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Hitchhiker's guide to migration: effects of experimental parasitic infection and other immune challenges on migratory traits of sparrows

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Abstract

Seasonal migration exposes animals to a variety of habitats and parasites, and if infected migratory birds migrate successfully there is great potential for birds to transport infectious diseases long distances. Our current understanding of whether birds contribute to the spread of disease relies upon observational field studies that are limited in their ability to discern cause from effect. Using captive and field-based experiments for my doctoral research, I answered three research questions: (1) are nocturnal migratory restlessness (*Zugunruhe*) and body condition affected by mounting an acute phase immune response during migration; (2) what are the impacts of parasitic infection on *Zugunruhe* and body condition compared to those of upregulating immunity, and (3) are the observed consequences of successful parasite infection in captive conditions also realized in nature? Contrary to expectations, song sparrows (*Melospiza melodia*) and white-throated sparrows (*Zonotrichia albicollis*) challenged with lipopolysaccharide (LPS) and/or keyhole limpet hemocyanin (KLH) in captivity during autumn migration exhibited increases in body condition and did not reduce *Zugunruhe*. Finding no negative impacts of short-term immune challenges on migratory traits suggests that short-term activation of the acute immune response does not constrain migratory activity or preparation in these songbirds. Successful *Plasmodium* infection in captive white-throated sparrows did not reduce body condition but did reduce *Zugunruhe* once peak parasite loads were reached, two weeks after inoculation. In contrast, birds exposed to *Plasmodium* that did not become infected (resistant) exhibited reduced *Zugunruhe* immediately after exposure but did not differ from controls (not exposed) two-week after inoculation. Resistant song sparrows exposed to *Plasmodium* had reduced lean mass 12 days post-exposure in captivity. Once released, radio-telemetry tracking indicated no significant

difference in the departure date between controls, infected, and resistant song sparrows. These results are the first to demonstrate that exposure to malaria can impact migratory traits, independent of infection. Contrasting captive and field-based results highlight the importance of using similar methodological timelines, hosts, and pathogens to draw concrete conclusions regarding the impact of disease on migration. These studies are the first to explore the impacts of upregulating immunity and parasitic infection in controlled captive conditions. In combination, these findings indicate that models predicting disease spread should consider resistant-induced effects on body condition and migration alongside infection-induced effects.

Keywords

Passerine, malaria, migration, immune upregulation, body condition, *Zugunruhe*

Co-Authorship Statement

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"You'll get mixed up, of course, as you already know. You'll get mixed up with many strange birds as you go. So be sure when you step. Step with great care and great tact and remember that Life's a Great Balancing Act."

- Dr. Seuss

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LPS: lipopolysaccharide

KLH: keyhole limpet hemocyanin

Chapter 1

1 General Introduction

Migration is widespread across the animal kingdom and varies from the daily movements of plankton in the water column to the long distance, seasonal migrations of mammals, reptiles, fish, and birds. The broad taxonomic, temporal, and geographic scale that migration occurs means that a definition of migration depends on the perspective it is being explored (Dingle & Drake 2007; Dingle 2014). Birds are an ideal taxon to study migration as their migration has been well studied in the last 100 years (Bairlein 2003) and their migratory strategies are diverse. I define avian migration for this thesis as the seasonal movement of individuals between their breeding and wintering grounds. These movements may entail crossing continental-scale geographic obstacles such as oceans and mountain ranges, ultimately exposing them to multiple habitats and thus multiple suites of pathogens. These predictable mass-movements create ecological connections between otherwise isolated sites and may allow pathogen transport along their journeys, thus having important ecological, biogeographical and evolutionary consequences (Bowlin et al., 2010; Viana, Santamaría & Figuerola 2016). For example, exposure of pathogens to naïve hosts has had detrimental effects on host populations, as seen in the introduction of malaria (*Plasmodium relictum*) to the Hawaiian Islands (USA) in the early 20th Century that resulted in population declines of several native bird species (Van Riper III et al., 1986).

Birds have been implicated in the spread of West Nile virus (Rappole et al., 2000; Malkinson et al., 2002; Peterson, Vieglais & Andreasen 2003; Owen et al., 2006), avian

influenza virus (Reed et al., 2003; Gilbert et al., 2006; Olsen et al., 2006), and in the spread of vectors, such as ticks (Scott et al., 2001; Ogden et al., 2008; Cohen et al., 2015), that can harbour a variety of diseases. However, the study of long-distance dispersal of pathogens by birds is hindered by the low frequency of events (Altizer, Bartel & Han 2011), the difficulty of tracking pathogens and vectors over large geographic scales, and the unpredictable nature of long-distance dispersal operating at the grand scale of animals moving over long distances (Viana et al., 2016). Understanding how migratory birds contribute to the spread of disease depends fundamentally on how pathogens affect migration preparation and timing, but the evidence to date is mixed, convoluted by observational studies that fail to discern cause and effect (Risely, Klaassen & Hoye 2018), making it difficult to conclude the extent to which birds contribute to the global spread of disease and leaving researchers with more questions than answers: If birds exercise at high intensity to complete migratory bouts, how is it possible for them to do so while infected? At what points during infection can a bird afford to migrate? Are infected individuals culled from populations during migration? Answering questions such as these are critical to our understanding of how birds contribute to the spread of infectious disease and our ability to model disease spread.

In the remainder of this chapter I will provide background information on migration and pathogens, summarize research examining their relationship to date, and contrast the findings to current hypotheses regarding the dynamics between migration and infectious disease. Summarizing research that examined relationships between disease and migration of birds is crucial to elucidate the role migratory birds play in the global spread of disease. This information is increasingly important due to the shifting

ranges of parasites and vectors in response to our changing climate (Garamszegi 2011; Zamora-Vilchis, Williams & Johnson 2012).

1.1 Why migrate?

From an ultimate perspective, organisms migrate to increase fitness (Dingle 2014). Seasonal changes in a habitat's ecology and climate are the basis for three central hypotheses explaining the evolution of bird migration: food limitation, direct climatic effects on physiological function, and risk of nest predation (Boyle & Conway 2007). Seasonally unreliable food (e.g., fruit, insects, or nectar) may force a species to migrate to a more abundant food source (Levey & Stiles 1992; Boyle & Conway 2007; Boyle 2010; Jahn et al., 2010). Second, abiotic conditions may induce migration if the changes indicate the individual cannot survive the thermal extremes (Kendeigh 1945; Ketterson & Nolan Jr 1976; Cristol, Baker & Carbone 1999; Jahn et al., 2010). This is proposed through two mechanisms: (1) the thermal tolerance hypothesis suggests migratory individuals are those that cannot survive thermal extremes (e.g., small individuals with unfavourable surface area to volume ratios) and/or (2) the fasting endurance hypothesis that predicts individuals at a greater risk of starvation (i.e., small individuals) will migrate (Ketterson & Nolan Jr 1976; Cristol et al., 1999). Last, migratory movement may be favoured if there is latitudinal or altitudinal variation in nest predation and moving to the breeding grounds enables migrants to reduce nest failure (Fretwell 1968; Zimmerman 1984; Cox 1985; Boyle & Conway 2007). It is important to note that these hypotheses might not be mutually exclusive; a small-bodied bird might migrate because suitable food resources are not available year-round in one location but also to avoid thermal extremes due to their small surface area to volume ratio. Because the ecological factors that have

driven the evolution of migration may affect species, or individuals, differently it is predicted that migration may vary at the species, population, or individual level.

1.2 Avian migration varies over time and space

1.2.1 Variation in migration distance

Regardless of the ultimate fitness consequences of migrating, natural selection has produced a variety of migratory strategies. Species differ in migratory distances, and similar differences can exist within species, typically among population classes such as sex and age classes (Ketterson & Nolan Jr 1985). For example, female and juvenile blue tits (*Parus caeruleus*) are more likely to migrate than males and adults (Smith & Nilsson 1987). This phenomenon, termed *partial migration*, includes species in which some, but not all, individuals migrate. Chapman et al. (2011) identify three categories of partial migration:

1. Non-breeding partial migration: All individuals breed in the same region but some individuals migrate in the non-breeding season while others remain on the breeding grounds.
2. Breeding partial migration: Individuals breed in different regions but overwinter in the same location.
3. Skipped breeding partial migration: While individuals must migrate to breed, some individuals do not do so every year, and remain on the non-breeding grounds during non-migration years.

Similar to partial migration, *differential migration* occurs when all individuals migrate between the breeding and non-breeding grounds but individuals vary predictably from one another in the distance travelled and/or the timing of migration, typically according to age or sex classes (Ketterson & Nolan Jr 1985; Terrill & Able 1988). Thus,

partial migration describes the situation where some individuals remain sedentary whereas in differential migration all individuals migrate, but migration distance varies.

Generally, if all individuals of a species or population are seasonal migrants, the benefits of migration should consistently outweigh the costs. Likewise, if some, but not all, individuals migrate, the relative advantages of migrating versus remaining year-round on the breeding grounds may vary across individuals and across age and sex classes (Ketterson & Nolan Jr 1985). Benefits of short migrations (or remaining resident) include avoiding flight costs such as the energy required to travel and physiological damage to tissues from exercise (McWilliams et al., 2004), but may also allow earlier return to the breeding grounds, a particular advantage to males competing to acquire high-quality breeding territories (Fudickar et al., 2013).

