadian range

2. Quantified the occurrence of blood parasites in space and compared this to land cover on local (bird home range) and landscape (site-level) scales

4. Investigated the relationship between hybridization and parasite prevalence

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Chapter 2. LITERATURE REVIEW

2.1. Vectors

2.1.1 Association between vectors and blood parasites

The association between avian haemosporidian parasites and their vectors is tight; genetic differences between parasites appear to be explained by an evolutionary shift in their vectors (Martinsen et al. 2008). There is a strong association between vector family and parasite genus for three genera of haemosporidian parasites that are being considered in this study: *Plasmodium, Haemoproteus,* and *Leucocytozoon.* Avian *Plasmodium* parasites are associated with mosquitoes (Culicidae, most often the genera *Culex, Aedes, Ochlerotatus,* and *Culiseta*) (Valkiūnas 2005). *Leucocytozoon* parasites are associated with black flies (Simuliidae) (Martinsen et al. 2008, Valkiūnas 2005). *Haemoproteus,* subgenus *Parahaemoproteus* parasites are associated with biting midges (Ceratopogonidae, most often the genus *Culicoides*) and *Haemoproteus* subgenus *Haemoproteus* parasites are associated with louse flies (Hippoboscidae). Hippoboscidae should not be important vectors in this system because the genus *Haemoproteus* subgenus *Haemoproteus* is generally limited to Columbiform birds (and no *Haemoproteus* subgenus *Haemoproteus* parasites were found in this study) (Valkiūnas 2005). Parasite-vector relationships are generally not well-resolved at finer taxonomic levels. There appears to be no relationship between mosquito species and parasite species, which indicates that significant vector switching or sharing occurs at that taxonomic level (Kimura et al. 2010). However, *Haemoproteus* haplotypes can be more strongly linked to specific *Culicoides* vector species (Martínez-de la Puente et al. 2011). It is important to understand the variety of vectors found in a parasite-host system, as the vectors' varied life history strategies may cause them to respond to environmental or biological

changes in different ways, which will influence the relationship between these environmental or biological changes and a host's blood parasite community.

2.1.2 Natural history

Feeding mechanism and larval habitat can differentiate the Haemosporidian vectors expected in this study. Mosquitoes, black flies, and biting midges tend to begin their lives as an egg on a wet or damp surface (standing water, running water, and damp soil, respectively) (Rozendaal 1997). Eggs of all species mature into a larva, then a larger pupa, before finally becoming an adult (Rozendaal 1997). Not all species within these families require a blood meal but it is a common strategy for females to require blood feeding to produce eggs. Mosquitoes acquire a blood meal through solenophagy, where they insert specialized tubular mouthparts into their host, extracting the blood cells with low levels of distraction or pain to the host (Black and Kondratieff 2005). Black flies and biting midges blood feed by lacerating the skin and lapping up the blood that is produced (Black and Kondratieff 2005).

Larval habitat for mosquitoes is species dependent: *Aedes* mosquitoes lay theirs eggs above the water-line on ponds, or anywhere the water level rises and falls, the eggs hatch when the water rises to the level that the eggs were laid; *Culex* females lay their eggs on the surfaces of water in clusters, and prefer still water that is filled with organic particles (Rozendaal et al. 1997). In Manitoba, temporary pools are important habitat for mosquitoes (Dixon and Brust 1972). Black flies, in contrast, prefer to lay their eggs in fast-flowing waters such as rivers and streams (Rozendaal 1997). Biting midges lay their eggs on wet soils with lots of organic matter, sometimes choosing swamps or marshes (Rozendaal et al. 1997).

2.1.3 Vectors in study sites

Of the 33 mosquito species that are likely to be present in Southern Manitoba (see distribution maps in Wood et al. 1979), four are known vectors of avian *Plasmodium: Aedes canadensis, Culex restuans, Culex tarsalis* and *Aedes vexans* (Kimuera 2010, Inci et al. 2012, Santiago-Alarcon et al. 2012). *Culex pipiens* is another *Plasmodium* vector that could be present, as it is has been seen, although rarely, in the past (Wood et al. 1979), and recent evidence shows that its range may be expanding into Manitoba (Hongoh et al. 2012). *Culex territtans*, will also blood-feed on birds but blood screens have yet to detect the formation of infectious sporozoites of *Plasmodium* within this species' blood (Wood et al. 1979, Santiago-Alarcorn et al. 2012). *Leucocytozoon* parasites may be transmitted by the black fly species *Simulium meridionalei* in Manitoba (Currie 2014). Two other species of black flies are also known to feed on birds in this area, *Ectemnia taeniatifrons* and *Simulium johannseni,* but are not known to transmit blood parasites (Currie 2014). Of the Ceratopogonidae found in the Prairie Provinces (see Lysyk and Galloway 2014), *Culicoides crepuscularis,* and *Culicoides haematopotus* are known to transmit *Haemoproteus* (Santiago-Alarcon et al. 2012)*,* and *Culicoides obsoletus* feeds on birds, but its transmission potential is not known (Santiago-Alarcon et al. 2013).

Potential vectors also exist across the rest of the range of the golden-winged warbler. The *Plasmodium* vector *Culex pipiens* is found in Ontario (Hongoh et al. 2012), Wisconsin and Kentucky (Farajollahi et al. 2011). *Leucocytozoon* vectors (black flies of the genus *Simulium)* are reported in Kentucky, Ontario, and Wisconsin (Rothfels et al. 1978, Rivera and Currie 2009, Weinandt et al. 2012**)**. Finally, either one or both of the *Haemoproteus* vectors *Culicoides haematopotus* or *Culicoides crepuscularis* occur in Kentucky, Ontario, and Wisconsin (Bennett 1960, Borkent and Spinelli 2000, Swanson 2012).

2.2 Avian blood parasites

2.2.1 Natural history

The haemosporidian parasites *Haemoproteus, Plasmodium,* and *Leucocytozoon* follow a similar life cycle. All three genera require two host species, a dipteran vector and a vertebrate host, with sexual reproduction taking place within the vector species, and asexual reproduction within the vertebrate (bird in this case) (Valkiūnas 2005). For transmission to occur: host, vector, and parasite need to be present in the same area; conditions need to be suitable for the host vector to persist; and a minimum temperature must be met for the parasite to replicate within the vector (Reisen 2010). When vectors acquire a blood meal, they transmit motile cells capable of infection (sporozoites) into the avian host; these cells produce asexually reproducing stages known as schizonts, which produce large amounts of uninucleated parasite cells known as merozoites (Valkiūnas 2005). This development occurs in the host's blood cells, and typically continues for several generations. Merozoites continue asexual reproduction and develop sexual stages (such as gametocytes) within the host's blood cells (Valkiūnas 2005). When an insect feeds on this infected bird, these sexual stages are picked up and initiate infection in the gut of the vector (Valkiūnas 2005). The resulting zygote then becomes a mobile ookinete, and it penetrates into the membrane of the gut, where it develops into a thick-walled oocyst that is able to produce many more motile infectious cells (sporozoites). The sporozoites then move through the vectors body and into the salivary glands, where they can be transmitted to a new host during blood feeding (Valkiūnas 2005).

The three parasite genera considered can be differentiated based on pigment presence and the locations of asexual reproduction and gametocyte development (Martinsen et al.

2008). *Haemoproteus* and *Plasmodium* both contain pigment during some part of their life cycle (sexual and asexual stages, respectively), while *Leucocytozoon* parasites do not (Valkiūnas 2005). All three parasites reproduce asexually in cells that line the bird's blood vessels or lymphatic cells, but *Haemoproteus* and *Leucocytozoon* may also asexually reproduce in certain white blood cells (Valkiūnas 2005). *Leucocytozoon* asexual reproduction is common in the liver, because their infectious cells are capable of penetrating host liver cells (Valkiūnas 2005). Lastly, sexual stages develop in the red blood cells for both *Haemoproteus* and *Plasmodium*, but may also develop in the white blood cells for *Leucocytozoon* (Valkiūnas 2005).

The stages of parasitic infection in birds are similar in all three genera. Infection begins with parasites developing in the blood, which build until the parasite load reaches 'acute infection' that is immediately followed by the peak concentration of parasites in the blood (Valkiūnas 2005). If the bird survives this stage, the infection becomes chronic (ie. present in low levels), and typically persists in the bird's body for years, with the ability to resurface in higher concentrations (Valkiūnas 2005). Recent evidence shows that these resurfacing times are associated with stress (Lachish et al. 2011), breeding, likely due to increased testosterone or stress of tending young (Asghar et al. 2011), and even vector presence (Cornet et al. 2014) (see section 6).

2.2.2 Phylogenetic Considerations

Morphological characteristics at different developmental stages are commonly used to differentiate genera under a microscope, but delimiting phylogenetically accurate genera and species relationships requires a molecular approach. Parasite divergence may arise when parasites switch vertebrate hosts (Bensch et al. 2000, Ricklefs and Fallon 2002), or (more

commonly) switch dipteran vectors (Martinsen et al. 2008, See 2.1). Recent phylogenetic

analysis indicates that *Haemoproteus* may be paraphyletic and more logically divided into

two separate monophyletic groups: *Haemoproteus* (transmitted by Hippoboscid flies) and

Parahaemoproteus (transmitted by biting midges) (Martinsen et al. 2008, Bennett et al. 1965).

Differences in *Haemoproteus* and *Parahaemoproteus* may be masked by their superficially

similar morphologies (Valkiūnas 2005). Differentiating morphospecies is very time-

consuming, and so it has become increasingly common to determine parasite haplotypes using

molecular techniques and phylogenetic analysis (see Gibb et al. 2005, Kimura et al. 2006).

Efforts are being made to link these phylogenetic species to their morphospecies through

projects such as MalAvi (Bensch et al. 2009). Bensch et al. (2004) determined a benchmark

for species divergence in avian blood parasites: parasites with more than 0.5% divergences in

their cytochrome *b* had different sequences at another locus (DHFR-T), indicating

reproductive isolation, whereas parasites with a 0.2% divergence of cytochrome *b* had

identical DHFR-T sequences (Bensch et al. 2004). Determining parasite species and genera is

important, because they may have different effects on their host (Asghar et al. 2011), and may

react differently to geographic and landscape structure due to their different vector

associations.

2.2.3 Distributions

 Blood parasites are common infections of avian organisms, and they are found in most parts

of the world (LaPointe 2012). *Haemoproteus* is generally considered the most common genus

of avian blood parasite in birds in North America, but some research indicates that

Leucocytozoon parasites may be most prevalent in the wood-warbler family (Greiner et al.

1975). One multi-species migration survey found a single golden-winged warbler to be

infected with *Haemoproteus,* while other warblers in their study were commonly infected with *Plasmodium* (Garvin et al. 2006). Many parasites are not well described to species level, and many studies identify and refer to smaller taxonomic units within a parasite genus as haplotypes or monophyletic clusters (see Gibb et al. 2005, Kimura et al. 2006). Several known species are widely distributed globally, including: *Plasmodium relictum, Haemoproteus passeris, P. circumflexum, P. vaughani,* and *Leucocytozoon fringillinarum* (Valkiūnas 2005, Kimura et al. 2006). However, each of these common species contains many distinct haplotypes, some of which show geographic segregation; for example, the lineage of *Plasmodium relictum* that has recently invaded Hawaii is most commonly found in other remote Oceanic islands, and almost entirely absent from the new world (Beadell et al. 2006).

The distribution of haplotypes across a species' range is important because virulence varies among parasite haplotypes within the same genus (Asghar et al. 2011), and this may make some populations more at risk than others. This is especially interesting for golden-winged warblers, as populations across Canada appear to have different fitness levels; for example, the Manitoba population appears to have lower survival and reproductive success than Eastern populations (Moulton, unpublished data, Vallender et al. 2007, Bulluck et al. 2013). Some suggest that the distribution of parasite haplotypes on the breeding ground may be a useful genetic marker that could be used to link breeding populations to wintering locations with matching parasite haplotypes (Webster et al. 2002). This idea is limited in practical application, however, as research on the black-throated blue warbler (*Setophaga caerulescens*) and common yellowthroat (*Geothlypis trichas*) indicates that parasites are often well distributed throughout the breeding range, probably due to extensive transmission on the wintering grounds (Fallon et al. 2006, Pagenkopp et al. 2008). Researchers often find several

well-distributed parasite haplotypes, with a few uncommon haplotypes that appear restricted to specific avian populations (Gibb et al 2005, Kimura et al. 2006). Furthermore, variance in prevalence or parasitemia across a host's geographic range is common (Gibb et al. 2005, section 2.6). According to known distributions of North American Haematozoa, any or all of the genera *Haemoproteus, Plasmodium,* or *Leucocytozoon* might be present across the range of the golden-winged warbler (Greiner 1975).

2.3. Golden- winged warblers

2.3.1 Ecology and natural history

Golden-winged warblers (*Vermivora chrysoptera*) are neotropical migrant songbirds in the wood-warbler family *Parulidae.* Their distribution is disjunct: one population follows the Appalachians in the north-eastern and north-central United States, and another spans Minnesota, Wisconsin, southern Quebec, Ontario, and Manitoba (Confer et al. 2011). Golden-winged warbler populations likely expanded into Ontario prior to the 1970s, and more recently into Manitoba, while contracting in the Midwestern United States and Appalachians (Buehler et al. 2007, COSEWIC 2006). They are a small songbird of approximately 13cm in length and 9g in weight, and are characterized by yellow wing bars, a bright yellow cap, and a black (dark gray in females) and white facial mask with a similar pattern to a chickadee (Confer et al. 2011). Golden-winged warblers are insectivorous, most commonly eating Tortricid (and other) moths and their larvae, as well as other insects and spiders (Confer et al. 2011). They typically forage by pulling insects out of leaf-rolls, and rarely catch insects in the air (Chandler and King 2011, Confer et al. 2011).

Their breeding habitat consists of patches of dense shrub patches with some tall trees, backing onto (or surrounded by) forest (Confer and Knapp 1981, Confer et al. 2011). They

require some degree of disturbance to maintain this shrub layer in their all-purpose breeding territory, and are often found at abandoned clear cuts and surface mine sites (Klaus and Buehler 2001, Bulluck and Buehler 2006, Confer et al. 2011). In these habitats males will select territories 0.4 to 6 hectares in size, which can be directly adjacent or overlap in space (Confer et al. 2011). Golden-winged warblers are socially monogamous with high rates of extra-pair copulation and extra-pair offspring; over 50% of golden-winged warbler nests in Ontario contained extra pair offspring (Vallender et al. 2007). They tend to have very low natal phylopatry and high nesting site fidelity (Confer et al. 2011), and this has been confirmed in Manitoba (L. Moulton, personal communication). They overwinter in Central and South America, including Nicaragua, Costa Rica, Panama, Colombia, and Venezuela (Confer et al. 2011). On the wintering grounds they are a microhabitat specialist, choosing habitat in large canopy gaps along rivers, steep slopes, or advanced second growth forest (Chandler and King 2011). Wintering habitat suitability appears to also be driven by precipitation (Chandler and King 2011). The golden-winged warblers' habitat specificity on the breeding and wintering grounds is the cause of one of the many conservation concerns for this species (Confer and Knapp 1981, Chandler and King 2011).

2.3.2 Conservation concerns

The Golden-winged Warbler has been designated as threatened under the Canadian Species at Risk Act (COSEWIC 2006, Species At Risk Act 2002, 2007). The population is declining throughout most of its range (Confer et al. 2011, Bulluck et al. 2013), but the population in Manitoba is thought to be large and possibly increasing (L. Moulton, unpublished data).

One threat to the golden-winged warbler appears to be hybridization with blue-winged warblers (*Vermivora cyanoptera*); historically, golden-winged warblers have been extirpated within 50 years of secondary contact with blue-winged warblers (Confer and Knapp 1981, Buehler et al. 2007, Gill 1980). The exact mechanism behind replacement of golden-winged warblers with blue-winged warblers is not yet elucidated, but may be related to the relatively general habitat requirements of the blue-winged warbler compared to the more specialized golden-winged warbler (Confer and Knapp 1981), and that hybrids individuals appear to face no direct reproductive fitness consequences (Vallender et al. 2007). Controlling blue-winged warblers or hybridization between the species is unfeasible because of the species' use of similar habitat and overlapping breeding ranges. Further study of habitat partitioning between the two species may reveal a method of creating or protecting habitat that is more suitable for golden-winged warblers than blue winged warblers, which may reduce hybridization rates (Environment and Climate Change Canada 2016). It has been hypothesized that hybridization began around the early 1900s, when many of the agricultural developments that had cleared late successional forest separating these two closely-related early successional species were abandoned; this created large quantities of early successional habitat that was suitable and accessible to both species (Gill 1980). However, recent research indicates that hybridization between the species predates European colonization; though it remains a possibility that human disturbance has accelerated the rate of hybridization (Toews et al. 2016). Additionally, though the two species are morphologically distinct, and there is a 3% divergence in the NDII gene within the mitochondrial genome (Gill 1997), their nuclear DNA differs in only a handful of loci, most of which appear to correspond to genes that code for plumage characteristics (Toews et al. 2016). Therefore, the threat of hybridization may

soon be considered less important than other factors, such as habitat loss, to the survival of the species complex.

Loss of habitat on the breeding grounds is a major threat to populations of the golden-winged warbler. Habitat loss primarily occurs as humans suppress disturbance and allow early successional habitat to mature into forest, and as humans directly remove suitable habitat through development (Hunter et al. 2001, Roth et al. 2012). This loss of early successional habitat is a known problem in North America that has been implicated in several extinctions and extirpations, including Bachman's warbler (*Vermivora bachmanii*), a close relative of blue-winged warbler and golden-winged warbler, and the only other species within the *Vermivora* genus (Hunter et al. 2001). Further, though poorly understood, their wintering habitat may be at risk as anthropogenic disturbances such as agriculture and human settlements cause a decline in montane oak forests in Central and South America (Buehler et al. 2007), and widespread degradation and loss of rainforests occurs worldwide (Asner et al. 2009). This habitat loss may be causing low over-winter survival (Chandler and King 2011).

Brown-headed cowbird (*Molothrus ater*) parasitism also affects golden-winged warbler breeding populations; brood parasitism has been documented in up to 30% of nests, and reduces fledgling rates by up to 17%, although there is great variability n the degree of parasitism across the golden-winged warbler's range (Confer et al. 2003, Confer et al. 2011). Golden-winged warblers have coexisted with cowbirds through much of their evolutionary history, but their ability to coexist may be hampered if agricultural cover and fragmentation increase the population of brown-headed cowbirds, which are edge specialists (Coker and Capen 1995, Confer et al. 2003, Environment and Climate Change Canada 2016). Climate change also poses a significant potential threat to golden-winged warbler; their northward

expansion is likely due to warming, but they will not find suitable habitat further north into the Boreal forest as further warming occurs because they will generally not use the coniferous forest habitat (Buehler et al. 2007). Finally, although the prevalence of blood-parasite infections in golden-winged warblers appears to be high in Manitoba, little is known about the risk that these parasites pose to the species (Vallender et al. 2012).