1.2.2 Variation in migration timing

In addition to distance, population classes may vary in the timing of their arrival to the breeding grounds, with ultimate consequences for individual fitness. Sexes differ in their investments in reproduction (Orians 1969) and these differences have resulted in differing ecological strategies relating to migration. Often observed is the arrival of males to the breeding grounds before females, known as *protandry* (Morbey & Ydenberg 2001). For most species, male investment in reproduction entails procuring and defending a breeding territory (Orians 1969), but high-quality territories are limited in supply. Late-arriving males run the risk of being left with poor-quality territories, if one at all, with detrimental effects on reproductive success for that season (Cristol 1995). In contrast, female investment requires production of gametes with a large amount of energy (Orians 1969), but this is not limited by their arrival to breeding grounds. Thus, while failure to

arrive early for males may result in failing to reproduce for a season, late-arriving females likely suffer only a delay in egg laying (Cristol 1995). Thus, differences between the sexes in the intensity of intrasexual selection might explain the shorter migration distances and earlier arrival of males (Cristol 1995; Cristol et al., 1999).

The arrival time hypothesis (Ketterson & Nolan Jr 1976; Fudickar et al., 2013), also known as the rank advantage hypothesis (Morbey & Ydenberg 2001), considers territory acquisition as the ultimate benefit for early arrival to breeding grounds. Simply, there is selection on the sex that establishes and defends breeding territories (typically males) to arrive earlier than other males. Similar to the arrival time hypothesis, the mate opportunity hypothesis (Wiklund, Wickman & Nylin 1992; Kokko et al., 2006) posits that the earlier arrival of males will maximize the number of opportunities for them to mate. Originally established in studies of insect emergence times, this hypothesis holds particularly well in populations with male-biased sex ratios (Kokko et al., 2006). The arrival time and mate opportunity hypotheses have the strongest support so far for migratory, territorial birds (Kokko et al., 2006), but Morbey and Ydenberg (2001) summarize additional hypotheses for protandry. Mechanisms of protandry likely differ across species and need not be mutually exclusive. Regardless of the ultimate factors for earlier arrival, individuals may adjust their arrival to the breeding grounds by wintering closer to them (Ketterson & Nolan Jr 1976; Cristol 1995), initiating migration and departing for spring migration earlier than conspecifics (Maggini & Bairlein 2012; Deakin 2017), and/or by increasing the rate of migration by making fewer stops during migration, stopping for shorter periods of time or flying at faster speeds (Dierschke, Mendel & Schmaljohann 2005).

1.3 Costs of migration

Of all phases of the annual cycle, those involving migration are arguably the most energetically demanding (Wikelski et al., 2003). The energetic cost of flight varies among and within species, due to differences in a bird's mass, mode of flight (e.g., soaring vs. flapping), and temperature of the air (Schmidt-Nielsen 1972; Hedenström 1993; Wikelski et al., 2003); regardless, the minimum oxygen consumption associated with flight can be over twice that of running by terrestrial mammals (Butler, Woakes & Bishop 1998). Moreover, migratory birds routinely fly for extended distances, up to thousands of kilometers, to cross geographic barriers. For example, bar-tailed godwits travel 29 000 km in only 20 days (Battley et al., 2012); great snipes (*Gallinago media*) fly up to 6800 km in three days (Klaassen et al., 2011); arctic terns travel up to 670 km in a single day (Egevang et al., 2010); and Swainson's thrush (*Catharus ustulatus*) and hermit thrush (*C. guttatus*) fly up to 600 km in a single flight (Wikelski et al., 2003). Unlike running mammals, birds cannot reduce energy costs of flight by flying more slowly as the relationship between power and speed is U-shaped, and the cost of flying is minimized at an intermediate speed (Hedenström & Alerstam 1997). Furthermore, migrating birds are heavily burdened with fat used to fuel their long flights, incurring additional energetic demands (McWilliams et al., 2004).

Migration can be treacherous not only due to the energy an individual must expend for its completion, but biotic and anthropogenic effects can impact the success of migration. Mortality is greatest during migration in in black-throated blue warblers (*Dendroica caerulescens*) compared to overwintering or breeding (Sillett & Holmes 2002). Furthermore, mortality is related to migration distance in dark-eyed juncos

(Ketterson & Nolan 1985) and the annual mortality of various raptor species is greatest during migration (Klaassen et al., 2014). Mortality during migration may be due to increased exposure to predators. Research focused on predation of birds during stopover found decreased fuel deposition rates (Schmaljohann & Dierschke 2005) as well as decreased foraging and movement (Cimprich, Woodrey & Moore 2005) when the risk of predation was high. Even if individuals evade predation, predatory threats still interfere with restoring depleted energy stores, a main purpose of stopover, and may ultimately impede the success of migration. Indeed, the nocturnal migration of passerines is thought to decrease the exposure to predators (Kerlinger & Moore 1989). Mortality may also be due to collisions with unfamiliar anthropogenic structures such as tall buildings (Gehring, Kerlinger & Manville II, Albert 2011), communication towers (Longcore et al., 2012) and/or wind turbines (Hüppop et al., 2006).

If migration is successful, optimal investment in migration can trade-off with reproductive success due to their adjacent timing, as inferred by the arrival time hypothesis described previously. This hypothesis is supported by early arriving male American redstarts (*Setophaga ruticilla*) being more likely to mate (Lozano et al., 1996) and early arriving females have heavier nestlings (Smith & Moore 2005). Furthermore, female snow geese (*Anser caerulescens atlanticus*) that arrive earlier than average have the greatest reproductive success (Bêty, Giroux & Gauthier 2004) and delayed arrival in female red-winged blackbirds (*Agelaius phoeniceus*) delays laying date (Cristol 1995). Despite the costs and inherent risks, migration is a dynamic life-history trait necessary for survival in many species that complete it, but which must be traded off against other life-

history traits. In addition to the costs describe above, there are other threats to the successful migration of birds, some of which are invisible to the naked eye…

1.4 Pathogenic hitchhikers during migration

Earth is home to a great diversity of pathogens that cause infection (growth of an organism in the body of the host) or disease (deviation from a normal state of health) in other organisms (Hoffman 2007). Most pathogens are parasites (representing about 40 % of known species [Dobson et al., 2008]) that reside in or on another organism (the host) which they exploit for resources. In addition to parasites, viruses, bacteria, prions, and fungi can also cause disease in a host (collectively termed and herein referred to as pathogens). Whether infection of a host with a pathogen will result in disease depends on a variety of physiological and ecological parameters such as the condition of the host, previous exposure of the host to the pathogen (immunity), and pathogenicity of the pathogen (Hoffman 2007). Successful invasion by a pathogen can have negative fitness effects on the host such as negative effects on host physiology (Merino & Potti 1995; Garvin, Szell & Moore 2006; Marzal et al., 2008; Shutler, Alisauskas & Daniel McLaughlin 2012; Schoenle et al., 2017), reproductive success (Rätti, Dufva & Alatalo 1993; Sundberg 1995; Richner & Tripet 1999; Marzal et al., 2005; Knowles, Palinauskas & Sheldon 2010), and survival (Brown, Brown & Rannala 1995; Merino & Potti 1995; Marzal et al., 2008; Knowles et al., 2010; la Puente et al., 2010).

Ideally, hosts would avoid exposure to pathogens throughout their lifetime to evade such negative fitness effects. However, the diversity of infectious agents and their prevalence on all continents (Schmid-Hempel 2011) means that few organisms can avoid exposure to parasites during their lifetime. Furthermore, migratory animals use multiple

environments during the annual cycle that can expose them to a greater number and diversity of pathogens compared to resident species (*migratory exposure hypothesis*; Møller & Erritzøe 1998; Figuerola & Green 2000; Møller & Szép 2010; Kelly et al., 2016). The high risk of pathogen exposure for migrants has raised concern regarding their ability to transport pathogens long-distances (Altizer et al., 2011) and, for pathogens limited in mobility, migratory animals are an opportunity to disperse greater distances than their physiology allows. Because migration can increase rates of contact between hosts and parasites, often while immune function is compromised due to trade-offs with sustained exercise (Owen & Moore 2008; Nebel et al., 2012; Dolan et al., 2016; Eikenaar & Hegemann 2016; Van Dijk & Matson 2016), it is reasonable to expect that migration enhances the spread of disease. However, in some systems migration may inhibit disease transmission, for example if infected hosts are unable to migrate successfully (*migratory culling hypothesis*; Bradley & Altizer 2005) or if migration allows hosts to escape from infected habitats *(natural enemy release;* Loehle 1995; *migratory escape* Bartel et al., 2011). Even in systems where infected hosts complete their migration, such individuals may delay departure from the breeding grounds or stopover sites (van Gils et al., 2007). Models of disease transmission predict that these infection-induced delays in migration should decrease infection rates by reducing contact between infected and uninfected hosts (*migratory separation*; Galsworthy et al., 2011). Evaluating this variety of different hypotheses regarding the relationships between pathogens, migration, and disease transmission has been the focus of several studies, mostly observational in nature. While I describe four hypotheses related to migration and disease to provide background knowledge, I only test the migratory separation hypothesis in this thesis.

Field studies on free-living animals provide some evidence that infection may affect migratory timing, potentially mediated through effects on body condition and stores. Risely, Klaassen, and Hoye (2018) compared 41 studies (85 observations across vertebrate and invertebrate species) to examine whether infection affects changes in body stores, refueling rates, movement capacity, phenology, and survival. Generally, host infection status was weakly associated with reduced body stores, delayed migration, and lower survival. This comprehensive analysis revealed there remains little understanding of how infections affect hosts during the migratory period, which is critical to our understanding of pathogen transmission. I have summarized 36 studies examining pathogenic effects on avian host traits during different migratory stages for a total of 51 hypotheses tests in Table 1-1. The studies are organized by the ecological pattern between a migratory trait and infection (e.g., infected individuals arrive or depart later than uninfected conspecifics) and then classified to the hypothesis they support (e.g., migratory separation). I evaluated whether the studies support, partially support, or do not support the hypothesis while noting the study design (observational or experimental; field or captive), the period of migration that the study took place, and state the host species and pathogen under examination. To be considered, the study must have taken place during migration or coincided with arrival or departure, except for studies investigating the prevalence of pathogens in relation to migration distance or wintering latitude that support migratory exposure.

Table 1-1 Summary of studies to date that investigate the effect of pathogens on their host during migration. Studies are organized by the ecological finding followed by the related hypothesis, support, and last by year of publication. Details from the study are also described: host species, pathogen, migratory stage the study took place, and study design.

ecological pattern	related hypothesis	support or not; reference	host	pathogen	migration stage	study design
infected individuals arrive / depart later than uninfected conspecifics	migratory separation; reduce disease spread	support; [1]	barn swallow (*Hirundo rustica*)	chewing louse (*Hirundoecus malleus*); *Haemoproteus prognei*	arrival to breeding	observational; field
		support; [1]	barn swallow (*Hirundo rustica*)	hematophagous mite (*Ornthonyssus bursa*)	arrival to breeding	experimental; field
		Support; [2]	tree pipit (*Ambus trivialis*); willow warbler (*Phylloscopus trochilus*); common redstart (*Phoenicurus pheonicurus*); dunnock (*Prunella modularis*); European robin (*Erithacus rubecula*); song thrush (*Turdus philomelos*)	*Haemoproteus, Plasmodium, Leucocytozoon*	stopover duration	observational; field
		partial; [3]	great reed warbler (*Acrocephalus arundinaceus*)	*Haemoproteus payevskyi* GRW1*; Plasmodium ashfordi* GRW2*; P. relictum* GRW4	arrival to breeding	observational; field

		partial; [4]	garden warbler (*Sylvia borin*)	*Haemoproteus, Plasmodium, Leucocytozoon;* intestinal parasites *(coccidians & spirurids)*	spring stopover	observational; field
		partial; [5]	blackcap (*Sylvia atricapilla*)	*Haemoproteus prarbelopolsky*	arrival to breeding	observational; field
		partial; [6]	barn swallow (*Hirundo rustica*)	fungi and bacteria	arrival to breeding	observational; field
		unsupported; [7]	purple martin (*Progne subis*)	*Haemoproteus prognei*	arrival to breeding	observational; field
		unsupported; [8]	great reed warbler (*Acrocephalus arundinaceus*)	*Haemoproteus & Plasmodium*	departure from wintering	observational; field
infected individuals move less than uninfected conspecifics	migratory separation; reduce disease spread	supported; [9]	Bewick's swans (*Cygnus columbianus bewickii* Yarrell)	low-pathogenic avian influenza A virus (LPAIV)	departure from wintering	observational; field
		unsupported; [10]	Bewick's swans (*Cygnus columbianus bewickii*)	LPAIV	fall migration	observational; field
		unsupported; [11]	white-fronted geese (*Anser albifrons albifrons*)	avian influenza virus	winter arrival	observational; field
		unsupported; [12]	mallard (*Anas platyrhynchos*)	LPAIV	fall migration	observational; field

		unsupported; [13]	mallard (*Anas platyrhynchos*)	LPAIV	fall stopover	observational; field
		unsupported; [2]	tree pipit (*Ambus trivialis*); willow warbler (*Phylloscopus trochilus*); common redstart (*Phoenicurus pheonicurus*); dunnock (*Prunella modularis*); European robin (*Erithacus rubecula*); song thrush (*Turdus philomelos*)	*Haemoproteus, Plasmodium, Leucocytozoon*	stopover duration	observational; field
reduced refueling rate in infected individuals	migratory separation; reduce disease spread	supported; [9]	Bewick's swans (*Cygnus columbianus bewickii* Yarrell)	LPAIV	departure from wintering	observational; field
		unsupported; [14]	blackcap (*Sylvia atricapilla*)	*Haemoproteus, Plasmodium, Leucocytozoon*	fall stopover	observational; field
		unsupported; [15]	yellow-rumped warbler (*Dendroica coronata*); magnolia warbler (*Dendroica magnolia*)	Haematozoa (*Haemoproteus, Plasmodium, Leucocytozoon, Trypanosoma*)	spring stopover	observational; field
immune challenge reduces	migratory separation; reduce	partial; [16]	common redstart (*Pheonicurus pheonicurus*); tree pipit (*Anthus trivialis*); willow	lippopolysaccharide	fall stopover	experimental; field

migratory movement	disease spread		warbler (*Phylloscopus trochilus*); dunnock (*Prunella modularis*); European robin (*Erithacus rubecula*); song thrush (*Turdus philomelos*)			
		unsupported; [17]	western sandpiper (*Calidris mauri*)	lippopolysaccharide	n/a	experimental; captive
immune challenge reduces condition	migratory culling and/or migratory separation; reduce disease spread	unsupported; [18]	Skylark (*Alauda arvensis)*	lippopolysaccharide	n/a	experimental; field
		unsupported; [17]	western sandpiper (*Calidris mauri*)	lippopolysaccharide	n/a	experimental; captive
infected individuals are in poor condition	migratory culling and/or migratory separation; reduce disease spread	support; [1]	barn swallow (*Hirundo rustica*)	*n/a*	arrival to breeding	observational; field
		support; [10]	Bewick's swans (*Motacilla flava feldegg; Motacilla flava flava*)	LPAIV	fall migration	observational; field
		supported; [19]	2 subspecies of yellow wagtails (*Motacilla flava feldegg; M. flava flava*)	*Haemoproteus motacillae, H. anthi, Plasmodium relictum, P. subpraecox, P.*	spring and fall migration	observational; field

		cathemerium, and Trypanosoma avium		
partial (findings differ between hosts); [20]	scarlet tanager (*Piranga olicacea*); summer tanager (*P. rubra*); rose-breasted grosbeak (*Pheucticus ludovicianus*); Baltimore oriole (*Icterus galbula*); red-eyed vireo (*Vireo olivaceus*); Swainson's thrush (*Catharus ustulatus*)	*Haemoproteus* & *Leucocytozoon*	spring stopover	observational; field
partial; [15]	yellow-rumped warbler (*Dendroica coronata*); magnolia warbler (*D. magnolia*)	*Haemoproteus, Plasmodium, Leucocytozoon, Trypanosoma*	spring stopover	observational; field
partial; [11]	white-fronted geese (*Anser albifrons albifrons*)	avian influenza virus	winter arrival	observational; field
partial; [21]	lesser snow geese (*Chen caerulescens caerulescens*)	nematode, trematode, cestode, and helminths	spring migration	observational; field
partial; [22]	great reed warbler (*Acrocephalus arundinaceus*)	*Plasmodium relictum* (pGRW04)	fall migration compared to breeding	experimental; captive

		unsupported; [23]	American kestrel (*Falco sparverius*)	*Haemoproteus*	arrival to breeding	observational; field
		unsupported in two inoculations; [24]	great reed warbler (*Acrocephalus arundinaceus*)	*Plasmodium ashfordi* (lineage GRW2); *P. relictum* (GRW4)	fall departure; fall migration	experimental; captive
		unsupported; [14]	blackcap (*Sylvia atricapilla*)	*Haemoproteus, Plasmodium, Leucocytozoon*	fall stopover	observational; field
		unsupported; [25]	gray catbirds (*Dumetella carolinensis*); common yellowthroats (*Geothlypis trichas*); western palm warblers (*Dendroica palmarum palmarum*)	*Haemoproteus, Plasmodium*	fall migration	observational; field
		unsupported; [12]	mallard (*Anas platyrhynchos*)	LPAIV	fall migration	observational; field
		unsupported; [8]	great reed warbler (*Acrocephalus arundinaceus*)	*Haemoproteus, Plasmodium*	departure from wintering	observational; field
death of infected individuals after	migratory culling; reduce	support; [26]	white stork (*Ciconia ciconia*)	West Nile virus	fall migration, blown off-route	observational; field; opportunistic

migratory bout	disease spread	partial; [23]	American kestrel (*Falco sparverius*)	*Haemoproteus*	arrival to breeding	observational; field
		unsupported; [3]	great reed warbler (*Acrocephalus arundinaceus*)	*Haemoproteus payevskyi* GRW1; *Plasmodium ashfordi* GRW2; *P. relictum* GRW4	arrival to breeding	observational; field
		unsupported; [27]	ruddy turnstones (*Arenaria interpres morinella*)	LPAIV	spring stopover	observational; field
few infected individuals post-migration	migratory culling; reduce disease spread	indirect support; [7]	purple martin (*Progne subis*)	*Haemoproteus prognei*	arrival to wintering	observational; field
		support; [28]	lesser black-backed gulls (*Larus fuscus*)	avian influenza seroprevalence	arrival to breeding	observational; field
longer migrations lead to increased pathogen exposure; migrants have more	migratory exposure; enhance disease spread	indirect support; [29]	Variety of closely related migrants and residents	n/a	n/a	observational; museum specimens
		support; [30]	Anseriformes (ducks, geese, swans)	haematozoa	n/a	observational; field
		support; [31]	Anseriformes, Accipitriformes, Turdidae	nematode	n/a	observational; field

pathogens than residents		supported; [32]	song sparrow (*Melospiza melodia*)	*Haemoproteus, Plasmodium*	arrival to breeding	observational; field
		unsupported; [28]	lesser black-backed gulls (*Larus fuscus*)	*Haemoproteus*, paramyxovirus, avian influenza seroprevalence	n/a	observational; field
		unsupported; [33]	European passerines	helminth species	n/a	observational; field
		unsupported; [34]	clay-coloured sparrow (*Spizella pallida*), chipping sparrow (*S. passerina*), black-chinned sparrow (*S. atrogularis*)	*Haemoproteus, Trypanosoma, Microfilariae*	fall migration (dry season)	observational; field
		unsupported; [35]	Charadriiform birds	helminth species	n/a	observational; field
greater prevalence of pathogens at lower latitudes	migratory exposure; enhance disease spread	support; [36]	wading shorebirds (Charadriiformes)	*Haemoproteus, Plasmodium*	n/a	observational; field
		partial; [37]	26 bird species	*Haemoproteus, Plasmodium*	n/a	observational; field