2.4. Effects of blood parasitism on songbirds

The direct physiological effects of blood parasitism on songbirds have been measured using experimental studies, largely on hosts that are infected with novel parasites. In the Hawaiian Islands, malaria causes high mortality and has been a driving force in the extinction, population decline, and range restriction of a variety of Hawaiian bird species (LaPointe et al. 2012). The effect is so extreme that the distribution of many Hawaiian honeycreepers is determined by their susceptibility to malaria relative to other species (Atkinson et al. 2000, LaPointe 2012). Amakihis that were experimentally infected with *Plasmodium* had an overall mortality of 65%, and the cause of death was anemia associated with common gross lesions of the liver and spleen, and watery and pale heart blood (Atkinson et al. 2000). These clinical signs were associated with concentration of parasites in the blood (parasitemia), and infected birds also reduced their food consumption and had lower overall body weight (Atkinson et al. 2000). Hosts that were infected with non-novel parasites are less likely to experience mortality associated with a malarial parasite, but are still likely to face fitness costs (LaPointe et al. 2012). One study found that medicating *Haemoproteus* infected blue tits (*Cyanistes caeruleus*) (a species that has evolved with blood parasites) lead to increased survival of females (Martinez-de La Puente et al. 2010). Further studies on non-novel malarial infections

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have also found parasitemia to be related to reduced body weight and fat scores (Buchanan et al. 1999, Garvin et al. 2006) as well as increased immune response (elevated ratio of white to red blood cells) (Buchanan et al. 1999). Additionally, parasitemia can cause birds to reduce their flight distance from impending threats, which may lead to mortality through increased probability of predation (Møller 2008).

Blood parasites also have been shown to have a marked impact on important aspects of fitness such as mate selection, reproductive success, and immune response (LaPointe et al. 2012). Medication experiments have revealed an increase in reproductive success for infected birds that are given antibiotics to combat malaria, through larger clutch sizes and higher hatching and fledging success (Merino et al. 2000, Marzal et al. 2005, Isaksson et al. 2013). Males with parasitic infections also tend to invest less in parental care, and females with parasites can have smaller broods (Buchanan et al. 1999). It may benefit female birds to select a male that is not parasitized; the Hamilton-Zuk hypothesis states that females may evolve to choose males with showy phenotypic traits that signify their parasite resistance, so that her offspring will inherit that resistance and go on to successfully reproduce and therefore propagate the mother's genes (Hamilton and Zuk 1982). Because parasite resistant males do not have to invest energy into combatting parasites or fighting infection, they can invest more in showy traits such as large body size or bright plumage pigmentation (Hamilton and Zuk 1982). An example of a showy trait is plumage brightness, which is considered an honest signal because parasitized birds cannot invest in it (Hamilton and Zuk 1982, Figuerola 1999 et al. 1999). Parasitemia can also reduce pairing success by causing reduced song length and flight display (Buchanan et al. 1999), reduced territorial song response to playbacks (Gilman

et al. 2007), late arrival on the breeding grounds (DeGroote and Rodewald 2010), and (or) reduced body size (Freeman-Gallant et al. 2001) (Figuerola et al. 1999*).*

However, not all studies have found pronounced effects of parasites. Studies on great reed warblers (*Acrocephalus arundinaceus*) have found that the probability of surviving to the next year, recruitment, and song repertoire, were not affected by parasitemia (Asghar et al. 2011, Bensch et al. 2007), and a multi species study on parasitemia and body weight and condition indicated that some birds face no obvious effects of parasites (Garvin et al. 2006). Parasitemia may also involve tradeoffs between reproductive success and energetic condition, therefore in testing for only one or the other researchers may miss the effect (DeGroote and Rodewald 2010). Importantly, when studying the effects of parasitism on mist-netted birds, we must assume that infected birds are available for capture. This assumption may not be met if birds with the highest parasitemia are too weak to fly into mist-nets, respond to a conspecific territorial song playback, or die before they are captured (Asghar et al. 2011). To improve detection of effects of infection, many different fitness characteristics could be studied at the same time, such as survival, reproductive success, body condition, and plumage brightness (DeGroote and Rodewald 2010). Experimental infection in a laboratory setting can also be used to examine the effects of parasites while keeping other factors constant (eg. Atkinson 2000, Palinauskas 2011, Martinez-de la Puente et al. 2010).

2.5 Factors affecting pathogenicity and distribution of parasites and their vectors

2.5.1 Biological considerations

Virulence is strongly affected by the biological characteristics of blood parasite species. Virulence is thought to vary by genus, with *Plasmodium* and *Leucocytozoon* being highly

virulent, and *Haemoproteus* much less so (Santiago-Alarcon et al. 2012). However, recent

work indicates that infection with *Haemoproteus* parasites strongly affect survival of their

hosts (Martinez-de la Puente et al. 2010). These trends are also often overshadowed by the

differences within parasite haplotypes (Santiago-Alarcon et al. 2012). For example: Asghar et

al. (2011) found that the haplotypes of blood parasites in their study behaved very differently;

one *Plasmodium* haplotype was widespread with high occurrence but little effect on birds,

while the *Haemoproteus* haplotype was less common but had high parasitemia levels in the

blood and large negative effects on the study species. Interestingly, several studies have found

co-infection of parasites to be more common than expected by chance, indicating that

infection by one parasite may lead an animal to be more susceptible to infection by another

(Cottontail et al. 2009, Asghar et al. 2011). When many parasite haplotypes occur together,

selection can favour the most virulent parasite, which can ultimately be detrimental to the host

species (Bensch et al. 2004). Parasites also tend to be most virulent after their initial switch to

a host species, with virulence decreasing after several generations of exposure (Bensch et al

2000, LaPointe et al. 2012). Infection is often most severe in the early stages (when it is

'acute'), tapering off as it reaches a chronic level of parasitemia in the blood, in which case

the infection may recrudesce during times of stress (Lachish et al. 2011).

Differences among host species have an important effect on parasite prevalence, because

different bird species may harbour different parasite concentrations or haplotypes of parasites

in their blood (Bonneaud et al. 2009, Ricklefs and Fallon 2002). These differences may be

caused by behavioural characteristics that vary among species, such as diet, foraging height,

habitat specialization and distributional ranges (Tella et al. 1999, Laurance et al. 2013). Host

density can increase parasite prevalence, and this density can be associated with bird

behaviour (Tella et al. 1999, Tella 2002; Isaksson et al. 2013), or landscape effects (see section 6). Density effects may be attributed to reduced immune responses from birds that are in intense competition (Isaksson et al. 2013), or because areas that have high bird density facilitate the transfer of parasites (Fourcade et al. 2014).

Within a species, populations and individuals have characteristics that may be differentially affected by parasites. Genetic diversity is commonly believed to improve organisms' fitness or ability to adapt to challenges such as disease, and varies among individuals and populations (Haig et al. 2011). Studies of host genetic diversity and parasite prevalence yield mixed results; some find genetic diversity to correlate with reduced prevalence (Bonneaud et al. 2006, Ferrer et al. 2014), and some find no effect (Vallender et al. 2012, Fourcade et al. 2014). Diversity in the major histocampatability complex (MHC) seems to be specifically important to a bird's response to parasites (Atkinson et al. 2000, Bonneaud et al. 2006, Westerdahl et al. 2005). Many studies have examined the infection susceptibility of individuals within hybrid species complexes, and the results are extremely variable. In one case, hybrid offspring between two pocket gopher species (*Geomys spp*) were released from the host-specific parasites of their parents (Heaney and Timm 1985). Conversely, hybrid offspring between two mouse species (*Mus musculus musculus* and *M. m. domesticus*) were more susceptible to gut parasites, possibly because hybridization could disrupt adaptive genetic material (Moulia 1991). It is also common for younger birds to have a lower prevalence of infection, but higher parasitemia than older birds, likely because young birds have been more recently infected and are more likely to be at an acute level of infection, and tend to have less competent immune systems; conversely, older birds have had more time to be exposed to infection throughout their lives, levels of parasitemia are lower because the

infection is in the chronic stage (Sol et al. 2000, Valkiūnas 2005, Wood et al. 2007, Ashgat et al. 2011, Vallender et al. 2012). Experimentally infected male Amakihis found were less likely to survive than females, which might be due to testosterone causing higher stress (Atkinson et al. 2000); this is consistent with other studies on sex and blood parasites (Buchannan et al. 1999, Wood et al. 2007), but some research indicates that females are more likely to be infected than males during breeding (Asghar et al. 2011). Host dispersal can also influence parasite prevalence; a dispersing host may either be released from its home parasites (Lewicki et al. 2015) or become exposed to novel parasites (Mølller and Szép 2011). Additionally, the stress and resource-depletion that results from long distance migration may increase a host's susceptibility to novel parasites (Clark et al. 2016). Because these host characteristics vary among individuals within a population, these factors may add considerable variation to the prevalence of parasites within a system.

2.5.2 Geographic considerations

The effects of local geography can be extremely important; one study found parasite prevalence to change from 10 to 60 percent infection prevalence over a few kilometres (Wood et al. 2007). This is perhaps not surprising, given that vector presence and population size is strongly tied to environmental variables, and vector density can be a stronger predictor of parasite prevalence than a host's parasite resistance (Sol et al. 2000). Minimum high and low temperatures appear to be some of the most important factors affecting parasite prevalence (Gonzalez-Quevedo et al. 2014), and this is likely because temperature affects vector density and the parasite incubation period within the vector (LaPointe et al. 2010, Wang et al. 2011). For example, in mosquitoes, temperature affects survival and the time delay between a female acquiring a blood meal and initiating egg development (LaPointe et al. 2012, Reisen 2010).

Moreover, incubation period of the pathogen within the vector is shortened at higher

temperatures, and parasites typically can only replicate (and therefore be transmissible) above

a minimum temperature threshold (LaPointe et al. 2010, Reisen 2010). This threshold is most

important in regions that hover around the minimum transmissible temperature for a given

parasite (which will vary by parasite lineage), because abnormally warm periods could lead to

transmission (Reisen 2010). Rainfall and indices of topographic moisture also often affect

parasite load, likely because they can increase the larval habitat available for vector species

(Wood et al. 2007, Chen et al. 2013). For example, studies on *Culex* mosquitoes show that

mosquito populations increase with both temperature and precipitation (Wang et al. 2011).

Similarly, birds breeding close to water edges often have higher prevalence of mosquito

transmitted *Plasmodium* infections (Wood et al. 2007), and calculations of topographic

wetness index (TWI) have also been positively correlated with mosquito prevalence (Ganser

and Wisely 2013).

Biological and geographic characteristics may also interact to affect the prevalence of

parasites. Because precipitation generally increases vector numbers, one might expect vector

availability and parasite transmission to decrease with drought, but the opposite can occur.

For example, though some vectors may be limited by biotic factors such as predation and

competition, drought may increase the abundance of large, potentially long-lived vectors by

reducing the population of competitors or predators (Chase and Knight 2003). Additionally,

drought can concentrate vector and host species around the remaining water sources, which

then enhances infection transmission (Day 2001). Lastly, low habitat quality for a host species

can also lead to higher parasite prevalence, because low resource availability can lead a host

to allocate resources away from immunity and into more pressing daily functions (Norris et al.

2000). These interactions add an extra layer of complexity that can make it difficult to estimate a general or typical parasite response to geographic change.

The effects of temperature and precipitation and their interactions on host and vector biology are important because it is likely that anthropogenic disturbance will affect these relationships. Climatic changes have already affected the mobility of parasites by facilitating movement of their vectors (Freed and Cann 2013). For example, warming at lower elevations in Hawaii caused vectors to disperse to higher elevations (Freed and Cann 2013). On a larger scale, Hongoh et al. (2012) found that suitable conditions for *Culex pipiens* are rapidly moving north, and that areas of Manitoba that have suitable climatic conditions may increase drastically in the coming years. This process is expected to increase greatly in the future and may even affect the phenology of vector species (Garamszegi 2011, Freed and Cann 2013, Atkinson et al. 2014).

2.6 Anthropogenic disturbance and blood parasites

Anthropogenic disturbance can also influence blood parasite prevalence, and is within the control of land-managers, but the results have been mixed and the mechanisms are not well understood. One hypothesis is that landscape changes alter host density, which in turn alters parasite prevalence: several studies have found increased avian density in undisturbed forests to correspond to an increase in parasitism (Isaksson 2013, Mendenhall et al. 2013, Fourcade et al. 2014). Another study on bird populations in South Africa found that blood parasite prevalence increased with bird density, but that bird density was also higher in anthropogenic than in undisturbed areas (Okanga et al. 2013). This result is similar to other research on bats in the Barro Colorado Nature Monument, and mammals in tropical Brazil

(Vaz et al. 2007, Cottontail et al. 2009). However, the density hypothesis is not fully supported, because another study on tropical birds indicated that avian density was higher in fragments, but parasitemia was still lower; the researchers suggested that in their study area, the population in disturbed areas might be less stable and therefore provide a less reliable host source to vectors (Laurance et al. 2013). Moreover, another study indicated that *Plasmodium* prevalence was higher in non-fragmented regions even when controlling for host density (Bonneaud et al. 2009). An alternate hypothesis is that anthropogenic disturbance affects the occurrence of blood parasites by altering vector habitat (Bonneaud et al. 2009, Chasar et al. 2009, Laurance et al. 2013). In a study in Thailand, deforestation reduced the diversity of *Anopheline* mosquitoes (Overgaard et al. 2003), but a study in Hawaii found an increased prevalence of *Culex quinquefasciatus* in fragmented zones (Reiter and LaPointe 2007). Additionally, preference for tree density can vary with mosquito species (see O'Brien and Reiskind 2013). Anthropogenic disturbance might also have a varied effect on larval habitat; farmers may reduce vector habitat by draining it to develop, or foster habitat by introducing ponds, irrigation systems, or poultry that may act as a parasite reservoirs (Patz et al. 2008, LaPointe et al. 2012, Gonzales- Quevedo et al. 2014).

It is clear that the effect of anthropogenic disturbance on blood parasite prevalence is driven by biological and environmental factors that vary with the type of disturbance and the ecology of particular species of blood feeders. Given that these factors are related to climate and water variables, it is worthwhile to investigate how these factors respond in temperate areas, where little work on fragmentation and blood parasites has been performed.

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Chapter 3. THE ROLE OF HISTORICAL DISPERSAL AND LANDSCAPE CONVERSION IN THE BIOGEOGRAPHY OF BLOOD PARASITES AFFECTING THE GOLDEN-WINGED WARBLER

Abstract

Blood parasites vary in time and space, and it is important to develop an understanding of what influences the distributions of these parasites to predict how they may respond to future global change. Two factors that are important in shaping parasite distributions are land cover and dispersal of host species. The response of parasites to land conversion in temperate climates is poorly understood. Impacts of land conversion may interact with historical dispersal patterns, because when hosts disperse they may be exposed to novel parasites, or they may be released from the parasites of their origin. The golden-winged warbler is a species of concern in the USA and Canada that may be at risk from blood parasitism. I screened golden-winged warblers from across the United States and Canada for blood parasites, and investigated if land use patterns or host dispersal affected the prevalence or haplotypes of parasites. Parasite prevalence varied strongly with study area, and areas with high cover of agriculture had significantly higher prevalence of *Leucocytozoon* and *Haemoproteus* parasites. Lineages of *Haemoproteus* and *Leucocytozoon* were significantly genetically differentiated among study areas, and the pattern of parasite prevalence and lineages indicated an increase in parasite prevalence and accumulation of unique parasite lineages from the Southeast to the Northwest. This matches the dispersal pattern of golden-winged warblers, and suggests that golden-winged warblers are exposed to novel parasites as they disperse. The high prevalence and diversity of parasite lineages in the Northwest population (Manitoba) indicates that this population may face unique pressures that should be considered when planning conservation efforts for the species.

3.1 Introduction

Avian blood parasites are infectious organisms that affect the fitness of bird species around the world (LaPointe et al. 2012). Three common and widespread genera of blood parasites are *Plasmodium*, *Leucocytozoon* and *Haemoproteus* (Greiner et al. 1975, LaPointe et al. 2012). The potential impacts of these parasites on their avian hosts are diverse, and can range from lower pairing success due to reduced plumage pigmentation, smaller body size, or ability to produce mating calls (Figuerola et al. 1999, Freeman-Gallant et al. 2001); to reduced clutch sizes, hatching rates, and fledging rates (Marzal et al. 2005, Asghar et al. 2011); and to

immunodeficiency, increased predation, and direct mortality (Asghar et al. 2011, Lachish et al. 2011, LaPointe et al. 2012). The fitness consequences of blood parasites may thus pose a conservation concern for many species, including those that are also at risk from a variety of anthropogenic impacts or other cumulative threats.

Baseline data describing global blood parasite communities is becoming increasingly important as environmental changes continue. Major anthropogenic disturbances such as climate change and land conversion can alter parasite communities, and there is concern that these could expose species to novel parasites or lead to new emerging diseases in animals or humans (Garamszegi 2011, Freed and Cann 2013, Oakgrove et al. 2014). Climate change has likely already altered the mobility of parasites by altering the distribution of their vector species, and this process is expected to increase as climate change progresses (Garamszegi 2011, Freed and Cann 2013, Atkinson et al. 2014). *Culex pipiens*, a *Plasmodium* parasite vector, is expected to shift its distribution north as climate change renders northern habitats more suitable (Hongoh et al. 2012). The effects of land conversion on parasite communities are variable and less well understood than climate; they appear to be related to how the conversion affects larval habitat for vectors, resource availability for hosts, or host population density (Mendenhall et al. 2013, Fourcade et al. 2014, Hernández-Lara et al. 2017). It is thus imperative that baseline parasite infection data be collected, so that effects of climate change and land conversion on parasite prevalence can be measured over the long term.

To understand the current and future risk of parasites to a species or region, we need species and location-specific data. Region-specific research is important because parasite communities are extremely variable over geographic space (eg. Lauron et al. 2014). The MalAvi database reveals a paucity of molecular parasite research in Canada compared to the USA; the

USA has 600+ parasite DNA records, covering 60 host species, whereas Canada has only has 34

records covering four host species (Bensch et al. 2009). Because parasite data in Canada are

severely lacking when compared to the United States, our ability to predict how parasite

communities in Canada will change over time is limited. Additionally, as parasite communities

can be host-specific, their response to change may vary with their hosts' response to change

(Bensch et al. 2000, Olsson-Pons et al. 2015). Therefore, it is important to characterize range-

wide parasite communities across hosts, especially if the fitness of that host is of particular

conservation concern.

When parasite communities are characterized, we can apply biogeographic theory to

better understand parasites' responses to environmental changes. For example, researchers in

Alaska noticed that ambient temperature limited the distribution of *Plasmodium* prevalence in

their system and predicted that climate change may cause the locally transmitted *Plasmodium*

haplotype to move northward (Loiseau et al. 2012). Similarly, in Eurasian blackcaps (*Sylvia*

atricapilla), *Haemoproteus* and *Leucocytozoon* parasites responded negatively to an increase in

temperature, while *Plasmodium* responded positively; this indicates that parasite genera may be

differentially affected by climate change (Pérez-Rodríguez et al. 2014). Land cover and

migratory status also influenced parasite prevalence and diversity in Eurasian blackcaps,

suggesting that anthropogenic land changes and disturbance-mediated changes in bird dispersal

may also have important effects on parasite communities (Pérez-Rodríguez et al. 2014). If we

can explain why parasites are found where they are, we can begin to hypothesize how parasite

distributions or prevalence may shift with global climate and land cover change.