References for Table 1-1:

[1] Møller, De Lope & Saino 2004; [2] Hegemann et al., 2018; [3] Asghar, Hasselquist & Bensch 2011; [4] López et al., 2013; [5] Santiago-Alarcon et al., 2013; [6] Rubaiee, Murayati & Møller 2018; [7] Davidar & Morton 1993; [8] Sorensen et al., 2016; [9] van Gils et al., 2007; [10] Latorre-Margalef et al., 2009; [11] Kleijn et al., 2010; [12] van Dijk et al., 2015; [13] Bengtsson et al., 2016; [14] Arizaga, Barba & Hernández 2009; [15] DeGroote & Rodewald 2010; [16] Hegemann et al., 2018; [17] Nebel et al., 2013; [18] Hegemann et al., 2012; [19] Shurulinkov, Chakarov & Daskalova 2012; [20] Garvin, Szell & Moore 2006; [21] Shutler, Alisauskas & McLaughlin 2012; [22] Dawson & Bortolotti 2000; [23] Dawson & Bortolotti 2000; [24] Zehtindjiev et al., 2008; [25] Cornelius, Davis & Altizer 2014; [26] Malkinson et al., 2002; [27] Maxted et al., 2012; [28] Arriero et al., 2015; [29] Møller & Erritzøe 1998; [30] Figuerola & Green 2000; [31] Koprivnikar & Leung 2015; [32] Kelly et al., 2018; [33] Bandelj et al., 2015; [34] Carbó-Ramírez & Zuria 2015; [35] Gutiérrez et al., 2017; [36] Clark, Clegg & Klaassen 2016; [37] Merino et al., 2008

The 37 articles (51 studies) included in Table 1-1 took place over 25 years and represent the different migration stages well, except for departure from the breeding grounds (Table 1-2). Half (54 %; 26 studies) of the studies examined blood parasites (Haematozoa) and avian influenza virus was examined in nearly one quarter (25 %; 12 studies) of the studies. The remaining 21 % (12 studies) leave helminth species, mites, West Nile Virus, fungi, bacteria, and immune challenges weakly represented (Table 1-2). Most ecological patterns had representation from a variety of hosts and pathogens, except for reduced movement of infected individuals that was only represented by low-pathogenic avian influenza virus and Anseriformes, and the greater prevalence of pathogens at lower latitudes was only represented by haematozoa. Evidence of migratory culling (Bradley & Altizer 2005) was evenly split between being supported, having partial support, and being unsupported (6 support, 6 partial, 8 unsupportive). Migratory separation (Galsworthy et al., 2011) attained weak overall support (7 support, 10 partial, 15 unsupportive) while migratory exposure (Møller & Erritzøe 1998) had more support than not (5 support, 1 partial, 3 unsupportive), albeit having the smallest number of studies examined.

Table 1-2 The number of investigations included in Table 1-1 sorted by the trait examined, pathogen, host family, host migration strategy, stage of migration examined, and study design. Studies using comparisons across families [28 – 30, 32, 34 – 36] are not included in family specifications.

variable	level		n
trait	body stores		15
	refuelling		3
	metabolic rate		2
	survival		6
	movement		22
host classification	Anseriformes		
		Anatidae	10
	Charadriiformes		
		Laridae	2
		Scolopacidae	2
	Ciconiiformes		
		Ciconiidae	1
	Falconiformes		
		Falconidae	2
	Passeriformes		
		Acrocephalidae	5
		Alaudidae	1
		Cardinalidae	3
		Hirundinidae	5
		Icteridae	1
		Mimidae	1

	Motacillidae	3
	Muscicapidae	4
	Parulidae	8
	Passerellidae	4
	Phylloscopidae	2
	Prunellidae	2
	Sylviidae	4
	Turdidae	3
	Vireonidae	1
host migration strategy	resident	1
	short distance (continental)	33
	long distance (intercontinental)	31
pathogen	protozoa	30
	virus	14
	mites	2
	helminths	5
	fungi / bacteria	1
	immune challenge	4
migration stage	fall departure	1
	during fall migration, including stopover	16
	arrival to wintering grounds	3
	spring departure	3
	during spring migration, including stopover	7
	arrival to breeding grounds	10

study design	observational; field	46
	experimental; field	2
	experimental; captive	4

The mixed support for the hypotheses above may suggest that the effects of pathogens on migratory traits of their host may be species-specific as a result of coevolutionary history between the pathogen and host. Alternatively, Table 1-2 reveals that observational studies dominate the literature (88 %) – consistent with Risely et al.'s (2018) meta-analysis (80 %) – with only six studies including an experimental approach. Studies relating natural variation in parasite load and prevalence to variation in body condition, refueling, and migratory movement and timing provide an important foundation to our understanding of interactions between parasites and migration. However, observational field studies of naturally occurring variation in infection status or parasite load are generally unable to detect individuals that do not survive infection and cannot determine how long a host had been exposed to the pathogen. Thus, the lack of control in these studies may underestimate pathogenic effects on condition and migratory performance. Moreover, even observational studies that find a relationship between a migratory trait and pathogenic infection remain limited in their ability to infer the direction of causation; naturally infected individuals may suffer reduced body condition or migratory delays due to the cost of parasitic infection, but an alternative explanation is that poor body condition or late departure timing increases susceptible to infection. Observational studies are also unable to quantify the effects of resisting pathogenic infection as it is difficult to assess whether an individual has been exposed to a pathogen, let alone ensure the encounter was recent. Pathogenic exposure will induce an immune response, but it remains unknown whether such upregulation trades-off with migratory traits independent of pathogenic effects. The literature review above highlights the need

for controlled experiments that manipulate pathogen exposure in order to discern the causal effects of infection, successful or not, on avian migration.

1.5 Balancing immunity with migration

Animals are unlikely to avoid pathogens throughout their lifetime and consequently have evolved a range of behavioural and physiological defences to help avoid, resist, or control pathogen invasion (Klasing 2004). The multiple environments migratory animals are exposed to in their lifetime has been associated with a greater likelihood of infection (Møller & Erritzøe 1998; Figuerola & Green 2000; Kelly et al., 2016). From the perspective of selection imposed by parasitism, animals are expected to match their investment in immune defence to the pathogenic threats they face and thus migrant birds are expected to invest more in immune defence than residents (Møller & Erritzøe 1998; Altizer et al., 2011). Indeed, the perspective of migratory culling and migratory escape predict selection for higher investment in mechanisms of disease resistance (Arriero et al., 2015). However, immune defence is costly, not only with respect to the metabolic and nutritional demands of 'surveillance' immune cells that must be continually maintained (phagocytes and lymphocytes; Klasing 2004; Buehler, Tieleman & Piersma 2010), but also due to the indirect consequences of immune upregulation paid in collateral damage to the host when the immune system harms the host as well as invaders (immunopathology costs; Raberg et al., 1998; Buehler, Tieleman & Piersma 2010). For example, during intense exercise, such as sustained flight for migration, muscle damage and heat-shock proteins stimulate the immune system in the same way as damage caused by infection, causing a response directed against the host (Raberg et al., 1998). Additional losses can include wasted lean tissue in migrants with

chronic infections as a result of excessive gluconeogenesis to fuel immune cells and anorexia during a period when the body requires nutrients to support the demands of mounting an immune response (Lochmiller & Deerenberg 2000). Thus, while investment in an immune defence is necessary to limit harmful pathogenic effects, immunity also incurs costs in many different forms.

Animals cannot maximize investment in all physiological traits at once, and the high metabolic and nutritional demands of immune upregulation and flight predict a trade-off between investment in either life-history trait (Altizer et al., 2011). If this is true, a trade-off prioritizing immunity should decrease migration efficiency, but this contradicts the predicted increased immune investment from the perspectives of migratory culling and migratory separation. Resources are limited during migration thus if migrants cannot invest appropriately in immunity during migration they may become vulnerable to infection and, if they survive, contribute to the spread of pathogens. Alternatively, migrants may opt to tolerate an infectious pathogen rather than clear the infection altogether in order decrease immunopathology costs (Raberg et al., 1998) and increase migratory efficiency.

A final consideration of how migrants may balance immunity with migratory flight is that immune function is complex and multi-faceted and different types of immunity have different costs and benefits (Klasing 2004; Demas et al., 2011). Thus, migrants may invest in one branch of the immune system over another rather than maximizing all immune traits at once. The immune response can be organized into two branches according to their effectiveness against foreign (innate immunity) or familiar pathogens (acquired immunity; Klasing 2004; Demas et al., 2011). Briefly, the innate

response is the initial defence against invading pathogens and consists of a standing array of rapid and nonspecific responses, including anatomical barriers (mucosal membranes and skin), humoral factors (lysozyme, complement, acute phase proteins) and cellular responses (phagocytic cells, inflammatory mediating cells, and natural killer cells; Klasing 1998, 2004; Demas et al., 2011). In contrast, the acquired response permits antigen-specific responses produced by B lymphocytes and creates a recognition system to prevent a second infection by a previously encountered pathogen (Demas et al., 2011). Innate responses incur high costs due to continual maintenance of protective cells (phagocytes) and proteins (natural antibodies; Klasing 2004), but the non-specificity of innate immunity allows quick development and provides defence against a diverse suite of pathogens. Acquired immunity uses antibodies to recognize re-encountered antigens, but the development of antibodies relies upon semi-random recombination and point mutations in their coding genes which rarely results in a functional antibody (Klasing 2004). Fortunately, random recombination does allow recognition of diverse antigens and once an antibody is created for an antigen, the antibody remains in the system for future recognition. Thus, in contrast to innate defences, the costs of acquired immunity arise from the long time needed to develop an appropriate antibody (Klasing 2004). It is possible, then, that individuals may strategically invest in one branch of immunity over another according to the familiarity of pathogens they encounter (Kelly et al., 2017).

Few studies have examined whether the additional parasites migrants are exposed to result in greater immunity of migrants, as predicted by migratory culling and migratory escape/separation, or whether energetic costs of flight impede immune investment. Table 3 summarizes the 13 studies that investigate the immunity of migrants during migration,

effects of endurance flights on immunity, contrast immunity of migrants across the annual cycle, and compare immunity of migrants to resident populations and/or closely related species. I evaluated whether the studies support, partially support, or do not support predictions from migratory culling (greater immunity), migratory separation (greater immunity), and migratory exposure (hampered immunity). The study species, parameter of immunity investigated, stage of migration, and study design are also noted.

Table 1-3 Summary of studies to date that investigate the relationship between immunity and migratory traits. Studies are organized by the ecological finding and by year of publication. Details from the study are also described: support for hypotheses describing the relationship between pathogens and migrants, species, parameter(s) of immunity examined, migration stage the study took place, and study design.

pattern	migratory culling; selection for greater immunity	migratory separation; selection for greater immunity	migratory exposure; low immunity enhances susceptibility to pathogens	species	immune function examined	migratory stage	study design
immunity is suppressed during migration			support; [37]	wood thrush (*Hylocichla mustelina*); Swainson's thrush (*Catharus ustulatus*); Veery (*C. fuscescens*)	IgY titres, leukocyte counts	compared migration to breeding	observational; field
			support; [38]	Swainson's thrush (*Catharus ustulatus*)	PHA challenge	compared migration to breeding	experimental; captive
	partial	partial	partial; [17]	Skylarks (*Alauda arvensis)*	natural antibodies, complement activity, haptoglobin, leukocyte counts	compared stages of annual cycle	observational; field

migratory / long-distance populations have stronger immunity than resident/ short distance populations	support	support; [28]		compared closely related migrant and resident species	size of bursa of Fabricius and the spleen	n/a	observational; field
	support	support; [39]		scarlet rosefinch (*Carpodacus erythrinus*)	MHC class I exon 3	n/a	observational; field
	partial	partial [2]		tree pipit (*Ambus trivialis*); willow warbler (*Phylloscopus trochilus*); common redstart (*Phoenicurus pheonicurus*); dunnock (*Prunella modularis*); European robin (*Erithacus rubecula*); song thrush (*Turdus philomelos*)	*Haemoproteus*, *Plasmodium*, *Leucocytozoon*	stopover duration	observational; field
	partial	partial	partial; [27]	subspecies of lesser black-backed gulls (*Larus fuscus* sp.)	natural antibodies, complement activity, IgY	breeding; population comparison	observational; field

				titres, haptoglobin, lysozyme, leukocyte counts		
	partial	partial	partial; [40]	common blackbirds (*Turdus merula*)	microbial killing capacity, haptoglobin activity, IgY titres	fall migration	observational; field
			support; [41]	song sparrow (*Melospiza melodia*)	natural antibodies, complement activity, macrophage activity, IgY titres	arrival to breeding	observational; field
	partial	partial	partial; [33]	clay-coloured sparrow (*Spizella pallida*); chipping sparrow (*S. passerina*); black-chinned sparrow (*S. atrogularis*)	leukocyte counts	breeding	observational; field
endurance flight affects immunity	support	support; [42]		red knot (*Calidris canutus*)	secondary antibody response to tetanus and diphtheria, PHA wing-web challenge	n/a	experiment; captive

partial	partial	partial; [43]	European starlings (*Sturnus vulgaris*)	haptoglobin, natural antibodies, complement activity, leukocyte profiles	n/a	experiment; captive
support	support; [16]		western sandpipers (*Calidris mauri*)	natural antibodies, complement activity, haptoglobin, bacterial killing ability	n/a	experiment; captive

References for Table 1-3:

[2] Hegemann et al., 2018; [15] Nebel et al., 2013; [16] Hegemann et al., 2012; [26] Arriero et al., 2015; [27] Møller & Erritzøe 1998; [32] Carbó-Ramírez & Zuria 2015; [36] Owen-Ashley & Wingfield 2006; [37] Owen & Moore 2008; [38] Promerová, Albrecht & Bryja 2009; [39] Eikenaar & Hegemann 2016; [40] Kelly et al., 2017; [41] Hasselquist et al., 2007; [42] Nebel et al., 2012

These studies were conducted over the past 12 years, except for Møller & Erritzøe (1998), and encompass many branches of the immune response (Table 1-4). In fact, most studies examined more than one parameter of immunity, a good thing considering the growing body of evidence that demonstrates that different aspects of immunity may be unrelated or negatively related (Martin, Hasselquist & Wikelski 2006; Buehler et al., 2008; Kubli & MacDougall-Shackleton 2014; Kelly et al., 2017). Only one study examined the acquired immune response. Evaluating immunity of animals is challenging. The simplest way to quantify immunity is to collect a single blood sample of blood from which plasma can be collected and later analyzed in a laboratory setting. This works effectively for innate responses, but assessing acquired immunity involves capturing and injecting individuals with a novel pathogen not likely to have been previously encountered in the wild, recapturing and re-injecting individuals with the pathogen after they have cleared the infection, then, within a restricted window of time, capturing individuals a third time to evaluate the specific antibodies produced in response to a pathogen (Demas et al., 2011). This difficulty has resulted in measures of delayed-type hypersensitivity (e.g., phytohemagglutinin-induced swelling responses; PHA) dominating the field of eco-immunology (Demas et al., 2011). However, within the scope of immunity and migration, only two studies used PHA (Table 1-4). Hemolysis-hemagglutination assay was the most common immunological techniques used (20.7 % each), followed by leukocyte counts and assessing IgY titres (17.2 % each; Table 1-4). These methods account for over half (55.1 %) of the immunological techniques implemented in investigations of migrant immunity (Table 1-4). Thus, while many

branches of immunity have been considered, only few are considered in multiple contexts.

Table 1-4 The number of investigations included in Table 1-3 sorted by the immunological trait examined, host family, host migration strategy, stage of migration examined, and study design. Studies using comparisons across families [27 & 28] are not included in family specifications.

Variable	Level		N
immunity	hemolysis-hemagglutination assay	natural antibodies & complement	6
	phagocytosis assay	macrophage activity	1
	phytohemagglutinin assay	T-cell mediated activity	2
	phagocytosis assay	macrophage activity	1
	IgY titres		5
	haptoglobin		3
	lysozyme activity		1
	microbial killing capacity		2
	leukocyte counts		5
	MHC characterization		1
	size of immune organs		1
	acquired immunity against diphtheria and tetanus		1
species classification	Charadriiformes		
		Laridae	1
		Scolopacidae	2
	Passeriformes		
		Alaudidae	1
		Fringillidae	1
		Motacillidae	1

		Muscicapidae	2
		Phylloscopidae	1
		Prunellidae	5
		Sturnidae	1
		Turdidae	6
host migration strategy	resident		2
	short distance (continental)		8
	long distance (intercontinental)		9
migration stage	fall departure		
	during fall migration, including stopover		2
	arrival to wintering grounds		
	spring departure		
	during spring migration, including stopover		
	arrival to breeding grounds		1
	breeding		3
	compared stages of the annual cycle		3
study design	observational; field		9
	experimental; field		
	experimental; captive		4
	within a species		9
	compared migrant/ long distance to resident/ short distance migrant		4

Table 1-4 reveals that only two studies took place during migration. This is understandable given the logistical challenges: what do you compare a snapshot of immunity to during migration? Either multiple timepoints are required or a comparison against a resident/ short-distance migrant at the same time is needed. Thus, most studies compared the immunity of migrants during migration and another stage of the annual cycle or compared immunity of birds varying in migration strategies. The support for whether migrants have greater immunity as a result of additional parasite exposure or poor immunity as a result of trading off against flight is equally split. The split evidence may be a result of an ever-changing balance of immunity and migration with respect to host-pathogen coevolution (van Dijk et al., 2015). Thus, the field remains unclear on understanding how immunity may interfere with host migratory traits. Captive studies examining immunity of birds after endurance flights found immunity to be maintained (Table 1-3), and immune challenges have not been found to affect flight endurance (Table 1-1); however, none of these studies took place while birds were in migratory condition, critical for our understanding of how upregulating immunity affects migratory traits. Without this understanding, it is difficult to anticipate how birds contribute to the spread of disease during migration.

1.6 Study species used in this thesis

Song sparrows (*Melospiza melodia*) and white-throated sparrows (*Zonotrichia albicollis*), family Passerellidae, are closely related and share similar ecologies. Both species are abundant in North America and complete short-distance migrations spanning from their breeding grounds in Canada to wintering grounds in the southern United States. White-throated sparrows exhibit two distinct morphs due to a chromosomal inversion

polymorphism (*ZAL2*^{m}): heterozygous, white-striped individuals (*ZAL2*^{m}*/ ZAL2*) and homozygous tan-striped individuals (*ZAL2/ ZAL2*) (Thorneycroft 1966, 1975). As a result, white-stripe birds are more aggressive than tan-stripes (Ficken, Ficken & Hailman 1978) but research examining differences in migratory tendencies are scant or are observational in nature. This is surprising considering their use as a model species to investigate migratory traits such as exercise physiology (Springer et al., 2011), navigation (Muheim, Phillips & Deutschlander 2009), and stopover refuelling (Brown et al., 2014). The size of these species make them ideal for captive experiments and migratory condition can be induced in captivity through manipulation of photoperiod (Robertson et al., 2014).