One of the most commonly cited determinants of blood parasite communities of birds is

variation in the presence of vectors due to habitat or climate (See Sol et al. 2000, Kimura et al.

2006, Pagenkopp et al. 2008, Chasar et al. 2009) and this pattern may help explain regional differences in parasite prevalence. This is likely because vector prevalence is strongly tied to environmental variables, and vector density can be a stronger predictor of parasite load than a host's parasite resistance (Sol et al. 2000). For example, vector density is strongly linked to temperature: warm temperatures improve survival and hasten egg development in female mosquitoes (up to a maximum), and the pattern is similar in black flies and *Culicoides* midges (Samuel et al. 2011, LaPointe et al. 2012, Bishop et al. 1996, Bernotiene and Bartkeviciene 2013). Additionally, parasites require a minimum temperature to replicate and become transmissible, and the rate of this development also increases with temperature (Reisen 2010). The relationship between temperature and parasite prevalence appears well resolved, at least on a local scale; warm temperatures seem to favour *Plasmodium* parasites, while *Haemoproteus* and *Leucocytozoon* appear to be more common at cooler temperatures and higher latitudes/altitudes, (Pérez-Rodríguez et al. 2013, Galen and Witt 2014, Oakgrove 2014). The temperature and latitude that this trend occurs at varies with study area, host species and parasite species, and likely results from different host availability with latitude and divergent thermal tolerances of parasites and their vectors (Valkiūnas 2005).

Climate is generally accepted as an important driver for parasite distribution, but it does not explain all of the variation in parasite prevalence, and land cover variables can also be important (Pérez-Rodríguez et al. 2013). For example, the association between *Leucocytozoon* and high altitudes may also be explained by the abundance of running water in these areas, which is required habitat for the *Leucocytozoon* vector (black flies) (Pérez-Rodríguez et al. 2013, Svoboda et al. 2015). Similarly, *Plasmodium* parasites may be more prevalent near shallow, still water, because it provides excellent mosquito larval habitat (Wood et al. 2007). *Haemoproteus* parasites

may be more prevalent in open areas because the common *Haemoproteus* vectors *Culicoides crepuscularis,* and *Culicoides haematopotus* are typically found in herbaceous areas with low tree cover and damp patches for larval development (Kardatzke and Rowley 1971, Gonzáles et al. 2013). Vector-parasite responses to land conversion are less well understood than climate, but appear to vary with the type of disturbance, location, and the species examined (Brearley et al. 2013, Reinoso-Pérez et al. 2016). However, most prior research has been conducted in tropical regions and further study is needed to determine whether general trends in temperate zones can be identified (see Laurance et al. 2013, Mendenhall et al. 2013, Okanga et al. 2013).

The biogeography of avian parasites may also be linked to the distribution and dispersal of their host species. Animal dispersal likely evolves because it allows individuals to escape competition, provides access to new resources when empty sites are reached, and reduces inbreeding depression (Johnson and Gaines 1990). Dispersal is very important to early successional species because their habitat is ephemeral, and locating new sites as the old ones progress past a useful state of succession is integral to the success of these species (Travis and Dytham 1999). This dispersal may also benefit the spread of the hosts' parasites; dispersing hosts can bring parasites from their home environment into a new location, if suitable vectors and transmission temperatures exist (Hoberg and Brooks 2008). The potential for host-parasite co-dispersal means that migratory birds may transmit parasites to isolated, non-migratory, resident populations (LaPointe et al. 2012, Clark et al. 2016). While transmission of many lineages of blood parasites appear to be limited to either the wintering or summer grounds, Haemosporidian parasites have repeatedly evolved full-year transmission mechanisms (Pérez-Tris and Bensch 2005, Hellgren et al. 2007). This allows them to be dispersed between temperate and tropical areas by migratory songbirds, and may facilitate parasite spread into new breeding locations

(Pérez-Tris and Bensch 2005). Parasites may even evolve to manipulate the behaviour of their hosts to facilitate their own dispersal, especially if the cost of this manipulation is low (Lion et al. 2006). Climate change is expected to increase host dispersal (Walther et al. 2002), and this may directly affect the distribution of parasites.

There are two conflicting hypothesis about how a dispersing bird is likely to respond to parasite communities in its new location: the Enemy Release Hypothesis, designed for invasive plants (Keane and Crawley 2002) but recently applied to animals as well (Lewicki et al. 2015) or the Novel Enemies Hypothesis (descried in Møller and Szép 2011 and named here). When a bird disperses into a new habitat it can benefit from being released from its home parasites (the Enemy Release hypothesis) (Lewicki et al. 2015). Based on the Enemy Release hypothesis, some hypothesize that migration evolved as a way to evade parasites (Clark et al. 2016); this may be particularly true in the Eastern Hemisphere, where parasites are commonly transmitted on the wintering grounds, and breeding in the North may provide a release from parasitism (Yohannes et al. 2008, Clark et al. 2016). The Enemy Release hypothesis may also contribute to the success of invasive or range-expanding species (Marzal et al. 2011, Lewicki et al. 2015, Ellis et al. 2016). Dispersers may also be resistant to new parasites that they encounter if these parasites are similar to those that were encountered on their natal grounds; if a novel lineage is similar enough to a previously acquired lineage, the bird's immune system may be well prepared to fend off the lineage before it establishes itself in the bird's body. Conversely, a dispersing host may experience increased susceptibility to novel parasites in a new location (the Novel Enemies hypothesis) (Møller and Szép 2011). The Novel Enemies hypothesis suggests that parasites are caught in an arms race with their hosts, and that host and parasite will evolve together in a specific geographic area (Møller and Szép 2011). In this scenario, host populations become

adapted to local parasites, and immigrants to an area are more susceptible than the local

individuals to the local parasites (Møller and Szép 2011). To avoid being exposed to novel

parasites, hosts may restrict their movement and increase site fidelity, and some researchers

suggest that this process maintains breeding and wintering site fidelity in migratory birds (Møller

and Szép 2011). For example, song sparrows (*Melospiza melodia)* were more likely to contract a

foreign parasite haplotype when they were inoculated with a parasite haplotype found ~500km

away than a local parasite haplotype from their home territory (Sarquis-Adamson et al. 2016).

Additionally, dispersal may simply expose birds to a wider suite of vectors and therefore

increase their probability of infection (Svoboda et al. 2015). Of course, birds will continue to

disperse if the benefit to accessing new resources outweighs any negative effects from increased

parasitism. Dispersal into a new territory may pay off two-fold for birds; they may benefit from

being released from their old parasites and be able to exploit new resources, but they also risk the

possibility of being exposed to a suite of novel, and potentially more virulent parasites.

A threatened species that may be at considerable risk from blood parasites is the golden-

winged warbler (*Vermivora chrysoptera*) (Species At Risk Act 2002, 2007). Little research has

been done on parasite infection in golden-winged warblers, but recent work suggests that the

occurrence of blood parasites increases from east to west across their Canadian range, which

ends in western Manitoba (Vallender et al. 2012). The Manitoba population is of great

conservation importance because, despite its large and potentially increasing population, the

population appears to have lower fitness than the populations in eastern Canada (Moulton

unpublished data, Bulluck et al. 2013). Additionally, an important threat to golden-winged

warblers appears to be hybridization with a closely related species, the blue-winged warbler

(*Vermivora cyanoptera*). Because the Manitoba population is allopatric to the blue-winged

warbler, it is the most genetically pure population of golden-winged warblers in the world, and its conservation can be considered to be of particular significance (Vallender et al. 2009). Classifying the parasite composition along the range of the golden-winged warblers will contribute much needed information about the landscape of parasitism in Canada, and help evaluate the risk that blood parasites pose to this threatened species (Species At Risk Act 2002, 2007).

Golden-winged warblers also provide an excellent opportunity to study host-parasite biogeography. Because parasite prevalence varies strongly across this species' range, it provides an opportunity to investigate how changes in physical geography across eastern North American relate to parasite prevalence. Additionally, golden-winged warblers have high natal dispersal (few young of year return to their natal grounds and many disperse across states and provinces (Vallender and Moulton, unpublished data)); their current and historical dispersal patterns are known; and they display strong migratory connectivity (breeding populations migrate and winter together (Kramer et al. 2017)), which means that parasites among different breeding populations should not become admixed on the wintering grounds. The combination of these factors allows us to test dispersal-related parasite prevalence hypotheses. Developing an understanding of what structures the golden-winged warblers parasite community is the first step to providing important information to managers about how the risk of parasitism may change over time.

I sought to investigate the prevalence and haplotypes of parasites across a significant portion of the golden-winged warbler's range, with the intention of investigating which populations may be at the greatest risk of parasitism, and of estimating what factors shape trends in parasite prevalence. I evaluated several hypotheses that might explain geographic segregation in parasite composition (where composition is the combination of parasite prevalence and

diversity): (1) the Land Cover Hypothesis – Where parasite composition is driven by land cover characteristics, which is shaped by vector availability or habitat quality, and (2) the Dispersal Hypothesis – Where parasite composition is driven by golden-winged warbler dispersal and exposure to new parasites, which may result from either (i) the presence of novel enemies or (ii) enemy release.

Under the land cover hypothesis, I predicted that areas with more vector habitat would have the highest prevalence of parasites associated with those vectors. At a landscape scale, I expected that regions that are highly anthropogenically disturbed would have higher parasite prevalence, because the dominant disturbance in the region is agriculture, and agricultural irrigation sometimes creates vector habitat (see Patz et al. 2008).

If golden-winged warbler dispersal results in Enemy Release, I expect the prevalence of parasites to be lowest in the areas most recently colonized by golden-winged warblers. The overall trend in golden-winged warbler dispersal from breeding grounds appears to be from the Southeast to the Northwest (Van Wilgenburg, unpublished data). Specifically, golden-winged warblers hatched in Kentucky appear to move to Wisconsin or Manitoba, golden-winged warblers hatched in Wisconsin appear to move to Manitoba, and golden-winged warblers hatched in Ontario appear to move to Manitoba (or much less likely, from Ontario to Wisconsin). Thus, under the Enemy Release hypothesis, golden-winged warblers in the Northwest should have the fewest parasites.

If golden-winged warbler dispersal results in an increase in parasite exposure to Novel Enemies, I predicted the prevalence and diversity of parasites to be highest in the areas most recently colonized by golden-winged warblers, i.e. the Northwest. If parasites co-disperse with golden-winged warblers during this natal migration, I would expect the parasite lineages in

Kentucky to be found in Wisconsin and Manitoba, the parasite lineages in Ontario to be found in Manitoba (and to a lesser extent Wisconsin), and the parasite lineages in Wisconsin to be found in Manitoba. Under this scenario Manitoba is the most likely to have unique parasites, as golden-winged warblers in Manitoba have not been found to natally disperse out of Manitoba.

3.2 Methods

My collaborators and I mist-netted and blood-sampled 257 golden-winged warblers from six study areas across their breeding range between 2009 and 2015 (Figure 3.1). Two of these study areas were in Manitoba, one in the West near Riding Mountain National park (49.764° N, 96.528° W) and one in the Southeast near the town of Richer (49.764° N, 96.528° W). Two study areas were in Southern Ontario, a western site near the town of Carden (44.683° N, 79.057° W), and an eastern site near Frontenac Arch Provincial Park (44.634° N, 96.332° W). Our two final study areas were in the USA, in Kentucky (36.810° N, 83.759° W) and Wisconsin (44.295382° N, 90.191257° W). We searched for golden-winged warblers in early successional aspen parkland or mixed forest ecosystems, and targeted the searches based on aerial surveys for available habitat and/or previous survey data (Artuso, personal communication).

Once a male golden-winged warbler was located, we identified the bird's territory based on where it was singing, cleared a 6m net lane within that territory, set up a 6m mist-net in the lane, and attracted the bird to the net with conspecific territorial playback. Upon capture, a ~20ul blood sample was obtained for each golden-winged warblers and stored in ethanol or Queen's lysis buffer, and the bird was released at the location of capture (Seutin et al. 1991). I assumed that the use of different blood storage mediums did not affect parasite detection. To my knowledge, no work has compared parasite detection ability between blood samples stored in lysis buffer and ethanol, but other studies have shown that using different types of buffers can

affect detection efficiency, so we must keep in mind that the different sample storage may have

introduced error into this study (eg. Freed and Cann 2006).
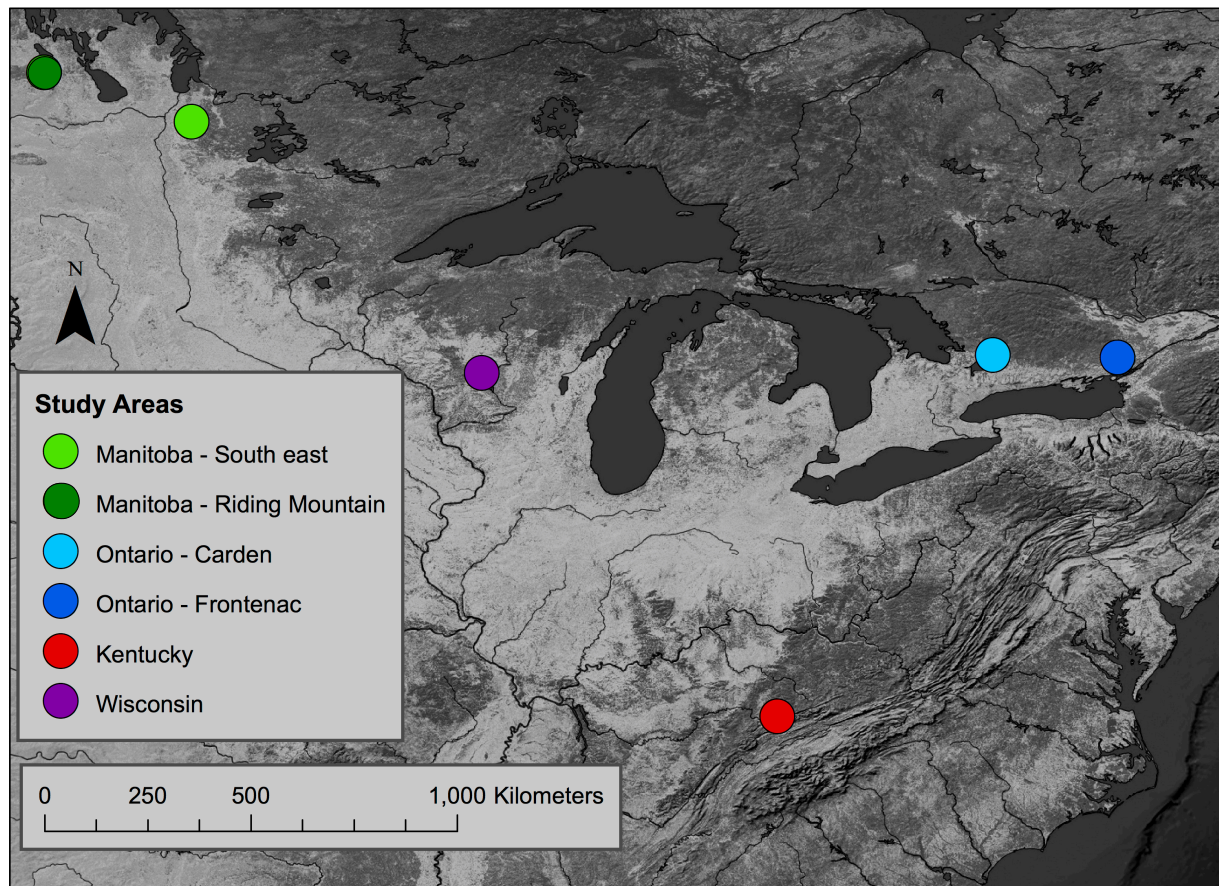
Figure 3.1. Map of study sites where golden-winged warblers were captured. Manitoba study sites are shown in green, where 100 birds were caught in the years 2009 and 2015. Ontario study sites are shown in blue and 71 birds were caught in 2015. Kentucky is shown in red where 38 birds were caught between 2005 and 2006, and Wisconsin is shown in purple where 48 birds were caught between 2009 and 2010. Created in ArcMap 10.2 with Natural Earth (Free vector and raster map data at naturalearthdata.com).

Knowledge of where parasites are transmitted is important both for monitoring parasite

communities' responses to change and understanding parasite biogeography. That said, I

attempted to determine which parasites were transmitted on the breeding grounds by sampling

resident black-capped chickadees (*Poecile atricapillus*) (Meixell et al. 2016). Resident species indicate breeding ground transmission because any parasites that they are infected with were acquired within the local dispersal distance of that species, and resident (non-migratory) species by definition do not leave the breeding grounds. I used the same method to capture black-capped chickadees as for golden-winged warblers. To identify locally transmitted parasites I captured a total of 14 black-capped chickadees in golden-winged warbler habitat in Manitoba. To determine blood parasite composition, my collaborators and I extracted DNA from all 232 golden-winged warbler and 14 black-capped chickadee blood samples using a homemade kit (Ivanova et al. 2006).

To screen for presence of blood parasites, I used two primer sets that amplified overlapping regions of cytochrome *b*. The first PCR protocol followed the methods of Vallender et al. (2012), which first looked at blood parasites in golden-winged warblers. Primers L15183 (Szymanski and Lovette 2005) and H15725 (Ricklefs and Fallon 2002) amplified a 550 bp region in cytochrome *b*, typically covering the two parasite genera *Plasmodium* and *Haemoproteus*. The second followed the nested PCR protocol outline in Hellgren et al. (2004), which detects parasite presence to a dilution of 1:10,000 parasite cell to blood cells. The first two primers (HaemNF and HaemNR2) initially amplified a 682-bp region of cytochrome *b*. I then performed a second and third PCR, each using the product from the first PCR. In the second PCR, nested primers (HaemF and HaemR2) isolated a smaller 480-bp region that is present and distinguishable in *Haemoproteus* and *Plasmodium* species. In the third PCR, nested primers HaemFL and HaemR3L isolated *Leucocytozoon* species. I tested for the presence of successfully amplified parasite DNA by running each PCR product through an ethidium bromide stained agarose TAE gel. I viewed these gels through UV light using a Kodak–Fisher Scientific Gel

Logic 100 Imaging System and scored birds as infected or not based on presence or absence of a PCR product band, respectively. All PCR assays were run with positive and negative controls, and assays that produced false negatives or false positives were run a subsequent time.