Hemosporidia (family Apicomplexa) are bloodborne protozoans that are transmitted between vertebrate hosts by insect vectors. Collectively, these parasites infect nearly 70 % of bird species, occur on every continent save Antarctica, and are expanding their range as well as the latitudes at which transmission can occur (Atkinson & Van Riper III 1991; Garamszegi 2011; Loiseau et al., 2012; Zamora-Vilchis et al., 2012). Genera *Plasmodium* and *Haemoproteus* associated with avian malaria can induce muscle wasting, anemia, fever, organ damage, and inflammation in their avian hosts (Booth & Elliott 2002; de Macchi et al., 2013). This is particularly prevalent during the first two weeks of infection (acute-phase) when the number of parasites circulating in the bloodstream is greatest. In extreme cases, these infections can result in the death of the host (de Macchi et al., 2013; Ilgūnas et al., 2016), but otherwise subside to chronic-phase infections associated with lower parasite burdens that may persist for months or years following initial infection (Asghar et al., 2012). *Plasmodium* have received particular

scrutiny, partly due to their broad distribution, high prevalence and harmful effects on host fitness, but also because *Plasmodium* is capable of proliferating in the peripheral blood of their vertebrate hosts (Atkinson & Van Riper III 1991). This trait makes *Plasmodium* highly suitable for experimental inoculations (Marzal et al., 2008), allowing infections to be transferred directly between host individuals in a controlled setting.

1.7 Thesis objectives and hypotheses

The over-arching objective of my doctoral research is two-fold. First, I will introduce controlled, experimental studies to the pre-existing observational body of research examining the relationship between harmful pathogens and migration. These experiments will (1; Chapter 2) determine the extent that migratory traits are reduced due to immune upregulation in contrast to harmful pathogenic effects; (2; Chapter 3) discern the causal effects pathogens have on the behavior and physiology of hosts during migration; and (3; Chapter 4) evaluate whether the results of captive experiments (Chapters 2 & 3) are consistent with results of field-based experiments. Second, I will contrast these results to the hypotheses regarding the relationships between pathogens, migration, and disease transmission outlined in the introduction of this thesis.

In general, I hypothesize pathogens will negatively impact migratory preparation and timing, more so than upregulating immunity. Understanding how birds contribute to the global transport of pathogens depends fundamentally on how pathogens affect migratory preparation and timing. Thus, my doctoral research provides essential information to model the anticipated spread of disease in our changing climate (Garamszegi 2011; Altizer et al., 2013; McKay & Hoye 2016). The three data chapters of this thesis (Chapters 2 – 4) were prepared as independent units for publication. One of the

data chapters has been published (Chapter 3), one is currently under review (Chapter 2), and the last is being prepared for submission (Chapter 1).

1.7.1 Effects of upregulating immunity on migratory traits

Chapter 2 evaluates the effects that mounting an acute-phase immune response has on migratory behavior and body condition of captive song sparrows and white-throated sparrows in migratory condition. Observational studies cannot discern the extent that immunity has reduced migratory traits from the harmful effects of the invading pathogen. I exposed migrant sparrows to lipopolysaccharide (LPS) and/or keyhole-limpet hemocyanin (KLH), both of which stimulate an immune response but cause no physical harm to the subject. Thus any changes to behaviour or body composition after inoculation are attributed to the effects of mounting an immune response (Kent et al., 1992; Harris & Markl 1999; Demas et al., 2011) without being confounded by pathogenic effects. I predict that stimulating the immune response will require energy stores otherwise allocated to migratory activity and body composition so that immunologically-challenged sparrows will exhibit reduced activity and body condition.

1.7.2 Effects of experimental malaria infection on migratory traits

Chapters 3 and 4 use controlled experimental inoculations with malarial parasites (*Plasmodium* sp.) to evaluate the causal effects parasites have on migratory traits. Controlled inoculations will also allow an investigation of whether unsuccessful infection impedes investment in migratory behavior and body condition, of which is currently unknown. I exposed captive white-throated sparrows in migratory condition to malaria to quantify if, how, and when malaria infection reduces migratory traits (Chapter 3). I predict malaria infection will reduce migration activity and body condition, but also

predict such reductions to differ with respect to acute and chronic stages of infection when the total number of malarial parasites differ. Additionally, I predict the impact on migratory activity and body condition to differ between birds that resist infection from successfully infected individuals, with the latter incurring greater reductions.

Captive conditions ensure that any impacts on migratory traits are due to malarial infection alone. However, conditions experienced in captivity differ vastly from field conditions and make it unreasonable to expect that identical effects would be observed in both environments. Therefore, Chapter 4 (Exposing migratory sparrows to *Plasmodium* suggests costs of resistance, not necessarily of infection itself; Kelly et al., 2018) utilizes a field-based experiment to determine if captive-observed reductions in activity are also observed in the field. I inoculated song sparrows with malaria parasites and released them immediately prior to fall migration, after confirming successful infections and evaluating body condition. I observed fall departure dates of controls, successfully infected birds, and birds that resisted infection and predict successfully infected birds will exhibit the greatest delay in fall departure. Chapter 3 and 4 in combination provide a comprehensive examination of if, how, and when malaria infection may affect migratory body condition and timing of songbirds, critical to identify the role migratory birds play in the long-distance transport of infectious disease.

1.8 References

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Chapter 2

2 Mounting an acute-phase immune response does not reduce migratory body condition or migratory restlessness in sparrows

2.1 Introduction

Migratory animals encounter multiple habitats and multiple parasite communities during their lifetimes, often resulting in increased risk of parasitic infection compared to year-round resident conspecifics (Figuerola & Green, 2000; Kelly et al., 2016; Møller & Erritzøe, 1998). Pathogen-mediated selection is thus likely to be particularly intense in migratory animals, promoting increased investment in immune defence (Altizer, Bartel, & Han, 2011; Møller & Erritzøe, 1998). However, immune defences are costly to develop, maintain, and deploy (e.g., energetic costs of maintaining "surveillance" cells (Buehler, Tieleman, & Piersma, 2010; Klasing, 2004); tissue damage associated with immune upregulation (Buehler et al., 2010; Raberg, Grahn, Hasselquist, & Svensson, 1998)), and these costs must be balanced against the costs of migration. Of all the phases of the annual cycle, migration may be the most energetically demanding (Wikelski et al., 2003), particularly for flying animals such as birds. The minimum oxygen consumption associated with flight can be over twice that of running (Butler, Woakes, & Bishop, 1998), and unlike terrestrial animals, flying animals cannot reduce these costs by flying more slowly (Hedenström & Alerstam, 1997). Furthermore, migratory birds routinely fly considerable distances to cross geographic barriers (up to 670 km/day in arctic terns [*Sterna paradisaea*; Egevang et al., 2010] ; up to 600 km in a single flight in Swainson's thrush [*Catharus ustulatus*] and hermit thrush [*C. guttatus*; Wikelski et al., 2003]). Thus, a plausible alternative to the hypothesis that migration is associated with upregulated

immunity (Altizer et al., 2011; Møller & Erritzøe, 1998) is that the costly nature of both immune defence and migration generates a trade-off between these two competing demands (Altizer et al., 2011).

Distinguishing between these two hypotheses requires determining whether and how mounting an immune response affects migratory preparation, migratory behaviour, and ultimately migratory success. Many studies have attempted to address this issue in migratory birds, largely by comparing body condition, stopover duration and migratory timing of naturally-infected versus uninfected individuals. *Haemoproteus*-infected barn swallows (*Hirundo rustica*; Møller, De Lope, & Saino, 2004) and female great reed warblers (*Acrocephalus arundinaceus*; Asghar, Hasselquist, & Bensch, 2011) arrive later than uninfected conspecifics, and intestinal parasite richness increased with later migration of garden warblers (*Sylvia borin*; López, Muñoz, Soriguer, & Figuerola, 2013). However, correlational studies are limited in their ability to discern whether delays were caused by infection or if delays made migrants susceptible to infection and cannot disentangle the costs of mounting an immune response from the costs of infection itself. The extent to which immune upregulation, as opposed to parasitic infection, reduce migratory success are likely to have important consequences for optimal investment in immunity, and for the spread of disease. Controlled experiments addressing the effects of endurance flights on immune function (Hasselquist, Lindström, Jenni-Eiermann, Koolhaas, & Piersma, 2007; S. Nebel et al., 2012) or conversely, the effects of mounting an immune response on endurance flight (Nebel, Buehler, MacMillan, & Guglielmo, 2013), represent a major advance over observational studies in that they permit disentangling costs of immunity from costs of infection. Of the studies reviewed above,

however, none were conducted on birds in migratory condition, and one used a non-migratory study species. Thus, we still lack a critical understanding of how mounting an immune response affects the migratory traits of birds.

I examined the effects of mounting an acute phase immune response on the body composition and nocturnal migratory restlessness (*Zugunruhe*) of two migratory songbird species, song sparrows (*Melospiza melodia*) and white-throated sparrows (*Zonotrichia albicollis*). I hypothesized that mounting an immune response interferes with migratory preparation (i.e., body composition) and behaviour (i.e., migratory restlessness), reflecting trade-offs between immune defence and migration. To determine the extent to which migratory traits are affected by immune response, as opposed to harm caused by parasitic infection, I challenged birds with one or both of two novel but non-infectious antigens, lipopolysaccharide (LPS; found in the cell well of Gram-negative bacteria) and keyhole-limpet hemocyanin (KLH; metalloprotein found in hemolymph of *Megathura crenulata*). I included a treatment group exposed to both LPS and KLH to evaluate whether exposure to multiple foreign entities entail additional expenses to immune upregulation. These substances elicit an acute-phase immune response in birds lasting no longer than 24 hours without inducing infection (Bonneaud et al., 2003; Dhabhar, 1998; Owen-Ashley, Turner, Hahn, & Wingfield, 2006). Thus, any changes to body composition or behaviour following these immune challenges can be attributed to the effects of mounting an immune response (Demas, Zysling, Beechler, Muehlenbein, & French, 2011; Harris & Markl, 1999; Kent, Bluthé, Kelley, & Dantzer, 1992).