I sequenced positive blood parasite infections by purifying the PCR product and using a Big Dye sequencing method. Some samples were purified twice: in the first purification, I added .02 µl Exo, 0.2 µlof Sap (Affymetrix), and 3.78 µlof DNA water (BioShop) to the PCR product of each positively infected sample, and held this mixture at 37°C for 15 minutes then at 80°C for 15 minutes in a thermocycler (Eppendorf Mastercycler ep gradient S; Eppendorf Canada, Mississauga, Ontario). When I did not use the first purification step, I diluted the PCR product depending on the brightness of the band, with a minimum of 0x dilution (weak PCR bands) and a maximum of 5x dilution (strong PCR bands). Following purification 1, or dilution, I used each successfully amplified PCR product in two separate sequencing reactions. The first reaction combined 1ul of PCR product with 1.5ul ABI buffer, 0.5 µlof forward primer, and 0.4 µlof Big Dye (Applied Biosystems Canada, Burlington, Ontario). The second reaction was identical but used the reverse primer. I then purified and precipitated DNA from the sequencing reaction by adding 1ul sodium hydroxide and 1ul Ethylenediaminetetraacetic acid (EDTA) to each sample, washing and drying the samples with 70% and 90% ethanol, drying the precipitate in a Vacufuge^TM (Eppendorf Canada, Mississauga, Ontario), and rehydrating the DNA with 15ul of HI-DI^TM Formamide (Applied Biosystems Canada, Burlington, Ontario) before loading the samples onto the sequencer. I sequenced the DNA-HIDI mixture on an ABI 3130 XL Automated Sequencer (Applied Biosystems Canada, Burlington, Ontario). I obtained the sequence data and cleaned and aligned sequences using Geneious version 10 (http://www.geneious.com, Kearse et al. 2012). I discovered the most similar reported parasite haplotypes by running the sequences

through MalAvi BLAST (Bensch et al. 2009) or NCBI BLAST (Altschul et al. 1990), and used this information to assign each infection to the appropriate genus (*Haemoproteus, Plasmodium,* or *Leucocytozoon*).

I tested for significant differences in parasite prevalence among study areas using generalized linear models (GLMs) with binomial distributions. I ran models with each genus of parasite as the response variable and study site as the predictor variable, comparing differences among all regions. These analyses were done in 'R' 3.1.4 (R Core Team, 2015), using the glm (generalized linear model) function, and the brglm (bias reduced generalized linear model) function in the package brglm (Kosmidis 2013); brglm was used to test for significant differences when perfect separation occurred in the data (ie. when any study area had zero infections of one parasite genus). Because the two study areas in Manitoba had similar infection prevalence of all parasites (*Haemoproteus: p* = 0.78, *Leucocytozoon: p* = 0.90*, Plasmodium, p* = 0.51), data from the two study sites were pooled; this was not true for the two study sites in Ontario (*p* < 0.1), so prevalence for these study sites was analyzed separately.

To test for genetic variation in Haemosporidian parasite haplotypes among geographic areas, I used analysis of molecular variance (AMOVA), executed in Arlequin 3.5 (Excoffier and Lischer 2010). AMOVA is a non-parametric test that looks for differences among groups by comparing the frequency and genetic distance of haplotypes (Excoffier et al. 1992). I used Kimura-2 to calculate sequence distances (Kimura 1980), and Monte Carlo simulation with 16,000 permutations to test for significance (as suggested by Guo and Thompson 1992). Because Kentucky only contained one *Plasmodium* and zero *Leucocytozoon* infections it was only included in the *Haemoproteus* analysis. Ontario samples were also pooled because Carden and Frotenac had low sample sizes of *Leucocytozoon* and *Plasmodium*, respectively, and

successful sequences revealed that these regions contained identical parasite haplotypes. The genetic differentiation we present here is likely a minimum estimate because AMOVA tends to underestimate differentiation, especially with the relatively small sample sizes of infected birds found here (Fitzpatrick 2009, Bird et al. 2011). I also used Arlequin to test for significance of paired FST values by permuting haplotypes among populations (Excoffier and Lischer 2010). I compared the *p* value of pairwise FST values among the populations to determine which populations differed. Because small differences in Haemosporidian cytochrome *b* sequences have been associated with rapid speciation (Bensch et al. 2004), I considered a haplotype distinct if it was one base pair different from others (eg. Lewicki et al. 2015), and defined a separate lineage as any that differed by more than 2 base pairs (e.g. Fallon et al. 2006).

Land cover variables were obtained at a site-level ('landscape') scale. I created a 50km buffer around the centre-point of each study area (Figure 3.1), and determined the proportion of different land covers within the resulting circle, using ArcMap 10.2 (ESRI 2011). For the Manitoba study areas, only the 2015 samples were used to calculate this centre-point. Land cover sources differed depending on the broad study area; for Kentucky and Wisconsin we used the USGS Geological survey data (US Geological Survey 2011); for Manitoba I used the Manitoba Land Initiative land use data (Manitoba Conservation 2005); and for Ontario we combined two data sets from the Southern Ontario Land Resource Information System (Ontario Ministry of Natural Resources 2000; Ontario Ministry of Natural Resources and Forestry 2011). Because GPS data was missing for one sample from Wisconsin, this sample was not included in this analysis.

I used binomial generalized linear mixed models to test for an association between one landscape level independent variable and the occurrence of three parasite genera. I chose

agriculture as a proxy to represent extent of human disturbance because agriculture is a large and relatively intensive human disturbance in all regions. I created three models, each one with the occurrence of a different parasite genus as the response variable, the above landscape variables as the fixed effects, and study area as the random effect. Analyses were performed in 'R' 3.1.4 using the package lme4 ('R' 3.1.4, Bates et al. 2015).

3.3 Results

3.3.1 Prevalence of parasites

I detected 99 infected individuals out of 257 sampled golden-winged warblers (232 males, 24 females, and one individual of unrecorded sex). Five positive infections from Manitoba produced weak PCR bands that either corresponded to *Haemoproteus* or *Plasmodium* but could not be sequenced and were removed from further analyses involving *Haemoproteus* and/or *Plasmodium*. Prevalence of infection varied significantly among study areas (Figure 3.2) Manitoba had significantly more overall infections than any other region, and this trend was driven by the significantly higher prevalence of *Haemoproteus* in Manitoba compared with the other five study sites (Figure 3.2). Manitoba and the eastern Ontario study site, Frontenac, had similar prevalence of *Leucocytozoon* parasites, which was higher than all remaining study sites. The western Ontario study site, Carden, had significantly more *Plasmodium* infections than any other site. Kentucky, on the other hand, had significantly fewer infections overall and tended to have fewer infections of every parasite genus (though not always significantly so) (Figure 3.2). Manitoba also had significantly more co-infections (infections of more than one genus of parasite) than the other study sites, probably because Manitoba has two very common genera of

parasites, *Leucocytozoon* and *Haemoproteus,* and therefore the probability of them occurring in one bird is high (Hellgren et al. 2004).
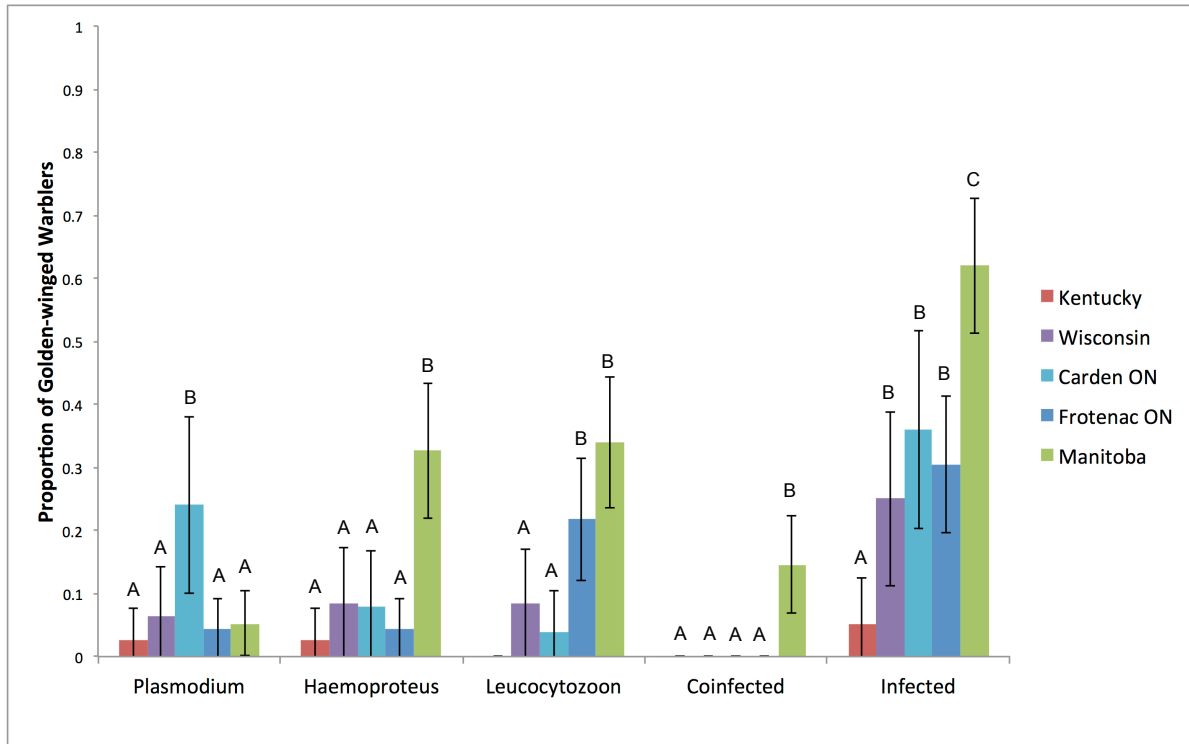
Figure 3.2. Prevalence of *Plasmodium, Haemoproteus, Leucocytozoon* infections in golden-winged warblers across five study sites across the golden-winged warbler's range. Different letters represent significant differences (*p* <0.1) within the parasite genus, not among parasite genera. Samples were collected as follows: Kentucky, *n* = 38 (2005, 2006), Manitoba, *n* = 100 (2009, 2015), Ontario, *n* = 71 (2015), WI = Wisconsin (2009, 2010), *n* = 48. Error bars denote 95% confidence intervals.

3.3.2 Parasite haplotypes and diversity

I found 20 unique Haemosporidian haplotypes, and these displayed some geographic structure. Of these, three belonged to *Haemoproteus,* 10 belonged to *Plasmodium,* and seven belonged to *Leucocytozoon.* AMOVAs revealed that *Plasmodium* was not genetically distinct among the study areas (*p* = 0.214), though FST analysis suggested that the Manitoba population of parasites is the most different from the Ontario population (Table 3.1). Manitoba also had one uncommon haplotype of parasite, BT7, which was not found in any other population (Figure

3.3). The AMOVA for *Leucocytozoon* displayed a different pattern: the populations were

significantly differentiated (*p* = 0.010), and FST testing suggested that the Manitoba, Ontario,

and Wisconsin populations were all significantly different (Table 3.2). Manitoba and Ontario

share one very common haplotype that is considerably less common in Wisconsin, and

Wisconsin and Manitoba share a complex of similar parasites, labeled Group A for clarity

(Figure 3.4). Further, all populations with *Leucocytozoon* infections had at least one rare

haplotype that was unique to that population (Figure 3.4). Interestingly, one of Manitoba's

unique *Leucocytozoon* haplotypes is nine base pairs different than any haplotype that has

previously been reported, which strongly suggests that it is belongs to an unsampled species

(Bensch et al. 2004). Lastly, the AMOVA for *Haemoproteus* also revealed that the populations

were significantly differentiated (*p* <0.001), which was driven by differences between Manitoba

and the other sites (Table 3.3). This is clear from the haplotype network, as Manitoba has one

unique and very common haplotype that is not found in the other study areas (Figure 3.5).

Table 3.1. Results from an FST test comparing frequency and genetic distance of haplotypes of *Plasmodium* among golden-winged warbler populations. Significance testing involved a Monte carlo estimation with 16,000 permutations. Samples were collected as follows: Manitoba, *n* = 100 (2009, 2015), Ontario, *n* = 71 (2015), WI = Wisconsin (2009, 2010), *n* =48.

	Manitoba	**Wisconsin**
Wisconsin	0 (*p* = 0.453)	-
Ontario	0.147 (*p* = 0.056*)	0 (*p* = 0.873)

Table 3.2. Results from an FST test comparing frequency and genetic distance of haplotypes of *Leucocytozoon* among Golden-winged Warbler populations. Significance testing involved a Monte carlo estimation with 16,000 permutations. Samples were collected as follows: Manitoba, *n* = 100 (2009, 2015), Ontario, *n* = 71 (2015), WI = Wisconsin (2009, 2010), *n* =48.

	Manitoba	**Wisconsin**
Wisconsin	0.18335 (*p* = 0.064*)	-
Ontario	0.092 (*p* = 0.093*)	0.791 (*p* = .005*)

Table 3.3. Results from an FST test comparing frequency and genetic distance of haplotypes of *Haemoproteus* among golden-winged warbler populations. Significance testing involved a Monte carlo estimation with 16,000 permutations. Samples were collected as follows: Kentucky, *n* = 38 (2005, 2006), Manitoba, *n* = 100 (2009, 2015), Ontario, *n* = 71 (2015), WI = Wisconsin (2009, 2010), *n* =48.

	Manitoba	Ontario	Wisconsin
Ontario	0.745($p = 0.010$*)	-	0 ($p = 1$)
Wisconsin	0.754($p = 0.002$*)	0 ($p = 1$)	0 ($p = 1$)
Kentucky	0.732 ($p = 0.030$*)	0 ($p = 1$)	0 ($p = 1$)
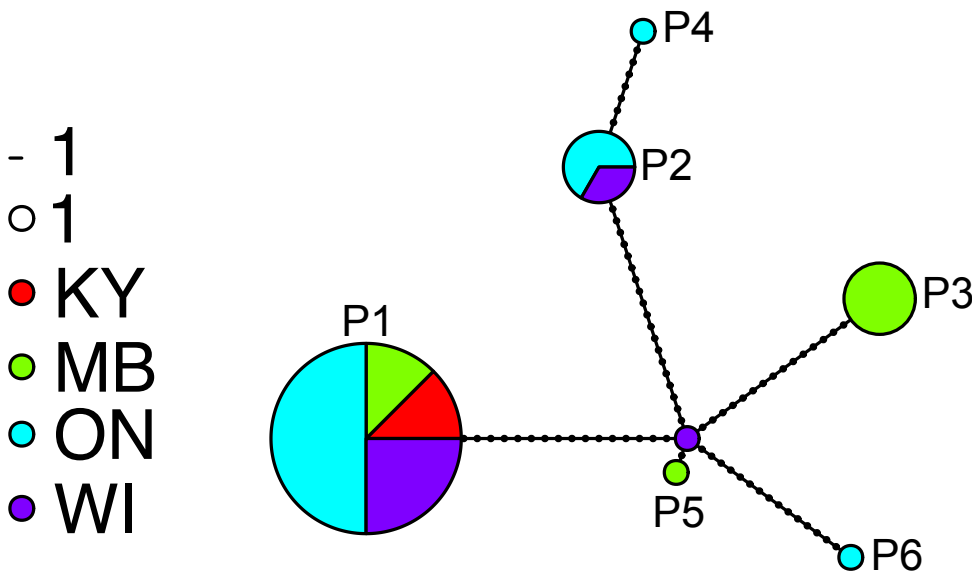
Figure 3.3. Haplotype network displaying frequencies and genetic distance among *Plasmodium* parasite haplotypes in different golden-winged warbler populations. KY = Kentucky, *n* = 38 (2005, 2006), MB = Manitoba, *n* = 100 (2009, 2015), ON = Ontario, *n* = 71 (2015), WI = Wisconsin, *n* = 48 (2009, 2010). Each circle is a unique haplotype (differs from other haplotypes by at least one base pair), the size of the circle corresponds to the relatively frequency of the haplotype, and the colour of the shape shows which study site it is found in. Labels denote unique lineages (haplotypes that differ from a central haplotype by more than 2 base pairs).
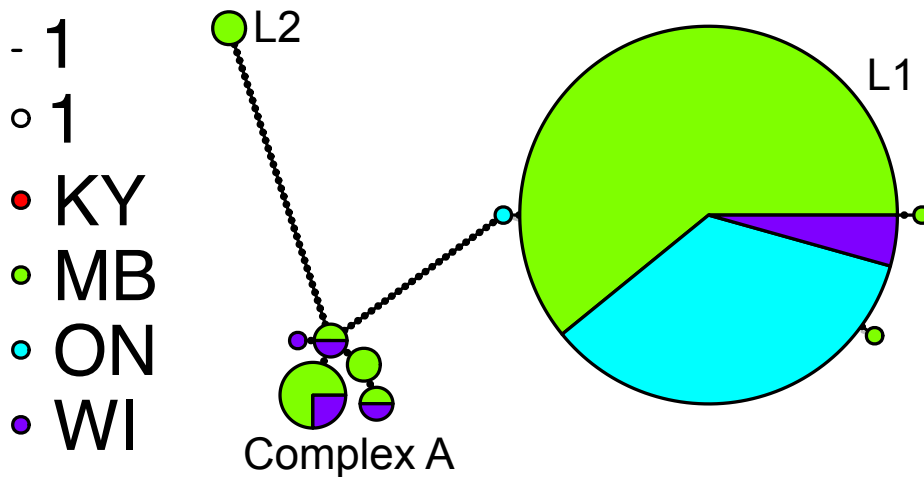
Figure 3.4. Haplotype network displaying frequencies and genetic distance among *Leucocytozoon* **parasite lineages in different golden-winged warbler populations. KY =** **Kentucky,** *n* **= 38 (2005, 2006), MB = Manitoba,** *n* **= 100 (2009, 2015), ON = Ontario,** *n* **= 71** **(2015), WI = Wisconsin,** *n* **= 48 (2009, 2010). Each circle is a unique haplotype (differs from** **other haplotypes by at least one base pair), the size of the circle corresponds to the** **relatively frequency of the haplotype, and the colour of the shape shows which study site it** **is found in. Labels denote unique lineages (haplotypes that differ from a central haplotype** **by more than 2 base pairs).**
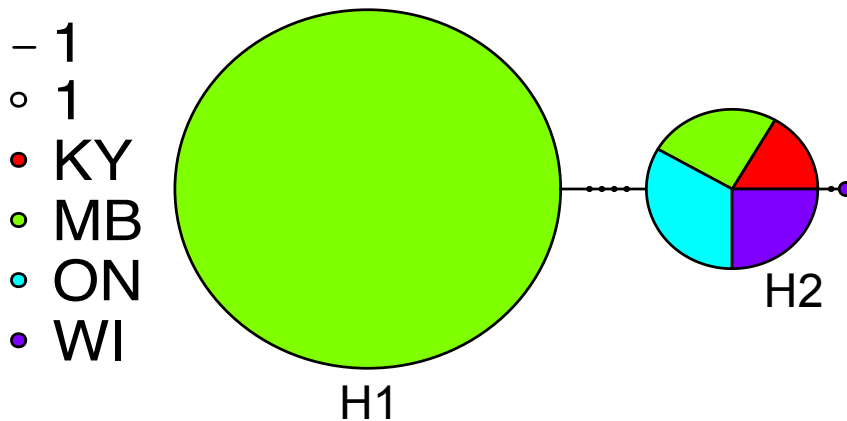
Figure 3.5. Haplotype network displaying frequencies and genetic distance among *Haemoproteus* **parasite lineages in different golden-winged warbler populations. KY =** **Kentucky,** *n* **= 38 (2005, 2006), MB = Manitoba,** *n* **= 100 (2009, 2015), ON = Ontario,** *n* **= 71** **(2015), WI = Wisconsin,** *n* **= 48 (2009, 2010). Each circle is a unique haplotype (differs from** **other haplotypes by at least one base pair), the size of the circle corresponds to the** **relatively frequency of the haplotype, and the colour of the shape shows which study site it** **is found in. Labels denote unique lineages (haplotypes that differ from a central haplotype** **by more than 2 base pairs).**

3.3.3 Evidence for local transmission

Of the 14 black-capped chickadees screened, eight were infected with *Leucocytozoon*, two of those were co-infected with *Plasmodium*, and one was only infected with *Plasmodium*. The *Plasmodium* haplotype that was present in this resident species was BT7 and the *Leucocytozoon* haplotypes all exactly matched one haplotype in the parasite lineage I named *Leucocytozoon* complex A (Table 3.4). No black-capped chickadees were infected with *Haemoproteus* parasites.