Song sparrows and white-throated sparrows are closely related and have similar ecologies, with both species occupying similar habitat and migrating short to medium

distances between breeding and wintering grounds (Arcese, Sogge, Marr, & Patten, 2002; Falls & Kopachena, 2010). Therefore, I predict that both species would respond similarly to immune challenges. I predict that exposure to LPS and/or KLH will negatively affect body composition and migratory restlessness, reflecting trade-offs between immunity and migration. Alternatively, if no reductions in *Zugunruhe* or body condition were observed, this could support the hypothesis that migratory birds can mount acute-phase immune responses without constraining migratory behaviours or preparation.

2.2 Materials and methods

2.2.1 Study animals and housing

Between October 7 and November 2, 2015, I captured song sparrows (n = 28) and white-throated sparrows (n = 27) during fall migration near Long Point, Ontario, Canada (42°554’ N, -80°174’ W). Birds were transported immediately to the Advanced Facility for Avian Research in London, Ontario, weighed to the nearest 0.1 g using a spring scale, measured tarsus (index of structural size) and unflattened wing length to the nearest 0.1 mm using dial calipers. I initially classified animals as male or female based on wing length, to distribute sexes equally within each treatment: I later confirmed sex of all birds using molecular techniques (2.2.3).

Animals were housed indoors in individual cages (39×34×42 cm) at 20 – 22 °C, with *ad libitum* access to water and food (a mixture of parakeet seed and ground bird chow (Mazuri Small Bird Maintenance). I manipulated the light schedule to mimic the natural photoperiod ranging from 11.5 h light: 12.5 h dark (11.5L:12.5D) on October 7 through 10L:14D on November 11) to maintain normal migratory schedules. Birds were

captured and collected under the required federal permits (Environment Canada Scientific Collecting Permit CA0244; Banding Permit 10691 E) and all animal procedures were approved by the University of Western Ontario's Animal Use Subcommittee (protocol # 2015-047).

2.2.2 Immune challenge

Three days after capturing an individual and two hours before lights-off, I exposed it to one of four different immune challenge treatments (Figure 2-1). I used a 2×2 experimental design, such that each individual was inoculated with either 0.9% saline (i.e., unchallenged controls); lipopolysaccharide (LPS); keyhole limpet hemocyanin (KLH), or both LPS and KLH. LPS is derived from the cell wall of Gram-negative bacteria and stimulates the production of pro-inflammatory cytokines, a component of innate immunity (Bliss, Dohms, Emara, & Keeler, 2005; Sijben et al., 2003). KLH is derived from a circulating glycoprotein of the mollusk *Megathura crenulata,* and elicits a localized, antigen-specific, T-cell-mediated response resulting in recruitment and activation of cytokines, macrophages, natural killer cells and cytotoxic T cells (Dhabhar, 1998). LPS (Sigma#L4005, serotype 055:B5) and KLH (Sigma#H7017) were each dissolved in 0.9% saline to 1 mg per mL. I injected doses subcutaneously over the pectoralis muscle.

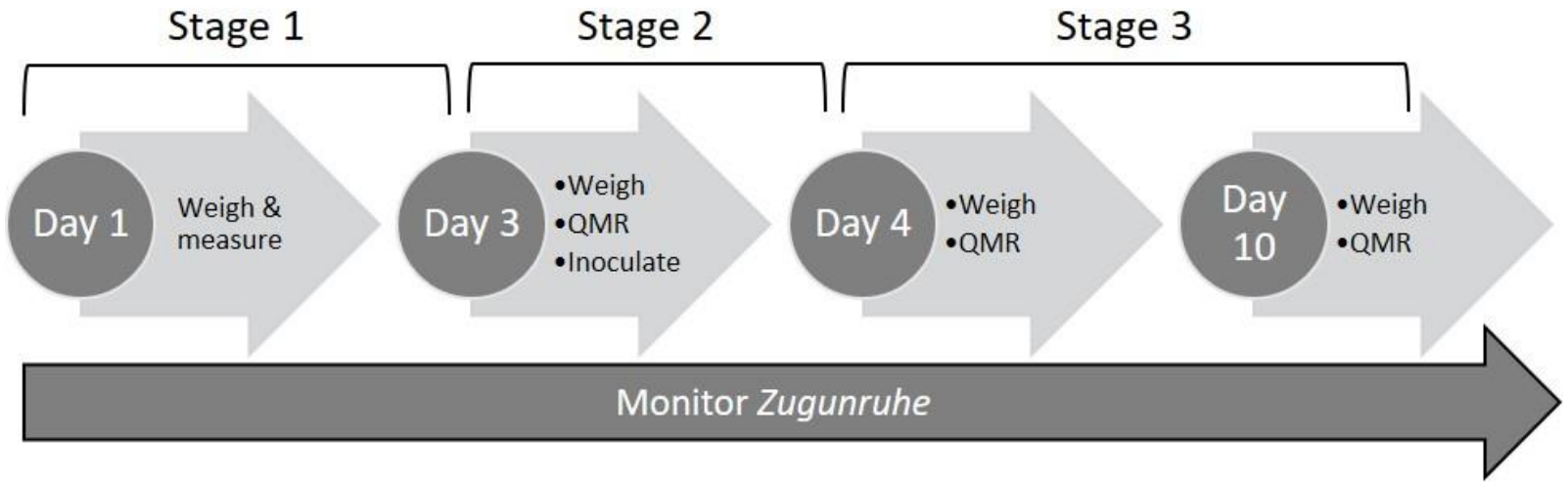

Figure 2-1 Experimental design. Depicts when song sparrows and white-throated sparrows received their treatment (saline, lipopolysaccharide (LPS), keyhole limpet hemocyanin (KLH), or LPS and KLH) and when body composition was evaluated. *Zugunruhe* was monitored each night.

Starting masses of song sparrows did not vary appreciably (17.8 – 23.8 g; 5 g range), thus I dosed each song sparrow with a consistent volume of 100 μL (saline, 1 mg/mL LPS in saline, or 1 mg/mL KLH in saline). These doses correspond to approximately 4 μg of LPS and/or KLH per gram of body mass (Nebel et al., 2013); animals in the LPS + KLH treatment received two separate 100 μL inoculations, corresponding to the full dose of LPS and of KLH. Starting masses of white-throated sparrows were higher and more variable (23.0 – 31.2 g; 8.2 g range) than those of song sparrows, so I adjusted dose volumes according to mass at capture. Each individual received a dose corresponding to 4 μg of LPS or KLH per gram of body mass, with individuals in the LPS + KLH treatment receiving 4 μg of each per gram of body mass.

2.2.3 Genetic determination of sex and morph

Upon capturing each animal, I collected a small blood sample via brachial venipuncture for genetic determination of sex (and in the case of white-throated sparrows, plumage and behavioral morph). I extracted DNA with an ammonium-acetate based protocol (Bruford, Hanotte, Brookfield, & Burke, 1998), and quantified DNA concentration using a NanoDrop 2000 spectrophotometer (Thermo Scientific), then used polymerase chain reaction (PCR) to confirm sex and plumage morph. To confirm sex, I used primers P2 and P8 (Griffiths, Double, Orr, & Dawson, 1998) to amplify portions of the CHD-W and CHD-Z genes. In birds, females are the heterogametic sex (ZW), so PCR product from females shows two bands (Z, W) on a gel while product from males shows a single band (Z). I conducted PCR reactions for sexing in a total volume of 20 μL, including 60 ng of genomic DNA, 1 μL 10 × buffer (Invitrogen; #Y02028), 1 mM/μL $MgCl_2$, 0.1 mM/μL

dNTPs, 0.1 μM/μL of each P2 and P8 primers, 0.025 U/μL Taq (Invitrogen; #10342-020). Thermocycling conditions included an initial denaturing step of 94 °C for 60 s, followed by 40 cycles of (94 °C for 30 s, 48 °C for 45 s, 72 °C for 45 s), and a final extension of 72 °C for 300 s. PCR products were separated by agarose gel electrophoresis, stained with RedSafe™ then visualized under ultraviolet light. As controls, on each gel I ran samples from a known male, a known female, and a no-template negative control.

2.2.4 Restriction fragment length polymorphism discrimination

White-throated sparrows have two discrete plumage and behavioral morphs, white-striped and tan-striped. Because these morphs cannot reliably be distinguished in the hand outside of the breeding season, I used a PCR-based genotyping assay for a *DraI* restriction fragment length polymorphism (modified from Michopoulos, Maney, Morehouse, & James, 2007) to confirm morphs of white-throated sparrows. I used primers PF and PR (Michopoulos et al., 2007) to amplify a 285 bp product: PCR was conducted in a total volume of 25 μL and included 1 μL 10 × reaction buffer (Invitrogen; #Y02028), 1.6 mM/μL $MgCl_2$, 0.16 mM/μL dNTPs, 0.16 μM/μL of both PF and PR, 0.06 U/μL of Taq polymerase (Invitrogen; #10342-020), and 50 ng of genomic DNA as a template. Thermocycling conditions included an initial denaturing step of 94 °C for 300 s, followed by 40 cycles of (94 °C for 30 s, 48 °C for 45 s, 72 °C for 45 s), and a final extension of 72 °C for 300 s. I then incubated PCR products with the restriction enzyme *DraI*: white-striped genotypes are heterozygous for a chromosomal inversion and for the *DraI* recognition site, so PCR product from white-striped individuals yields three bands (285 bp, 200 bp, and 85 bp) after digestion and electrophoresis, while tan-striped

genotypes lack this site and yield a single band at 285 bp (Michopoulos et al., 2007). I digested PCR products for 2 h at 37 °C in a total volume of 25 μL, containing 10 μL of PCR product, 0.8 U/μL of *DraI* (New England BioLabs; #RO129S), and 5 μL of 1× CutSmart® Buffer (New England BioLabs). Digestion products were separated by electrophoresis on an agarose gel, stained with RedSafe™ and visualized under ultraviolet light to determine morph.