Table 3.4. Parasite lineages** present in golden-winged warblers in Kentucky (2005, 2006), Manitoba (2009, 2010, 2015), Ontario (2015), and Wisconsin (2009, 2010).

Genus	Lineage**	Study Sites*	Mal Avi Name	Earliest Mal Avi Reference
Haemoproteus	H1	MB	Pasil01	Walther et al. 2016
	H2	MB, ON, WI, KY	N / A	N / A
Leucocytozoon	L1	MB, ON, WI	CNEORN	Oakgrove et al. 2014
	Complex A	MB, WI	EMPALN02	Ricklefs and Fallon 2002
	L2	MB	N / A	N / A
Plasmodium	P1	MB, ON, WI, KY	GEOTRI09	Pagenknopp et al. 2008
	P2	ON, WI	CATUST05	Loiseau et al 2012
	P3	MB	BT7	Beadell et al. 2006
	P4	ON	WW3	Durrant et al 2007
	P5	MB, WI	RWB01	Ricklefs and Fallon 2002
	P6	ON	N/A	N/A

*MB = Manitoba, ON = Ontario, WI = Wisconsin, KY = Kentucky
**Lineages consist of more than one haplotype. All parasites in each lineage fall within 2 BP of a central haplotype.

3.3.4 Land cover

The glm revealed that site-scale cover of agriculture was positively associated with the prevalence of *Haemoproteus* ($\beta = 8.22$, SE = 2.26, $p < 0.001$) and *Leucocytozoon* parasites ($\beta = 6.93$, SE= 3.38, $p = 0.0407$), but was not a determinant of *Plasmodium* prevalence ($p = 0.717$).

3.4 Discussion

The presence of potentially virulent parasite genera and high parasite prevalence in some populations of golden-winged warblers may pose a conservation concern. I discovered a third genus of parasite that had not been found in this species before (*Leucocytozoon*), and revealed that the most prevalent blood parasites among golden-winged warblers populations are *Leucocytozoon* and *Haemoproteus. Leucocytozoon* parasites are thought to be highly virulent,

and therefore may be of particular concern (Atkinson and Van Riper 1991). *Haemoproteus* parasites are typically thought to be benign, but they appear to be linked with reduced body condition in blue tits (*Cyanistes caeruleu*s), and survival of this species is negatively correlated with parasitemia of *Haemoproteus* (Martinez-de la Puente et al. 2010). My results confirmed previous work that suggested that the Manitoban population of golden-winged warblers has the highest prevalence of blood parasitism compared to other populations (Vallender et al. 2012), and revealed that parasite prevalence generally increases to the north and west across their range. More than half of all Manitoba's golden-winged warblers carried at least one genus of blood parasite, and the prevalence of parasites in Manitoba is roughly 10 times higher than the site with the lowest prevalence, Kentucky. Manitoba also has the highest number of co-infections, which can have synergistic fitness consequences (Palinauskas et al. 2011). The Manitoba population appears to be at a fitness disadvantage in terms of lower survival and reduced reproductive output (Moulton et al. unpublished data) and it is possible that high parasite prevalence is exacerbating this trend. The extensive variation in parasite prevalence suggests that populations may face differential fitness consequences as a result of parasitism, and this might be a conservation concern for the highly parasitized Manitoba population.

Although the level of variation I found in golden-winged warbler parasite prevalence is frequently reported in the literature, I found much greater genetic structure to parasite communities than is typically observed; most studies find little to no genetic structure to parasite populations, with apparent mixing of parasites throughout a birds' range (e.g. Gibb et al. 2005, Kimura et al. 2006, Fourcade et al. 2014). Golden-winged warblers are infected with many population-specific lineages, and two of the three parasite genera are significantly genetically differentiated among populations. *Haemoproteus* and *Leucocytozoon* parasites were genetically

differentiated among study areas, while *Plasmodium* was not. This pattern likely exists because *Haemoproteus* and *Leucocytozoon* lineages are more likely to be geographic specialists than *Plasmodium* lineages, which are typically widespread and host generalists (Olsson-Pons et al. 2015). Common yellowthroats (*Geothlypis trichas*) and black-throated blue warblers (*Setophaga caerulescens*) also show strong geographic variation in the prevalence of their blood parasites, but both show low genetic differentiation of parasites across their range (Fallon et al. 2006, Pagenkopp et al. 2008). Because golden-winged warblers display strong migratory connectivity (Kramer et al. 2017), parasite communities may have little opportunity to mix and become homogenized. This pattern is also seen in American redstarts (*Setophaga ruticilla*); the populations of American redstarts that migrate together harbour the same parasite lineages (Durrant et al. 2008). The extensive variation in parasite prevalence and lineages present in golden-winged warblers provides an excellent opportunity to investigate factors that influence the distribution of parasite lineages.

The Land-Cover Hypothesis

Golden-winged warbler populations in sites with high cover of agriculture had more infections of *Haemoproteus* and *Leucocytozoon*. Agriculture and associated irrigation create wet and open habitat, which may be suitable larval habitat for the two species of biting midges that transmit *Haemoproteus* (Patz et al. 2008, Kardatzke and Rowley 1971). Similarly, irrigation and drainage typically create running water that can create larval habitat for black flies, which transmit *Leucocytozoon* parasites (Patz et al. 2008, Fredeen and Shemanchuck 1960). However, not all running water is appropriate for black flies, and the pesticides and other runoff associated with agriculture could actually be detrimental to these organisms (Overmyer et

al. 2005). Because I did not explicitly examine vector habitat or vector populations, I cannot be sure that the trend for higher parasite prevalence in heavily cultivated areas is due to vector abundance.

Suitable vectors for all three common blood parasite genera are reported in all of the states and provinces studied. I obtained mosquito data from the Manitoba study sites at the same time as the golden-winged warbler blood sampling (in 2015), and those data revealed that the *Plasmodium* vectors *Aedes canadensis, Culex restuans,* and *Aedes vexans* were present in the study sites we sampled (Enslow et al. unpublished data). This is informative because it indicates that these vectors are present in the type of habitat the golden-winged warblers use, and habitat type is one of the most important local determinants of mosquito presence (Reiskind et al. 2017). The near global distribution of the *Plasmodium* vector *Culex pipiens* includes Ontario (Hongoh et al. 2012), Wisconsin and Kentucky (Farajollahi et al. 2011). Black flies of the genus *Simulium* are a common *Leucocytozoon* vector, and reported in all four study states/provinces as well (Harding et al. 2006, Rivera and Currie 2009, Weinandt et al. 2012). Finally, either the *Haemoproteus* vector *Culicoides haematopotus* or *Culicoides crepuscularis* are expected to occur in Wisconsin, Kentucky, and Ontario (Bennett 1960, Borkent and Spinelli 2000, Swanson 2012, Lysyk and Galloway 2014). Our knowledge of the midge community in Manitoba is limited, but *C. crepuscularis* has been reported as abundant throughout the grasslands of the Canadian Prairies (Lysyk and Galloway 2014), and composes 4-8.8% of the midges near cattle facilities in Alberta (Lysyk 2006). It is important to note that dominant vector species in an area can change dramatically over time; for example a recent survey of the mosquitoes of Alberta found that at least one of the dominant species had changed from previous (decades old) studies, several new species were identified, and several previously uncommon species had disappeared

entirely (Lysyk 2010). Thus, though previous recordings suggest that capable blood parasite vectors should be present in all study areas, this cannot be determined with absolute certainty without more recent vector community sampling.

My results suggest that the association between agriculture and blood parasites indicates a relationship between suitable vector habitat and blood parasite prevalence. However, other patterns may also explain these relationships. It is possible that high quantities of agriculture reduce the resources available to golden-winged warblers, either through reducing the number of trees to forage on or reducing forage-insect populations through non-target pesticides. Reduction in food resources may restrict the birds from allocating resources to immune defenses, which may lead to higher parasite prevalence (Lochmiller et al. 1993, Norris and Evans 2000, Ludtke et al. 2013).

Due to the small number of study areas, I was only able to test one site-level variable, but there are a few other variables that may affect the parasite distribution in golden-winged warbler populations. *Leucocytozoon* parasites are most successfully transmitted at higher latitudes, and this might explain the complete absence of *Leucocytozoon* parasites in low latitude Kentucky, the moderate level of infection in mid-latitude Wisconsin, and the high prevalence of *Leucocytozoon* in higher latitude Manitoba (Pérez-Rodríguez et al. 2013). Similarly, *Leucocytozoon* and *Haemoproteus* may be more likely to be transmitted in cooler climates, and Manitoba's relatively cool spring temperatures may partially explain its high prevalence of *Haemoproteus* and *Leucocytozoon* infections. However, I do not have evidence that *Haemoproteus* is transmitted on the breeding grounds, and the latitude/temperature explanation does not hold unless breeding ground transmission occurs. It is possible that the resident species that I used to examine local transmission is resistant to Haemoproteus or not commonly fed on by biting midges. Overall,

latitude and temperature have well-known impacts on parasite communities, and it is likely that these have additional important effects in this system.

The Dispersal Hypothesis i) Enemy Release

Our knowledge of parasite prevalence in golden-winged warblers does not support the Enemy Release hypothesis, because the most recent region to be colonized (Manitoba) has a high level of parasitism; in Canada, Ontario and Quebec were colonized before Manitoba, which were all colonized after regions in the United States (COSEWIC 2006). If golden-winged warblers were escaping parasitism through dispersal, we would expect the more recently colonized sites to have lower parasite prevalence. Thus, it is considerably more likely that golden-winged warblers were susceptible to novel parasites as they colonized new areas.

The Dispersal Hypothesis ii) Novel Enemies

The pattern of parasite prevalence observed supports the predictions of the Novel Enemies hypothesis. As golden-winged warbler's dispersed north and west (Van Wilgenburg, unpublished data, COSEWIC 2006), they may have encountered new parasites that the individuals were not adapted to, and therefore may have low resistance to (Mølller and Szép. 2011). This is supported by the increasing trend in parasite infection from the Southeast to the Northwest across the golden-winged warbler's range. These results are consistent with other studies, which revealed that local birds typically have fewer parasites than new dispersers. For example, parasitemia of nonlocal male white-crowned sparrows (*Zonotrichia leucophrys*) was over 30x higher, on average, than in local males (Macdougal-Shackleton et al. 2002). Interestingly, in barn swallows, the ectoparasite that was more common in dispersers varied by

sex: non-local male barn swallows (*Hirundo rustica*) had significantly more chewing lice on their bodies than local males, and non-local females had almost twice as many louse flies (*Ornithomya biloba*) on average than local females (Saino et al. 2014).

The pattern of parasite haplotypes observed also supports the Novel Enemies hypothesis. Dispersing golden-winged warblers may bring novel parasites into their new locations from their environment of origin. Because *Haemoproteus* and *Leucocytozoon* showed significant genetic differentiation among study areas, I will focus this discussion on lineages of these two genera. Golden-winged warblers appear to have historically expanded their range from Southeast to the Northwest (Confer et al. 2011, Van Wilgenburg, unpublished data), and this fits the observed pattern in parasite haplotypes. Golden-winged warblers in our most southeastern study site, Kentucky, had the fewest haplotypes of parasites, and the one haplotype of *Haemoproteus* in Kentucky was also present in every other study area. Golden-winged warblers from Ontario disperse into Manitoba, and occasionally into Wisconsin. Consistent with this dispersal pattern, golden-winged warblers in Ontario and Manitoba share an extremely common *Leucocytozoon* lineage that is present but much less common in Wisconsin. Golden-winged warblers from Wisconsin are most likely to disperse to Manitoba, and these two populations share a complex of very similar parasites that are not found in other study areas. Lastly, Manitoba has at least one unique and distinct parasite lineage from each parasite genus. This supports the Novel Enemies hypothesis because if parasites are spread through dispersal, unique haplotypes should be most common in regions where golden-winged warblers do not disperse into the other study areas (ie. Manitoba). When other study areas had unique haplotypes they were similar (less than 2 BP different) to haplotypes found in other locations. Overall, the pattern observed among parasite haplotypes corresponds closely to the dispersal pattern of golden-winged warblers.

In contrast to the other two genera, the distribution of *Plasmodium* haplotypes appeared relatively random. The sporadic distribution of unique *Plasmodium* haplotypes may be a result of 'parasite spillover' from other species (Bensch et al. 2007, Woolhouse et al. 2001). Because *Plasmodium* parasites are able to infect many hosts, golden-winged warblers may be incidentally infected with parasite strains of other nearby hosts (Bensch et al. 2007). These haplotypes may differ because bird community composition differs slightly among study areas.

It is also possible that the distribution of vector species is affecting the haplotypes of parasites that occur. Some parasites, particularly *Haemoproteus*, are very closely associated with their vectors (Martínez-de la Puente et al. 2011). If certain species or subspecies of vectors were reasonably geographically isolated, then these closely associated parasites would be as well. Unfortunately not enough is known about the phylogeography of the parasites and vectors in this system to address how strongly this contributes to geographic structure in parasite communities.

Differences in parasite communities between the two Ontario study sites are best explained by the Novel Enemies hypothesis. Interestingly, the study sites in Ontario, though close in proximity, records of their historical distributions indicates that they were colonized by different populations: the Carden population from the North and the Frontenac population from the East (Rondel, personal communication). Otherwise, these two sites are geographically proximal, have similar land use characteristics, sit at similar latitude, and are climactically similar. Consistent with the dispersal hypothesis, the dominant parasite genus is different between the two sites; *Plasmodium* is significantly more abundant in the western site and *Leucocytozoon* is significantly more abundant in the eastern site. If those populations had historically been exposed to different parasites, their parasite resistance might be different, and the two Ontario study areas may have divergent susceptibility to parasite genera. Thus, the

unique colonization history of the golden-winged warbler populations may have been more important than landscape and geography in determining parasite communities in Ontario. Despite the two sites geographic similarities it remains possible that this trend is driven by different vector communities between the two regions; vector populations vary strongly over short geographic distances (Reiskind et al. 2017) and thorough comparison of site level vector habitat and/or vector communities would be needed to fully rule out this possibility.

Breeding ground transmission

The landscape hypothesis assumes birds are being infected where I analyzed the proxies for vector habitat: the breeding grounds. If birds are only infected on the wintering grounds, proxies for vector habitat on the summer grounds are meaningless, and the correlation between land cover and parasite prevalence would have resulted from something other than vector prevalence. For example, if birds are already infected (from the wintering ground) when they arrive on the breeding grounds, the infected birds may arrive on the breeding sites later (eg. DeGroote and Rodewald 2010) and be forced to settle in the only free landscapes, which may be more likely to be anthropogenically disturbed.

Conversely, both dispersal hypotheses are robust to violations of the assumption of breeding ground transmission. Migratory connectivity between golden-winged warbler populations is extremely strong (Kramer et al. 2017). If a parasite lineage is transmitted on the wintering ground, the only aspect of the dispersal hypothesis that changes is that the lineage disperses between wintering ground locations instead of breeding ground locations. This introduces a new assumption that when a bird disperses from its natal ground it migrates with its

new population. This assumption is likely valid because natal dispersers would rapidly erode migratory connectivity if they did not migrate with their new population.

To examine local transmission potential I screened parasites of resident black-capped chickadees, but there are limitations to comparing parasites between different species. Black-capped chickadees and golden-winged warblers may have different parasite communities because they are not closely related and use different habitat types. Even though they were caught in the same general areas, golden-winged warblers nest on the ground near open areas, and black-capped chickadee nest in trees (Foote et al. 2010, Confer et al. 2011). Because different vectors use different habitat types and have different feeding preferences, these two species may be differentially exposed to vector species (Hassan et al. 2003, Reiskind et al. 2017). This may particularly affect the prevalence of *Haemoproteus* parasites, as golden-winged warblers with large quantities of rangeland in their territories are more likely to have *Haemoproteus* parasites (Ch. 4). Additionally, although both species are Passeriformes, they belong to different superfamilies and shared a common ancestor approximately 44.7 million years ago (Jetz et al. 2012). Because host-parasite relationships can correspond with phylogeny, not all parasite lineages that infect golden-winged warblers will necessarily be compatible with black-capped chickadees, and vice-versa (Bensch et al. 2000).

Despite these limitations, by comparing parasite lineages infecting golden-winged warblers to those infecting resident black-capped chickadees I was able to ascertain that at least some lineages are transmitted on the breeding grounds. The *Plasmodium* lineage that was only found in Manitoba, BT7, was also found in black-capped chickadees. Interestingly, this lineage is widely reported in the literature, and appears to be nearly globally distributed (Bensch et al. 2009). BT7 has been found in Alaska, Vermont, and California in the US, and in many regions

across Eurasia. Evidence of transmission for this parasite in North American is limited to California (Walther et al. 2016), Alaska (Oakgrove et al. 2014), and now Manitoba. Black-capped chickadees in Manitoba were also infected with the group A *Leucocytozoon* parasite lineage, demonstrating that this lineage is transmitted on the breeding grounds in Manitoba.

Black-capped chickadees harboured no *Haemoproteus* parasites, and lacked one *Plasmodium*, and one *Leucocytozoon* lineage of golden-winged warblers. Thus, it is possible that these lineages are transmitted on the wintering ground. This hypothesis is supported by the result that Julian date was not a significant predictor of *Haemoproteus* presence, which would be unexpected if parasites were being transmitted as the season progressed. However, Julian date was also not a significant predictor of *Leucocytozoon* prevalence, despite evidence that *Leucocytozoon* can be transmitted on the breeding grounds, and it is possible that birds harbouring persistent infections from previous summers reduce our ability to detect an effect of Julian date. Additionally, the common and Manitoba-specific *Haemoproteus* lineage has been found in hatch year fox sparrows (*Passerella iliaca*) that were caught on their breeding grounds in California (Walther et al. 2016); because hatch year birds have not yet made it to the wintering grounds, this provides evidence that this lineage is transmittable in breeding grounds in North America. Additionally, though evolution of year round transmission mechanisms in parasites is common (Pérez-Tris and Bensch 2005), most parasites in the Western hemisphere are either transmitted in North America or South America, and not both (Hellgren et al. 2007). Warblers (Parulidae) are more closely related to sparrows (Passeridae), than chickadees (Paridae), which may explain why this parasite was identified in fox sparrows and not black-capped chickadees (Jetz et al. 2012). The widespread lineage, H2, has not been reported elsewhere, but it is closest to the previously reported Ictleu01 (Outlaw and Ricklefs 2009). Ictleu01 was also found in two

resident birds in the tropics: bananaquits (*Coereba flaveola*) and Jamaican orioles (*Icterus leucopteryx*) (Outlaw and Ricklefs 2009). Because H2 is closely related to a lineage transmitted in the tropics, it is likely that this lineage is transmitted in the wintering grounds. The transmission locations of the *Leucocytozoon* lineages CNEORN and L2, and the *Plasmodium* lineage Geotrio9 are unknown (Bensch et al. 2009, Oakgrove et al. 2014, Walther et al. 2016).

Because I cannot be certain of the transmission locality of all lineages, transmission on the wintering grounds may affect my ability to determine land cover-parasite relationships. However, because some of these lineages are transmitted on the breeding grounds, and because North American lineages are generally transmitted on the breeding grounds instead of the wintering grounds (Hellgren et al. 2007), the associations I noticed between land cover and blood parasites likely represent a true correlation between suitable vector habitat and blood parasitism.

It appears that land-cover characteristics and golden-winged warbler dispersal both affect the community composition of blood parasites across the species' range. Increasing agricultural land use, especially in areas of low agricultural intensity, may increase the prevalence of parasites in the species. Additionally, the southern portion of the golden-winged warbler's range appears to be contracting, while the north is expanding (Buehler et al. 2007). This renders the conservation of the Manitoba population of golden-winged warblers extremely important, and unless the species rapidly evolves to tolerate these parasites, the high prevalence and diversity of parasites in this region is a conservation concern.

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Chapter 4. BODY FAT AND BLOOD PARASITES REVEAL THAT ANTHROPOGENIC DISTURBANCE MAY DEGRADE HABITAT QUALITY FOR A THREATENED SONGBIRD

Abstract

Early successional habitats are in rapid decline. This leads early successional species to use anthropogenically disturbed habitat, which may negatively impact the health of the species that use it by decreasing resource availability or by increasing susceptibility to infections. I investigated whether being near different human disturbances negatively impacted the health of a threatened early successional species, the golden-winged warbler. Golden-winged warblers that had large quantities of rangeland in their home-ranges were body fat-depleted and more likely to be infected with *Haemoproteus* parasites, and those near roads, trails, or rail lines also had lower body fat scores. Increased parasite infection and depleted body fat near these disturbances may decrease avian fitness, and thus is a conservation concern for this threatened species.

4.1 Introduction

Early successional habitats are tracts of land that are in the early stages of regeneration after a disturbance, such as a clear-cut or a fire. These diverse, shrubby, herbaceous ecosystems are declining around the world (Litvaitis 1993, Hunter et al. 2001, Swanson et al. 2011). In North America, early successional habitat increased rapidly after farm abandonment by the early 1900s, but has decreased in recent decades (Litvaitis 1993) and is now at a historical low (Hunter et al. 2001). Early successional habitat is continuing to be lost due to land conversion, development, and forest succession as a result of disturbance suppression (Askins 2001). Because of this loss

of habitat, many early successional species have become threatened, extirpated, or extinct; for example, early successional habitat loss likely caused the extinction of the Bachman's warbler, a small songbird in the genus *Vermivora* (Litvaitis 1993, Hunter et al. 2001).

The Bachman's warbler's close relative, the golden-winged warbler (*Vermivoria chrysoptera*) is another at-risk early successional species. It was designated as threatened under the Canadian Species at Risk Act in 2007, and the main threats to this species are recognized as being habitat loss and hybridization with a closely related species: the blue-winged warbler (*Vermivora cyanoptera*) (COSWEIC 2006, Species at Risk Act 2002, 2007). However, genomic analysis recently revealed that there is very little nuclear divergence between the two species, and that hybridization between the species predates Europoean colonization (Toews et al. 2016). This indicates that hybridization may be a less important threat than originally expected, and reinforces the potential role of early successional habitat loss. Both golden-winged warblers and blue-winged warblers are early successional species that are in decline; overall population estimates have dropped by an average 2.95% and 1.24% per year, respectively, since 1965 (Sauer et al. 2017). Thus, identifying and retaining high-quality early successional habitat is expected to benefit both *Vermivora* species.

Although some anthropogenic activities destroy golden-winged warbler habitat, others create it. Anthropogenic disturbances followed by natural succession pathways can create shrubby edge habitat that is used by golden-winged warblers (Bulluck and Buehler 2006, Moulton, unpublished data). However, being near anthropogenic disturbances might negatively affect bird health by reducing forage availability, increasing exposure to infections, or introducing stressors such as noise (Ewers and Didham 2006, O'Brien and Dawson 2016). Of course, the presence of poor quality habitat is better than the absence of any habitat, but it is important to identify the

relative quality of different habitats to ensure that land managers prioritize habitats where the birds will be most successful. The recovery strategy for the golden-winged warbler indicates the need to identify and maintain high quality habitat for this species, and to identify and mitigate anthropogenically caused ecological traps (Environment and Climate Change Canada 2016). An ecological trap occurs when species are drawn to habitat that reduces their fitness (Battin 2004). For example, golden-winged warblers will nest in utility right of ways, but nesting success is low here due to higher nest predation rates (Bulluck and Buehler 2006, Kubel and Yahner 2008). Monitoring individual health, such as the absence of infection and the presence of good body condition, provides one useful metric of habitat quality because habitats with plentiful resources and low levels of vectors should lead to healthier birds. Birds with good body condition and without infections are often more capable of surviving migration and providing for their young (see Bonneaud 2009, Martinez-de la Puente et al. 2010, Labocha and Hayes 2012). Therefore, it is important to monitor avian health in both anthropogenically modified environments and natural habitats. If anthropogenic disturbances negatively impact the health of golden-winged warblers, this suggests that this type of habitat is poor quality and may function as an ecological trap.

Human disturbance may affect body condition of birds either by reducing the availability of important resources or by causing birds to re-allocate resources to fight or flight responses in the presence of more predators or noise (Frid and Dill 2002, Quinn et al. 2006, Liker et al. 2008). For example, urban house sparrows (*Passer domesticus*) in Hungary were in poorer body condition in the city than in rural areas, and this appeared to be associated with poor development as nestlings (Liker et al. 2008). Blood parasites are another potentially informative health metric as they tend to negatively affect birds and their presence may indicate a

compromised immune system (Lee et al. 2006, LaPointe et al. 2012). There are many different

avian blood parasites, and *Plasmodium* and the closely related genera *Leucocytozoon* and

Haemoproteus are widespread and abundant (LaPointe et al. 2012). These parasites may

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). The Manitoba population of golden-winged warblers has the highest

prevalence of infection across its range, perhaps due to high levels of anthropogenic disturbance

on the landscape or the recent colonization by golden-winged warblers (Vallender et al. 2012,

Chapter 1). The Manitoban golden-winged warbler population is significant because this

population has the lowest rate of genetic introgression with the blue-winged warbler (*Vermivora

cyanoptera*), a species that hybridizes with and frequently replaces golden-winged warblers

(Vallender et al. 2009). If anthropogenic disturbances are correlated with increased parasite

prevalence in Manitoba's golden-winged warblers, this is may pose a conservation concern to

this important population.

Research examining the consequences of human disturbance 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). Parasite prevalence does not respond uniformly to human

disturbance: some researchers noticed that parasitism increased in areas surrounded by human

disturbance (eg. Vaz et al. 2007, Cottontail et al. 2009, Okanga et al. 2013), and some noticed the

opposite trend (eg. Bonneaud et al. 2009). Variable responses to human disturbance are

unsurprising because there are many different types of human disturbance, 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 anthropogenic disturbances (Brearley et al. 2013). This is especially important in northern environments, where the few studies that have examined the link between human disturbance and blood parasites have generally restricted their analysis to a single variable such as urbanization or agriculture (eg. Evans et al. 2009, Fourcade et al. 2014, Bailly et al. 2016).

In this study, we used three bird health metrics to infer the effect of being surrounded by human disturbance on the health of the golden-winged warbler. These metrics were blood parasite occurrence, body fat, and body weight normalized to date and size. Golden-winged warblers were caught in areas ranging from heavily disturbed to mostly forested. We compared the parasite prevalence and body condition of birds with territories that contained various quantities of rangeland (land grazed by cattle), aggregate (gravel) mines, and linear disturbance features (roads, trails, and rail lines). Rangeland was predicted to be positively associated with parasite prevalence, as this disturbance may create vector habitat through cattle footprints and drinking areas (González et al. 2013). Body condition indexes were predicted to show a negative relationship with gravel mines and linear disturbances, as these disturbances directly remove foraging habitat and may distress birds from traffic or loud equipment (Francis et al. 2009, McClure et al. 2013). This study contributes to the growing body of work examining the response of parasitic infections to anthropogenic disturbance, while also providing information to managers regarding habitat quality for this threatened species.

4.2 Methods

4.2.1 Study areas

Assistants and I surveyed and captured golden-winged warblers in southeastern Manitoba and Riding Mountain National Park, in western Manitoba. The southeastern Manitoba study area was

situated approximately 60 km southeast of Winnipeg, Manitoba, within a 15 km radius around the town of Richer (49.764° N, 96.528° W). The Riding Mountain National Park study area was concentrated on the eastern border of the park and regions immediately inside and 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 centre-west near Riding Mountain National Park, in the Southeast, or in a population in the centre of the province (the Interlake) (Environment and Climate Change Canada 2016, Artuso, personal communication). Overall, my study areas should encompass the majority of the blood parasite variation for the species in Manitoba. The study areas are broadly similar; both areas contain large quantities of aspen parkland and boreal transition zone forest, which contain tracts of undeveloped forest that is interspersed with anthropogenic disturbances of various sizes (from small country roads to large acreages of farmland). Anthropogenic development is similar in these two study areas, and is dominated by rangeland, cropland, transportation features, or aggregate mines. Golden-winged warblers in this study were all caught in low elevation, flat terrain, but a large, higher elevation escarpment bordered the Riding Mountain National Park study area. Specific sampling locations were chosen based on previous survey data 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, Confer et al. 2011). This type of forest is characterized by large aspen (*Populus tremuloides*) and birch trees (*Betula papyrifera*), interspersed spruce (*Picea*) or pine (*Pinus*), and considerable shrub cover of hazel (*Corylus cornuta*), raspberry (*Rubus occidentalis*), Saskatoon berry (*Amelanchier alnifolia*), and pincherry (*Prunus pensylvanica*) (L. Moulton, personal communication).

4.2.2 Field methods

My field team and I captured 31 adult golden-winged warblers in Riding Mountain National Park (28 males and 3 females) and 44 birds in Southeast Manitoba (40 males and 4 females) using 6-m long mist-nets and territorial song playbacks. I also created a golden-winged warbler decoy out of Sculpy© clay and acrylic paint, which we deployed when the birds were responding to playback but could not be captured. When caught, the birds were extracted from the mist-net and immediately processed. Processing included banding each bird with a Canadian Wildlife Service marked aluminum band, sexing and aging by plumage according to Pyle (1997), weighing in a bird bag with a Pesola scale, measuring wing chord (Ralph et al. 1993), assigning a furcular fat score based on a 0-7 scale (Ralph et al. 1993), and taking ~20 µL of blood via the brachial vein. The blood was immediately placed into Queen's Lysis Buffer and stored at room temperature (Seutin et al. 1991).

4.2.3 Infection prevalence

I compared a non-nested PCR protocol and a nested PCR protocol that are both commonly used to detect Haemosporidia. I extracted DNA from the blood samples with 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, I 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 (Figure 4.1). 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. I then performed a

second and third PCR, each using the product from the first nested PCR step. In the second PCR,

nested primers (HaemF and HaemR2) isolated a smaller 480-bp region of cytochrome B in

Haemoproteus and *Plasmodium* parasites. In the third PCR, nested primers HaemFL and

HaemR3L only isolated *Leucocytozoon* parasites. I screened for positive infections using

electrophoresis, where visible bands of PCR product were scored as positive infections. I

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). I cleaned and aligned all successful

sequences using Geneious version 10 (http://www.geneious.com, Kearse et al. 2012), and

identified infections to genus using BLAST (Altschul et al. 1990).

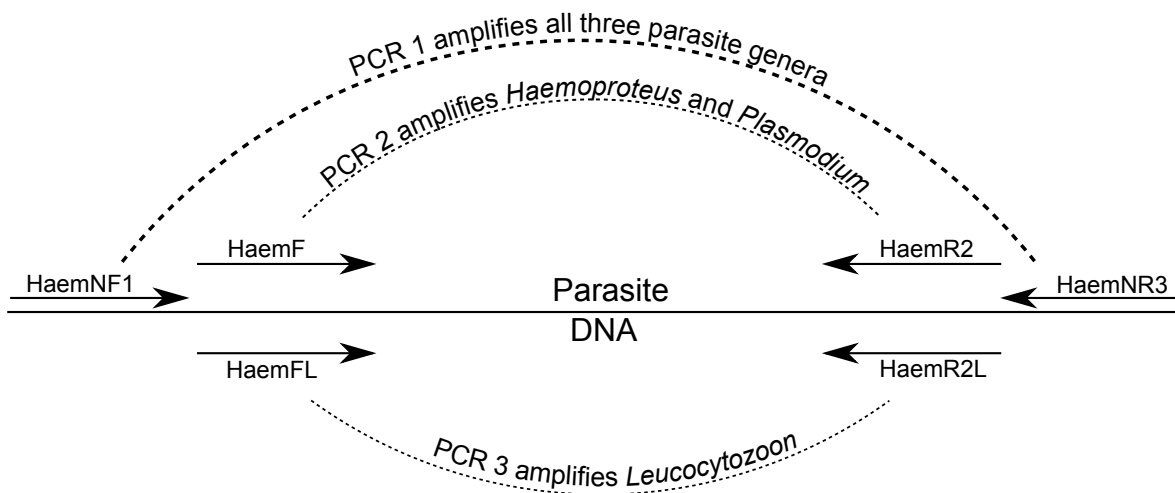**Figure 4.1. Schematic diagram of the nested PCR sequence used to amplify *Haemoproteus, Plasmodium,* and *Leucocytozoon* parasites in golden-winged warblers in Manitoba. Adapted from Hellgren et al. 2004.**

4.2.4 Geographic analyses

I estimated percent composition of five kinds of land cover within 200 metres of each bird:

percentage of total area covered by linear disturbance features (roads, trails, rail lines),

rangeland, wetland, and aggregate pits (sand and gravel). These analyses were performed in ArcMap 10.2 (ESRI 2011). I obtained land-use data from the Manitoba Lands Initiative, and overlaid the GPS position of each golden-winged warbler sampled (Manitoba Conservation 2005). I created a 200-m buffer around each point to select an area approximately the size of an average home range of golden-winged warblers in this region (Moulton, personal communication), and intersected this buffer with the land-use layer. I visually verified the land-use classifications around each bird by comparing the land-use layer to field observations and an orthographic base map. I also calculated distance from each bird to the nearest river, which included both intermittent and permanently flowing watercourses (Harrison 2005).

4.2.5 Statistical analyses

To detect an effect of local land-use on prevalence of infection, I created three binomial generalized linear models in R 3.1.4 with each parasite genus (*Plasmodium, Haemoproteus,* and *Leucocytozoon*) as the response variable in separate models (R Core Team 2015). I evaluated effects of three anthropogenic predictor variables on parasite prevalence: percent of buffer zone around each bird that is covered by rangeland, percent of area covered by aggregate pit, percent of area covered by linear disturbances (roads/trails/rail lines), and four additional factors that might affect parasite occurrence: distance to river, and percent of buffer zone around each bird covered by marshland, Julian date, and bird age. I used Akaike information criterion (AIC) to determine whether or not to include the additional variables age and Julian date in each model; for all other analyses I took a null-hypothesis significance testing (frequentist) approach (Mundry 2011). Removing nuisance variables when they were not useful improved sample sizes because these data were missing for some birds.

I also tested if parasite occurrence or anthropogenic disturbance 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 $n = 70$ and $n = 73$, respectively. First, I created a body condition index in R by taking the residuals (R1) of a regression between body weight and Julian date, and then the residuals ('body condition index') of a regression between R1 and wing length (R Core Team 2015, Whittingham and Dunn 2000). Diagnostic graphs combined with a Shapiro-Wilk test indicated that this body condition index was normally distributed. I performed a linear regression in 'R' with body condition index as the response variable, and included presence of *Haemoproteus* and *Leucocytozoon* and the above anthropogenic and natural variables: proportion rangeland, proportion aggregate pit, proportion linear disturbances (roads/trails/rail lines), distance to river, proportion marshland, Julian date, and bird age as fixed independent variables. I tested for effects of the presence of parasites and these land cover 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 'loglog' link function best fit this model.

4.3 Results

Overall parasite infection of golden-winged warblers in Manitoba was 55.7%: 27.0% were infected with *Haemoproteus*, 5.4% were infected with *Plasmodium*, and 32.9% were infected with *Leucocytozoon*. Overall, 12.7% of golden-winged warblers were infected with more than one genus of parasite. Due to the low prevalence of *Plasmodium*, only *Haemoproteus* and *Leucocytozoon* were used in the rest of the analyses. Two positive infections produced weak PCR bands that either corresponded to *Haemoproteus* or *Plasmodium* but could not be sequenced and were removed from further analyses involving *Haemoproteus*.

The nested PCR detected a *Plasmodium* or *Haemoproteus* infection in 36.8% of our samples; this was higher than the single-PCR method, which detected an infection of one of these two genera in 26.3% 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 bases after cleaning. The regions amplified by the two methods overlapped considerably, and individuals could accurately be assigned to the same parasite haplotype if different primers were used.

A golden-winged warbler's odds of infection with parasite genus *Haemoproteus* increased by 1.27 times as the proportion of rangeland in their estimated home-range increased by 10% ($\beta = 2.36$, SE = 1.18, $p = 0.028$), but there was no effect of the other anthropogenic or natural variables ($p > 0.293$) (Figure 4.2). There was also no effect of age class ($p > 0.341$) or Julian date ($p = 0.982$) on *Haemoproteus* presence, and because these variables increased the AIC value of the model, they were removed. *Leucocytozoon* occurrence did not vary significantly with any anthropogenic variables, but when marsh-cover in a golden-winged warblers home-range increased by 10%, the bird's odds of being infected with *Leucocytozoon* increased by 9.47 times ($\beta = 22.5$, SE = 1.01 $p = 0.054$). Conversely, being one kilometre closer to a river decreased a birds odds of infection by 1.56 times ($\beta = 0.443$, SE = 0.238, $p = 0.063$). Once again, neither age ($p = 0.921$) nor Julian date ($p = 0.218$) significantly predicted *Leucocytozoon* prevalence or improved model fit, and they were removed from the model.
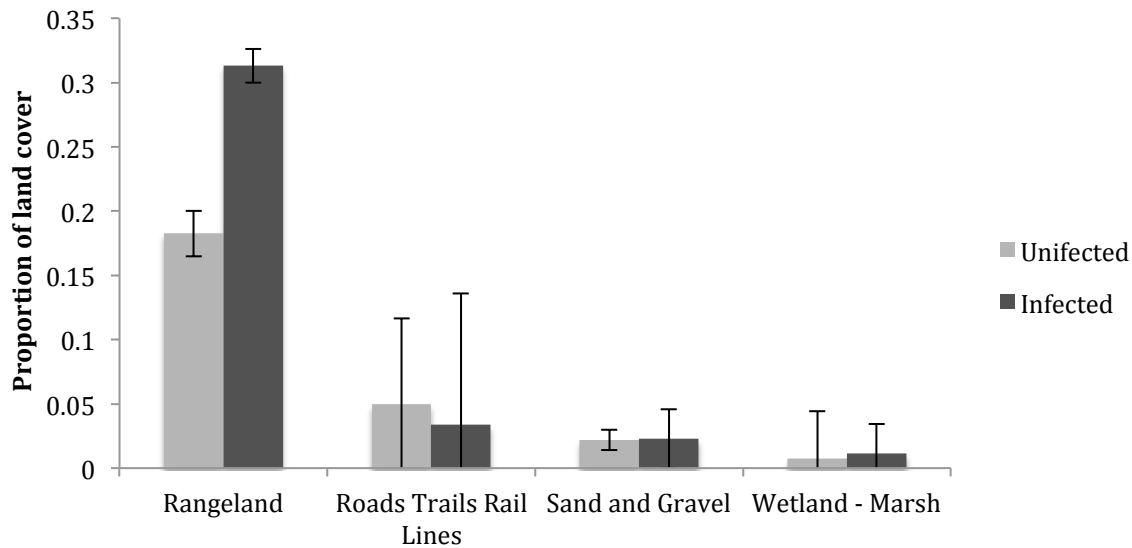
Figure 4.2. Proportion of land-use within a 200m radius of *Haemoproteus*-infected and uninfected golden-winged warblers sampled in Southeastern and Southwestern Manitoba in 2015, *n* = 75. Error bars denote 95% confidence intervals. Bars with non-overlapping confidence intervals are significantly different.

Bird body fat, but not body condition varied with land cover characteristics. Golden-winged warblers with home-ranges with larger proportions of rangeland (β = -1.34, SE= 0.664, *p* = 0.044) or linear disturbance features (β = -6.23, SE= 3.58, *p* = 0.081) had lower body fat scores, while golden-winged warblers that we caught nearer to a river had higher body fat scores (β = - 0.281, SE = 0.149, *p* = 0.060). Bird age (*p* = 0.672) and Julian date (*p* = 0.769) were not significant predictors of body fat score, did not improve model AIC, and were thus removed from the body fat model. The type of land cover within a golden-winged warbler home range had no significant effect on body condition (*p* > 0.363).

Body fat and body condition also responded differently to the two parasite genera. *Haemoproteus*-infected golden-winged warblers were of equal body condition (*p* = 0.202) and body fat (*p* = 0.651) compared to uninfected warblers. Conversely, *Leucocytozoon*-infected golden-winged warblers were significantly more fat (β = 0.924, SE = 0.390, *p* = 0.012) and in significantly better body condition (β = 0.362, SE = 0.158, *p* = 0.026) than uninfected birds

(Figure 4.3). None of the 10 golden-winged warblers with a body fat score of zero were infected with *Leucocytozoon.*
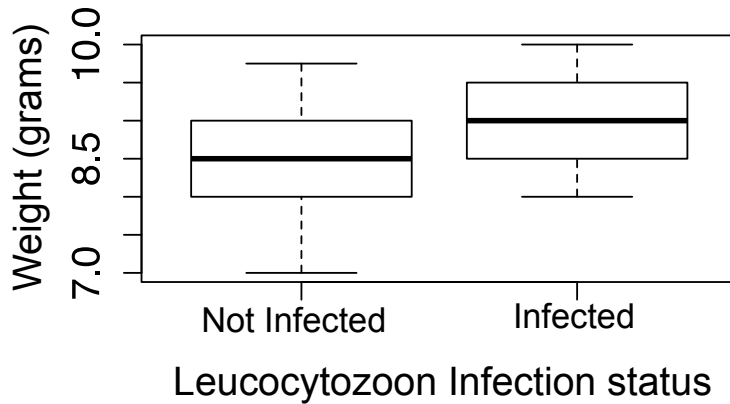
Figure 4.3. Weight distribution of *Leucocytozoon* infected and uninfected golden-winged warblers sampled in Southeastern and Southwestern Manitoba in 2015, *n* = 75.

4.4 Discussion

My results provide some support for the hypothesis that golden-winged warblers that are surrounded by certain anthropogenic disturbances may be in poorer health. Rangeland and linear disturbances may deplete resources or increase actual or perceived threat levels in a bird's home range, and this may lead to lower immune competency and/or fat stores in individuals near these disturbances. Closer proximity to a river was associated with increased health in golden-winged warblers. Rivers may provide access to more resources, and allow birds to allocate more resources to body fat storage and immune function. This suggests there are negative impacts of anthropogenic but not natural habitat features on this early-successional species.

Nested PCRs are more likely to detect low parasitemia infections than non-nested PCRs. Therefore, the result that Hellgren (2004)'s nested PCR detected more infections than the non-nest PCR is not surprising, and suggests that at least some golden-winged warblers in this system have relatively low parasitemia. Using the nested PCR, we found a 36.8% combined prevalence

of *Haemoproteus* and *Plasmodium* in Manitoba, which is slightly higher than the 30% combined

prevalence found by Vallender et al. (2012), which might simply be explained by year-to-year

variation. However, because previous research on this species used a non-nested PCR, they may

have slightly underestimated the infection prevalence. Furthermore, the nested PCR also screens

for *Leucocytozoon* parasites, which the non-nested PCR does not. By screening for

Leucocytozoon parasites we revealed that the majority of birds in this population are infected by

blood parasites. Interestingly, work on red-billed gulls (*Chroicocephalus scopulinus*) in New

Zealand found that the Hellgren (2004)'s nested PCR was not usable in their study because the

sequences it produced contained many ambiguous bases (Cloutier et al. 2011). In contrast, I

noticed that the nested PCR produced cleaner sequences with fewer ambiguous bases than the

non-nested PCR, which suggests that the best practices for parasite detection and sequencing

may vary by host-parasite system.

Of the anthropogenic disturbances that we examined, only rangeland was associated with

parasite prevalence, and it was positively associated with the presence of *Haemoproteus.*

Culicoides midges are the vectors of the parasite genus *Haemoproteus*, and cattle ranging can

increase larval habitat quality for *Culicoides* midges (Martinsen et al. 2008). Cattle manure and

drinking water can provide suitable larval habitat for *Culicoides*, and cattle hoof-prints alter soil

substrate to increase favourable larval habitat (Schmidtmann et al. 1983, González et al. 2013).

Additionally, the *Haemoproteus* vectors in this region, *Culicoides haematopotus* and *Culicoides*

crepuscularis (Atkinson et al. 2009, Lysyk and Galloway 2014), also feed on mammals (Becker

et al. 2010), and these vectors may be in higher concentrations near rangeland if cattle are

providing blood meals (Schmidtmann et al. 1983). Conversely, an abundance of mammal hosts

may provide zooprophylaxis, a process where transmission potential of a parasite is diluted when

vectors feed on hosts that the parasite cannot complete its life cycle in. Although the

Haemoproteus vector *Culicoides crepuscularis* is found near cattle facilities in Alberta (Lysyk

2006), without abundance data for *Culiciodes* midges in this region, I cannot be certain that

rangelands increase the prevalence of this vector.

Lower body fat scores in golden-winged warblers near rangeland may be due to

decreased resource availability in grazed areas. Although standardized dietary studies for golden-

winged warblers have yet to be conducted, they are known to forage for Tortricid moth larvae in

leaf-rolls, typically near the tops of trees (Confer et al. 2011). Because cattle grazing suppresses

tree growth, the foraging locations for golden-winged warblers may be reduced near rangeland

(Bond 2008). Additionally, forage quantity may also be reduced in open areas; for example one

Tortricid, the grape berry moth (*Endopiza viteana*), is most abundant in woody crops near

deciduous forest and least abundant in crops near grassy areas (Botero-Garces and Isaacs 2004).

This may have conservation implications because birds with low fat may not have adequate

stored energy to survive migration, tend to their young, or respond to threats in their environment

(Pfister et al. 1998, Scheuerlein et al. 2001, Davis et al. 2005).

Most migratory birds are expected to have low body fat during breeding, either due to the

stress of breeding or to facilitate easier flight (Merkle and Barklay 1996), but other work has also

noted an impact of anthropogenic disturbance on body fat of breeding birds. For example, seven

migratory bird species had lower body fat scores in a forest treated with Dimilin®, than near-by

untreated forests (Whitmore et al. 1993). The pesticide is used to control moth populations, and

therefore may reduce forage quality for insectivourous birds (Whitmore et al. 1993).

Low body fat may also exacerbate the effect of parasites in golden-winged warblers. If

infected birds have reduced access to high-quality food sources, they may not be able to allocate

resources adequately to combat infections (Lochmiller et al. 1993, Ludtke et al. 2013, Hernández-Lara et al. 2017). Poor-quality habitat has been shown to influence parasitemia; for example, blue tits in areas with higher oak density (a measure of forage quality) had significantly reduced parasitemia when compared to blue tits in areas of low oak density (Knowles et al. 2011). Similarly, research in the Mexican highlands revealed that goat abundance was positively correlated with parasitemia in canyon towhee (*Melozone fusca*) (Reinoso-Pérez et al. 2016). These researchers suggested that it was the lack of resources around goat grazed habitat, rather than a change in vector community, that rendered their population more susceptible to parasites (Reinoso-Pérez et al. 2016). Interestingly, they also noticed that moderate grazing reduced parasitemia when compared to intense grazing, suggesting that reducing grazing intensity may help resource abundance improve (Reinoso-Pérez et al. 2016). However, if immunodeficiency due to poor resource quality was the only explanation for increased *Haemoproteus* prevalence around rangeland in this system, we would expect the parasite *Leucocytozoon* to also be more common around rangeland. Because this is not the case, it is likely that both resource availability and vector habitat are important factors affecting parasite occurrence in this system.

Linear disturbance features, such as roads, trails, and rail lines, are associated with reduced body fat in golden-winged warblers, and this may be due to resource availability, predator abundance, or noise (Forman and Alexander 1998, Frey and Conover 2006, McClure et al. 2013). Like rangelands, linear disturbances can reduce tree density and increase tree death (Prasad 2009), potentially decreasing foraging habitat for golden-winged warblers. Birds surrounded by linear disturbances may also allocate more time and resources to responding to humans, vehicles, or predators, and thus have less time to allocate to foraging (Frid and Dill 2002, Quinn et al. 2006). Moreover, habituation to these stressors is generally only partial,

because the cost of underestimating danger is greater than the cost of overestimating it (Frid and Dill 2002).

Contrary to our expectations, we did not find any effects of aggregate (gravel) pits on body condition, body fat, or parasite prevalence in golden-winged warblers. This is also contrary to previous studies on tree swallows (*Tachycineta bicolor*) (O'Brien and Dawson 2016). Though the exact ages of gravel pits in this region were not available, land cover analysis will only register gravel pits that remain open and have not yet regrown vegetation. This lack of vegetation may explain why few golden-winged warblers had large proportions of gravel pit in their territories, and it is possible that the species is avoiding negative effects of gravel pits by not establishing territories directly beside them.

There are two possible mechanisms for increased infection prevalence and decreased body fat scores in some anthropogenic habitats. One mechanism may be that birds randomly select territories in the spring and then gain or lose fat or parasites depending on the qualities of the territory. An alternate mechanism is that immune-deficient (or already parasitized) or fat-depleted birds are poorer competitors, and are forced into more disturbed territories. My data do not allow us to reliably distinguish between these two possibilities, but both mechanisms would lead to the conclusion that the habitat in question is not of high quality.

Leucocytozoon parasites displayed an unexpected relationship with land cover. *Leucocytozoon* parasites were significantly less prevalent near rivers, which are known breeding habitat for their black fly vector (Valkiūnas 2005). However, black flies can also reproduce in extremely small sources of running water, and many of these were too small to be included in the resolution of our landscape cover layer (Harrison 2005). *Leucocytozoon* parasites were positively associated with marsh cover, and these wet marshy areas may contain small sources of running

water that contribute to development of rivulets. Additionally, I included both intermittent and permanently flowing streams in this analysis, and the spatially and temporally patchy rain patterns in Manitoba may have rendered some of these streams dry (ie. not black fly habitat), and therefore reduced my ability to detect an effect of real black fly habitat. Golden-winged warblers also had higher body fat scores near rivers, so it is possible that rivers provide increased resources for the birds, which may improve their ability to fight off infections (Lochmiller et al. 1993, Isaksson et al. 2013, Hernández-Lara et al. 2017). Proximity to river may also be a proxy for proximity to protected habitat; the government of Manitoba requires a 30m buffer of retained habitat around natural riparian zones, which includes retention of trees (The Planning Act: 5.1.3, 2011). However, if resource availability near rivers were the only driver of this trend, we would expect improved immunity to also lead to a decreased *Haemoproteus* prevalence near rivers, but this is not the case.

There was no significant effect of *Haemoproteus* infections on body condition or body fat of birds. *Haemoproteus* has historically been considered a common and non-virulent parasite, and it is possible that the evolutionary history between golden-winged warblers and *Haemoproteus* parasites has reached an equilibrium that causes little damage to the host (Toft and Karter 1990, Santiago-Alarcon et al. 2012). However, it is also possible that golden-winged warblers are allocating resources in a way that maintains their body condition but reduces their fitness in other ways. There may be an energetic trade-off, where some species retain the same body condition to the detriment of their survival or nesting success (Garvin et al. 2006, LaPointe et al. 2012, Marzal et al. 2005).

Surprisingly, *Leucocytozoon* parasites were more common in golden-winged warblers that were fatter and in better body condition. This result is especially surprising because

researchers often assume that *Leucocytozoon* parasites are especially virulent (Santiago-Alarcon 2012). In other organisms, such as mosquitoes, when a specific size class has significantly fewer infections, it is assumed that this size class cannot tolerate infection and do not survive (see Anderson et al. 2000, Lyimo and Koella 1992). If size-dependent mortality is occurring in this system, then smaller birds may die or be less mobile following infection, and thus be less likely to be mist-netted. Alternatively, higher testosterone may be linked to more exaggerated sexually dimorphic traits (such as large body size), and testosterone can suppress immune function and lead to greater parasitism (Folstad and Karter 1992). Thus, females selecting for these traits will be selecting truly high quality males that can handle both infections and investing in these sexual characteristics at the same time (the Handicap Hypothesis; Folstad and Karter 1992). Lastly, it is possible that good foraging habitat for golden-winged warblers is also good habitat for black flies (the *Leucocytozoon* vector). Golden-winged warblers will feed on a variety of insects, and quality habitat for black flies may be quality habitat for some of their food sources as well. The result that golden-winged warblers are fatter near rivers supports this hypothesis, as black flies require running water for their larvae. Stomach content analysis or other dietary studies on golden-winged warblers in Manitoba are needed to test this hypothesis.

Human disturbance variables are an important local-scale determinant of body fat and parasite infection in golden-winged warblers. Rangeland and linear disturbance features (roads, trails, and rail lines) appear to increase parasite infections and decrease body fat of golden-winged warblers. The impact of parasites on the body condition of these birds was mixed, but the negative relationship between parasite prevalence and fitness is strong enough in the literature to warrant concern. Abundance of rangeland and extent of linear disturbances may pose conservation threats to this species at risk.

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Chapter 5. ELEVATED PREVALENCE OF *LEUCOCYTOZOON* PARASITES IN

GOLDEN-WINGED WARBLER X BLUE-WINGED WARBLER HYBRIDS IN

ONTARIO, CANADA

Abstract

Parasites often influence the evolution of their hosts. In hybrid systems, parasites may facilitate

hybridization if hybrids are resistant to host-specific parasites of their parents; conversely,

parasites may resist hybridization and maintain species boundaries if hybridization breaks down

adaptive resistance to parasites and renders hybrids more susceptible to infection. The Ontario

population of golden-winged warblers provides an excellent opportunity to investigate the effect

of parasites on hybridization systems as the population contains a high prevalence of both

parasites and golden-winged x blue-winged warbler hybrids. I screened adult golden-winged

warblers and adult hybrid warblers from Ontario, and compared the prevalence of three genera of

parasites (*Leucocytozoon*, *Haemoproteus*, and *Plasmodium*) to hybrid status. Hybrid individuals

were more likely to be infected with the parasite genus *Leucocytozoon*, suggesting that blood

parasites may help maintain species boundaries in this hybrid system.

5. 1 Introduction

Avian blood parasites can have strong impacts on the evolution of their hosts. The

relationship between host and parasite is so strong it may be one of the driving factors of

migration, site fidelity, and sexual dimorphism (Hamilton and Zuk 1982, Møller and Szép 2011).

An important way that parasites affect the evolution of their host is by supporting or eroding

species-boundaries. Differential resistance to parasites can promote and maintain speciation,

especially if the factors that cause differential resistance are also linked to mate choice (Maccoll

2009). Conversely, parasites may erode species boundaries if hybrids are genetically superior at combatting parasites (Hedgecock 1995, Wiley et al. 2009).

 If hybrid birds display 'hybrid vigour', they may be better able to resist parasites than either host species, and the force of parasitism would be towards homogenizing species. Hybrid vigour as a concept has been demonstrated in several cases, and appears especially relevant when hybrids are exposed to novel or extreme environments (See Arnold and Martin 2010 for a review). For example, hybrids between two populations of *Geospiza fortis* on the Galapagos were selected for when novel anthropogenic disturbances altered the available resources on an island (Hendry et al. 2006). Additionally, hybrids between two species of *Laurus* gulls outcompete both of their parent species by selecting nest sites that have lower predation risk and feeding their chicks higher quality food (Good et al. 2000). Hybrids might be more resistant to parasites because heterozygosity can enhance vigour, and can release the host from host-specific parasites of the parents (Hedgecock 1995, Wiley et al. 2009). For example, a study on highly species-specific pocket gopher (*Geomys spp*) parasites revealed that hybrid pocket gophers were infected with neither of the louse species (*Geomydoecus spp*) of the parent species (Heaney and Timm 1985). When hybridization promotes parasite resistance, hybrids may be more successful than genetically pure individuals and species boundaries may erode.

Alternatively, parasites might facilitate and maintain speciation if diverging hosts have differential resistance to parasites. Hybridization can reduce fitness by disrupting adaptive genetic material of the parent species, and low viability, mating success, or survival of hybrid offspring can select against hybridization and maintain species boundaries (Templeton 1986, Arnold and Hodges 1995). Parasitism may also decrease survival of hybrids if hybridization disrupts species-specific immune adaptations through genetic recombination (Moulia 1991,

Moulia 1999). For example, hybrid mice (European mice *Mus musculus musculus* and *M. m. domesticus*) have more gut parasites than their parents, and this force may limit the extent of their hybrid zone (Moulia 1991). This can even occur in populations that are not distinct species; for example, largemouth bass (*Micropterus salmoides*) that were outbred between two distinct populations had a 14% decline in fitness compared to their parental stocks, and suffered increased mortality when exposed to a novel pathogen, suggesting that hybridization compromised their immune systems (Goldberg et al. 2005). If hybrids are more susceptible to parasites than their parent species, the force of parasitism may help maintain species boundaries.

The golden-winged warbler (*Vermivora chrysoptera)* and blue-winged warbler (*Vermivora cyanoptera)* hybridization complex offers an interesting opportunity to examine relationship between hybridization and parasite prevalence. Golden-winged warblers and blue-winged warblers have almost identical genetic composition (Toews et al. 2016), and this low genetic divergence implies that genetically based immunity should not be greatly disrupted when the species hybridize. Despite this genetic similarity, golden-winged warbler populations are often rapidly replaced by hybrid and blue-winged warblers in regions where the two species have recently come into contact; it has been suggested that this could lead to a serious conservation concern for this species (Gill 1980**).** The mechanism by which hybrids and blue-winged warblers replace pure golden-winged warblers is unclear (Vallender et al. 2007b), and resistance to parasites could be an invisible force that promotes hybridization. "Pure" golden-winged warblers (those with golden-winged warbler mitochondrial DNA instead of blue-winged warbler mitochondrial DNA) seem to be more likely to choose wetland habitats, which may be good habitat for vectors, and thus habitat selection might contribute to this mechanism (Valkiūnas 2005, Confer et al. 2010, Chapter 4).

I compared three hypotheses to describe the role of blood parasite prevalence in the golden-winged and blue-winged warbler hybrid complex: 1) Hybrid golden-winged x blue-winged warblers will display hybrid vigour and be more resistant to parasites. 2) Hybrid golden-winged x blue-winged warblers will suffer from outbreeding depression and be less resistant to parasites. 3) Golden-winged warblers and their hybrids are too genetically similar to face immune related vigour or depression as a response to parasitism and will have similar parasite prevalence.

5.2 Methods

My collaborator (Emily Rondel) captured 72 golden-winged warblers and golden-winged warbler x blue-winged warbler hybrids from two field locations in Ontario. Both study sites were in Southern Ontario, one near Frontenac Provincial Park (44.634° N, 96.332°) and one near the town of Carden (W 44.683° N, 79.057° W). To locate golden-winged warblers they searched appropriate habitat and previously occupied locations by sight and sound. Appropriate habitat consists of deciduous-dominated parkland forest with early successional gaps interspersed (Confer et al. 2011). Once a golden-winged warbler was identified they set up a 6m net lane within its territory and used conspecific playback to lure the bird into a six-metre mist-net. Once captured, the bird was immediately extracted, banded with a CWS aluminum band, and had a ~20μl blood sample taken from its brachial vein.

In the laboratory, my collaborator and I screened each blood sample for hybrid status and parasite prevalence. First, we extracted DNA from each blood sample using a homemade DNA kit (Ivanova et al. 2006). To determine the hybrid status of each bird we amplified a conserved portion of mitochondrial DNA (NDII) that occurs in both golden-winged warblers and blue-winged warblers. We used gel electrophoresis to confirm the presence of PCR product in each

sample, which also confirmed that all blood samples successfully yielded avian DNA. We then combined 1 µl of a restriction enzyme MAEII and 2 µl of buffer (HpyCHIV; New England Biolaboratories, Ipswich, Massachusetts, USA), 4 µl of PCR product, and 13 µl of DNA-grade water (Fisher Scientific, Hampton, New Hampshire, USA) and placed the resulting mixture into a thermocycler (Eppendorf Mastercycler ep gradient S; Eppendorf Canada, Mississauga, Ontario) under the following conditions: 95°C for 3 minutes, 34 cycles at 95°C for 1 min, 1 min at 53°C, and 1 min at 72°C, and then 72°C for 5 min (Vallender et al. 2009). The restriction enzyme cuts blue-winged warbler mitochondrial DNA type (mtDNA) in half and leaves golden-winged warbler mtDNA type at the length of the original PCR product (which is approximately 280 base pairs long). To check the length of the product, we used gel electrophoresis and compared the length of all resulting PCR bands to a DNA ladder. Samples with short bands (of approximately 150 base pairs) indicated the presence of blue-winged warbler mtDNA and were scored as cryptic hybrids: birds that have a golden-winged warbler phenotype but blue-winged warbler mitochondrial DNA type.

To screen for prevalence of parasites I used a nested PCR approach that is capable of detecting parasites at a concentration of 1:100,000 (Hellgren et al. 2004). In the first PCR reaction I amplified a 682 base pair region of parasite DNA that is present in *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* parasites using the primers HaemF and HaemR2 (Hellgren et al. 2004). In the second PCR, primers HaemF and HaemR3 amplified *Plasmodium* and *Haemoproteus* DNA. Lastly, in the third PCR primers HaemFL and HaemR2L amplified *Leucocytozoon* DNA. I screened the PCR products that resulted from the second and third PCR with electrophoresis, and scored each sample for presence or absence of parasite DNA. I considered presence of parasite DNA to indicate a positive infection. Positive infections were

sequenced using Big Dye and an ABI 3130 Automatic Sequencer (Applied Biosystems). I

aligned the resulting sequences in Geneious version 10 (http://www.geneious.com, Kearse et al.

2012), and identified the sequences to genus using MalAvi Blast (Bench et al. 2009).

To compare hybrid status to infection prevalence we created binomial generalized linear models

in R 3.1.4 (R Core Team 2015). Two models were created with each parasite genus

(*Haemoproteus* or *Leucocytozoon*) as the response variable and hybrid status and study site as

fixed effects. I considered a bird a hybrid if the genetic analysis described above revealed it had

blue-winged warbler mtDNA, or if it had phenotypically hybrid characteristics. Phenotypic

hybrids displayed some or all of the characteristics of the Brewster's Warbler phenotype: a dark

eye line in place of a mask, a white throat patch in place of the black throat patch, and yellow on

the chest in place of clear white (Gill 1980). This definition of hybrid for golden-winged

warblers has been commonly employed (see Vallender et al. 2009), but is limited, because

mitochondrial DNA is only passed from the female parent and many hybrids are likely missed

with this criteria (Vallender et al. 2007a); that some Brewster's or otherwise phenotypically

hybrid individuals in this study had golden-winged warbler mitochondrial DNA reinforces this

notion. Age and Julian date were not included in this model because we found these factors to be

unimportant in our Manitoba study area, which covered the same possible ages and the entire

breeding season (Chapter 4).

5.3 Results

Of the 72 golden-winged warblers screened (Table 5.1), 24 were infected with blood parasites.

Four were infected with *Haemoproteus,* 11 were infected with *Leucocytozoon* and eight were

infected with *Plasmodium.* One sample repeatedly produced relatively weak bands on an agarose

gel, in comparison to controls, and that I could not sequence, and therefore could not be assigned accurately to genus. Hybrids were more likely to be infected with *Leucocytozoon* parasites (E = 1.38, SE = 0.74, p = 0.063), but were equally likely to have *Plasmodium* (p = 0.483) and *Haemoproteus* parasites (p = 0.994) (Figure 5.1). The two sites had equal *Haemoproteus* prevalence (p = 0.376), Carden had significantly higher *Plasmodium* prevalence (E = 2.03, SE = 0.88, p = 0.021), and Frontenac Arch had significantly higher *Leucocytozoon* prevalence (E = 2.28, SE = 1.13, p = 0.044), which is consistent with our results in Chapter 1.

Table 5.1. Number of hybrid and pure golden-winged warblers blood sampled from two study sites in Ontario in 2015.

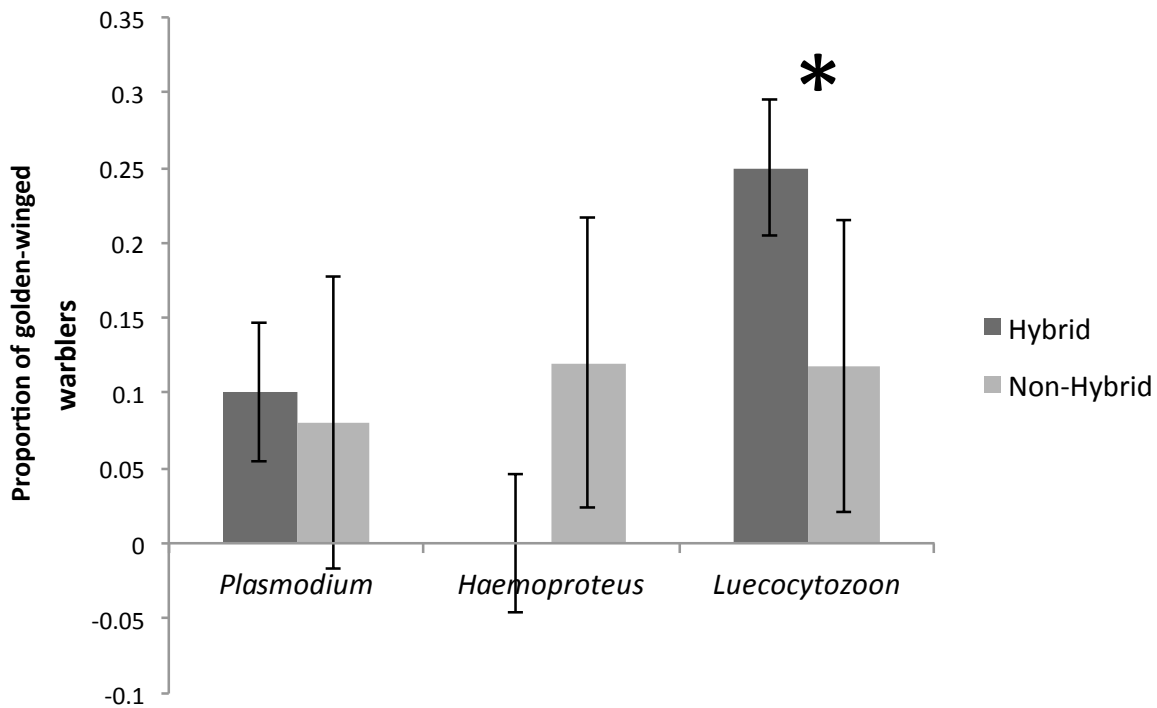| Study Site	Pure	Hybrid
Frontenac Arch	26	10
Carden	15	10

Figure 5.1. Proportion of blood parasite infected and uninfected golden-winged warblers that are genetically pure (Non-hybrid) or hybrids sampled in Southern Ontario in 2015, *n* =

72. Asterisks (*) indicate significant differences (*p* < 0.1). Error bars denote standard error.

5.4 Discussion

The higher prevalence of *Leucocytozoon* parasites in hybrid individuals compared to pure golden-winged warblers supports the hypothesis that outbreeding depression is occurring in this system. Golden-winged warblers may be more resistant to parasites than hybrid warblers, which could occur via several mechanisms. The other parent species (blue-winged warblers) may have poor genetic resistance to parasites, causing hybrids to inherit some poorly adaptive genes. Alternatively, the two parent species may have adapted to *Leucocytozoon* parasites through different genetic mechanisms, and the recombination of these genes could break down adaptive resistance. However, for either of these to be true, parasite resistance must be related to one of the few genes that differ between pure golden-winged warblers and blue-winged warblers, which is possible as the functionality of these genes is not entirely known (Toews et al. 2016). Additionally, to prove differential resistance we would need to control for exposure to infectious vectors, and given the mobility of birds and vectors, this could only effectively be done in a laboratory. However, habitat is a strong predictor of vector communities (Reiskind et al. 2017), and research in Manitoba suggests that golden-winged warblers are their hybrids select similar habitat types (Moulton et al. 2017); therefore, pure golden-winged warblers and their hybrids should be exposed to similar vector communities.

Conversely, it is also possible that hybrid golden-winged x blue-winged warblers have more *Leucocytozoon* parasites because they are better able to cope with and survive infections. If pure golden-winged warblers die or have reduced activity or aggression towards playback due to infection we may be less likely to capture them (Asghar et al. 2011). Cornwell encountered this problem in the black duck x mallard hybridization system, and suggested that monitoring the

success of hybrids versus genetically pure individuals in an area of parasitism should indicate whether increased prevalence of parasitism in hybrids is harming or facilitating the hybrids (1963).

I did not monitor the fitness of the birds in this study, but the pattern of lower hybrid presence in sites with high *Leucocytozoon* prevalence suggests that hybrids are more susceptible to parasites rather than more successful at surviving parasitism. Frontenac Arch, the site with the higher prevalence of *Leucocytozoon*, also has significantly fewer hybrids, which could indicate that parasites are fatal to hybrids (Rondel, personal communication). *Leucocytozoon* parasites are generally thought to be quite virulent, and our research indicates that golden-winged warblers in poor body condition may not be able to tolerate *Leucocytozoon* infection (Chapter 4). If fitness is reduced by *Leucocytozoon* infection, the lower prevalence of infection in pure golden-winged warblers may provide them a fitness advantage over hybrids in areas where *Leucocytozoon* parasites are common. Therefore, it seems that likely that *Leucocytozoon* parasitism is a force that is resisting hybridization, but further research should be done to compare the fitness of infected hybrids and infected pure golden-winged warblers in this area, and to test the role of differential exposure to parasite vectors.

Leucocytozoon parasites are more common in hybrid warblers in Ontario, and therefore this parasite genus may be promoting differentiation between the golden-winged and blue-winged warbler. Because hybrids persist, and because golden-winged warblers still appear to be replaced by blue-winged warblers and hybrids, the force is not strong across populations. However, in regions where *Leucocytozoon* parasites are common, hybrids are less common, and so this force might be locally important and should be explored as a possible mechanism explaining hybridization patterns among warblers and other species. I would suggest monitoring

the survival, reproductive success, and parasite status of a population of hybrid and pure

individuals over several years to determine the strength of this effect on hybridization.

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Chapter 6. Conclusions

The parasite community in golden-winged warblers appears to be shaped by host dispersal, land cover characteristics, and avian hybridization. The pattern of parasite prevalence and distribution of parasite lineages across the range of the golden-winged warbler suggests that parasites were transported with the individuals as they migrated north and west from their natal grounds. On a landscape scale, golden-winged warbler populations that were situated in a landscape with high cover of agriculture appeared to be more likely to be infected with both *Haemoproteus* and *Leucocytozoon* parasites. On a local scale, golden-winged warblers with estimated home ranges that had high cover of rangeland and linear disturbance features seem to be in poorer health, with lower fat scores and/or higher parasite prevalence, while birds near rivers seemed to be in better health, with higher fat scores and lower parasite prevalence. These trends might be due to increased vector prevalence near rangeland (González et al. 2013), decreased resource availability near rangeland and linear disturbances (Bond 2008, Prasad 2009), and increased resource availability near rivers. Lastly, hybrid golden-winged x blue-winged warblers had more *Leucocytozoon* parasites than genetically pure golden-winged warblers, suggesting that hybridization may erode natural adaptation to parasites (Moulia 1991), and that blood parasitism might be a force that resists hybridization between the two species when *Leucocytozoon* parasites are common.

The patterned distribution of golden-winged warbler parasites implies that the population is at risk of an overall increase in parasite prevalence in the future. Parasite prevalence in golden-winged warblers steadily increases to the north and west across the species' range, and different parasite lineages also appear to increase along the same trajectory, with novel lineages steadily appearing to the north and west. Golden-winged warblers are known to generally disperse north

and west from their natal grounds (Van Wilgenburg, unpublished data) and if birds dispersing from the southeast are susceptible to local northwestern parasites, this implies that the low prevalence of parasites in the southeastern proportion of the birds' range is not due to greater immune capabilities of these populations. The southern population of golden-winged warbler's range is shrinking, while the more parasitized northern population appears to be growing (Buehler et al. 2007). Thus, the future population of golden-winged warblers will likely be more parasitized overall. Additionally, if a novel parasite is introduced into the species' range, it is possible that dispersing birds will spread that parasite into the northwestern portion of the range.

It is also possible that Manitoban golden-winged warblers are not heavily affected by the parasites they harbour, and if they are less affected than other, similar species, it may provide the species a competitive advantage. For example, if golden-winged warblers facilitate the introduction of novel parasites to other early successional warblers, the other species may be more affected by the parasites, and less able to allocate energy to obtaining food resources or nesting sites. To test this hypothesis we would need to gather and compare parasite, foraging, and nest-site selection data among early successional warblers in the area.

The result that *Leucocytozoon* parasites were more common in hybrid than pure golden-winged warblers is important because hybridization with the blue-winged warbler has repeatedly been listed as a conservation concern for the species (Gill 1980, COSEWIC 2006, Species At Risk Act 2002, 2007, BirdLife International 2016, Environment and Climate Change Canada 2016). When hybrids appear, golden-winged warblers often disappear within a few decades (Gill 1980). Interestingly, the study area with significantly higher *Leucocytozoon* prevalence also had significantly fewer hybrids, and this suggests that *Leucocytozoon* parasitism could be helping to

maintain species boundaries in this system. This may help to explain the relatively low frequency of hybrids that we see in the Manitoba population (Vallender et al. 2009).

Conversion of forests to agriculture and rangeland appears to negatively affect golden-winged warbler health. These findings agree with a paradigm shift that is occurring in golden-winged warbler management; that focusing on preserving early successional habitat alone is not enough, and retaining natural forests is critical (Thogmartin 2010, Streby et al. 2014). Of course, replacing all agriculture with reforested habitat is not feasible. Instead, managers could prioritize habitat reserves for golden-winged warblers in areas with low landscape levels of agriculture land and low local cover of linear disturbances and rangeland. Retaining forest habitat would also benefit the many forest dependent species that co-occur with golden-winged warblers, such as ovenbirds (*Seiurus aurocapilla*) (Streby et al. 2014), and cerulean warblers (*Setophaga cerulea*) (Hamel et al. 2005).

The negative effect of landscape and local land cover on bird health is likely applicable to other bird species. I have shown that golden-winged warblers share parasites with passerines from other families (eg. Emberizidae, Paridae); therefore, these trends may be applicable beyond this taxonomic level. Because vectors are habitat specific (Reiskind et al. 2017), it is likely that these trends are most applicable to other early successional species in the same study areas. Therefore, increasing agriculture may increase parasite prevalence in other early successional species in the Eastern USA, and increasing cover of rangeland may affect various early successional species in Manitoba. Similarly, any bird that requires trees or shrubs to forage might experience a similar deterioration of body fat around rangeland or linear disturbances.

Historical grazing by bison might have had a much smaller effect on blood parasite prevalence. Though bison may have created larval habitat through wallowing depressions or

hoof prints, humans provide extra water sources for cattle, which provide larval habitat that would not be present with free ranging bison (Hartnet et al. 1997). Additionally, bison grazed nomadically and their populations were limited by drought and other natural constraints, which would have diluted the effects of grazing (Hartnett et al. 1997). This would likely have mitigated the negative effects of grazing on parasitemia; lower grazing intensity reduced parasitemia around grazed areas in the canyon towhee (*Melozone fusca*) (Reinoso-Pérez et al. 2016).

Two of the most pressing conservation concerns for the golden-winged warbler (habitat loss and hybridization with the blue-winged warbler) affect blood parasite prevalence. Examining host-parasites relationships has improved our understanding of this threatened species, and because these trends are likely not unique to the golden-winged warbler, it would be worthwhile to investigate the role parasites in the conservation of other species.

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