2.2.5 Body composition

I measured total body mass to the nearest 0.1 g with a spring scale and used quantitative magnetic resonance (QMR) to assess lean and fat mass, at three timepoints: experimental day 3 (i.e., the morning before inoculation with saline or LPS/KLH), day 4 (~ 15 hours after inoculation), and day 10 (after 7 days of recovery post-inoculation; Figure 2-1). QMR provides a noninvasive measure of fat mass, lean mass, and total body water in about 3 minutes (Guglielmo, McGuire, Gerson, & Seewagen, 2011; Seewagen & Guglielmo, 2011), with animals gently restrained in a ventilated holding tube of 4.5 cm diameter. I calibrated the QMR instrument (Echo-MRI-B™, Echo Medical Systems Houston, TX, USA) using standards of canola oil to ensure accurate readings to the nearest 0.001 g (Guglielmo et al., 2011; Seewagen & Guglielmo, 2011). For each animal, I conducted two replicate scans, each using four primary accumulations, and calculated their average values.

2.2.6 Behavioural analysis

Song sparrows and white-throated sparrows are nocturnal migrants, so I measured migratory restlessness (*Zugunruhe* behavior) each night. The setup included two racks with four shelves each placed facing each other, ~ 1 metre apart. I covered the back and

outer sides of the racks with white plastic corrugated board to increase the contrast between the bird and the background for more effective tracking. Four cages (39x34x42 cm) fit on a single shelf and a final piece of board was placed between the center cages so each bird could see a neighbour as well as the birds across from them. Infrared-sensitive cameras (PC182XS) were attached directly to the racks opposing the cages to be recorded. Each camera was capable of recording four cages (2 x 2), and four cameras were set up to record the opposing rack. The four camera feeds were directed to a quad processor (model ADQUAD77) that joined the four feeds into a single feed and was then connected to the computer running Noldus software. Thus, each rack had the capability of housing and recording 16 birds. An infrared light (850 nm Smart B-Series, model AT-35-B) was positioned above the camera, so it would illuminate the same cages recorded by the paired camera without disrupting the natural photoperiod of the birds. Painter's tape was used to decrease the glare of the light in the opposing camera as birds were sometimes lost behind the glare. This did not impede the ability of the light to illuminate the view. There were two pairs of racks set up in this manner. Using this setup, it would be possible to record activity of up to 64 birds.

For simplicity, I recorded and saved the videos to analyze after the experiment was complete. Each video began with the researcher holding a piece of paper with the date and a clock was placed in lieu of a cage, so the proper time was always known in the video. Each cage was labelled with a numbered piece of paper to identify each bird. The total recording time each night varied per the natural photoperiod; video recording began two hours before lights-off and ended 30 minutes after lights-on the following morning.

I used the video tracking software package Noldus Ethovision XT (version 10.0.826) to quantify nocturnal migratory restlessness. Sparrows were identified using the grey-scaling technique that identifies the darkest image in the frame and tracks from its center. Detection settings were optimized using static subtraction, which uses a static reference image as background. I used a unique reference image for each trial with birds digitally removed from a captured frame of the video. Specific settings varied each night to optimize the automatic detection of the dark bird against a light background but ranged as follows: dark contrast 10 – 147, subject size 3 – 264, sample rate 29.9706, low video pixel smoothing, track noise reduction on, contour erosion 1, and contour dilation 1. Distance units were calibrated for each video by using the calibrate scale.

The software extracted movement variables in two ways. First, the software identified a centre-point of the bird (dark bird against light background) and tracked time [s; *time moved*] and distance [cm; *distance moved*] for which the centre-point of the bird changed location. Second, the software identified changes in the contour of the bird as mobility and tracked the time the bird was mobile [s; *time mobile*], regardless of centre-point movement (e.g. a stationary bird flapping its wings). An individual was classified as mobile when the total body area changed by a user-defined threshold of >10 % and, for centre-point movement, movements had to be greater than a used-defined threshold of >2 cm/s. Centre-point movement tracking captured short flights, hopping and/or jumping behaviours while mobility tracking captured wing whirring, short flights, hopping and/or jumping.

2.2.7 Data analysis

To determine how immune challenges affected body composition, I constructed four sets of linear models, one for each dependent variable: whole-body mass, fat mass, and lean mass. I used the percent change in mass as the dependent variable, calculated using the following formula:

$$\%\ change = \left(\frac{(mass\ at\ time\ 2 - mass\ at\ time\ 1)}{mass\ at\ time\ 1} \right) \text{x}\ 100\ \%.$$

These linear models compare the immediate effects of immune upregulation (morning after: time 1 = day 3 mass, time 2 = day 4 mass) and effects after one week of recovery (recovery: time 1 = day 4 mass, time 2 = day 10 mass). An information theoretic approach (Anderson & Burnham, 2002) and Akaike's information criterion corrected for small sample sizes (AICc) was used to compare support for 51 (100 for white-throated sparrows) alternative models predicting each physiological parameter. Candidate models in each set differed in the presence and absence of terms for tarsus length, capture date, sex, LPS, KLH, sex × LPS, sex × KLH, and LPS × KLH. Models for white-throated sparrows also differed in the presence and absence of morph as a main effect. However, there was an uneven distribution of white- and tan-striped birds between treatments such that testing for interactions involving morph would have low statistical power (Table 2-1). If the top two models fell within two AICc units of each other, I compiled model-averaged parameter estimates from the full set of AICc-ranked candidate models using the conditional averaging method (Anderson & Burnham, 2002) implemented in *model.avg* in the R package MuMln (Bartoń, 2018).

Table 2-1 The number of birds in each treatment split by species, sex, and morph. Birds were brought into captivity at Western University during fall migration from Long Point, Ontario, between September 15 and November 10, 2015.

	song sparrow		**white-throated sparrow**			
			male		**female**	
treatment	**male**	**female**	**tan**	**white**	**tan**	**white**
control	4	5	4	1	1	1
LPS	2	5	1	2	0	4
KLH	2	5	2	2	0	2
LPS+KLH	3	3	5	2	0	1
total:	11	18	12	7	1	8

I used Pearson's correlation to characterize relationships among the three measures of migratory restlessness (*distance moved, time moved, time mobile*). I calculated hourly averages of each measure, for each bird, on each night. These activity measures were significantly and positively correlated (Table 2-2a & 2-3a), so to reduce dimensionality, I conducted principal component analysis (PCA) using the *prcomp* command in the *ggfortify* package (Tang, Horikoshi, & Li, 2016). Analyses were conducted in R (version 3.4.1; (R Core Team, 2017)). For each species, I retained the first principal component as it was the only component with an eigenvalue > 1. This component explained 73 % of the variation in activity for both species (Table 2-2b, 2-3b). Positive values of PC1 were associated with greater distances moved, more time spent moving, and more time spent mobile (Table 2-2c & 2-3c). I rotated the PC1 using varimax rotation and term this PC1 factor as *Zugunruhe intensity*.

Table 2-2 Creating principal components to analyze song sparrow *Zugunruhe*.
a. Correlation matrix of activity variables (total distance moved [cm], total time spent moving [s], and total time mobile [s]) obtained from Noldus. Pearson's r (r), sample size (n; the number of correlations, one for each night recorded), significance values (p) are reported. Distance moved (cm) represents the total distance the center-point moves, time moved is the total time the center-point moved, and time mobile represents wing movement while the subject is stationary (no center-point movement).
b. Summary statistics for the principal components analysis of activity variables.
c. Factor loadings for each principal component.

a.	**distance moved (cm)**	**time spent moving (s)**	**time mobile (s)**
time moved (s)	r = 0.93 n = 271 p < 0.0001		
time mobile (s)	r = 0.41 n = 271 p < 0.0001	r = 0.40 n = 271 p < 0.0001	

b.	**PC1**	**PC2**	**PC3**
eigenvalue	2.20	0.66	0.07
proportion of variance	0.73	0.24	0.02
cumulative proportion	0.73	0.97	1.00

c.	**PC1**	**PC2**	**PC3**
distance moved	0.639	-0.297	0.268
time moved	0.637	-0.313	0.024
time mobile	0.431	0.976	1.000

Table 2-3 Creating principal components to analyze white-throated sparrow *Zugunruhe*.
a. Correlation matrix of activity variables (total distance moved [cm], total time spent moving [s], and total time mobile [s]) obtained from Noldus. Pearson's r (r), sample size (n), significance values (p) are reported. Distance moved (cm) represents the total distance the center-point moves, time moved (s) is the total time the center-point moved, and time mobile (s) represents wing movement while the subject is stationary (no center-point movement).
b. Summary statistics for the principal components analysis of activity variables.
c. Factor loadings for each principal component.

a.	**distance moved (cm)**	**time spent moving (s)**	**time mobile (s)**
time moved (s)	r = 0.90 n = 264 p < 0.0001		
time mobile (s)	r = 0.31 n = 264 p < 0.0001	r = 0.37 n = 264 p < 0.0001	

b.	**PC1**	**PC2**	**PC3**
eigenvalue	2.20	0.73	0.07
proportion of variance	0.73	0.24	0.03
cumulative proportion	0.73	0.97	1.00

c.	**PC1**	**PC2**	**PC3**
distance moved	0.643	-0.319	0.695
time moved	0.655	-0.239	-0.716
time mobile	0.396	0.917	0.055

To determine how immune challenges affected *Zugunruhe intensity*, I constructed a series of candidate models predicting PC1. The experiment days were split into stages that I predicted, *a priori*, would differ in activity: pre-inoculation (stage 1, days 1 – 3), morning after inoculation (stage 2, day 4), and recovery (stage 3, day 4 – 10; Figure 1). White-crowned sparrows (*Zonotrichia leucophrys gambelii*) show the greatest change in behaviour three hours post-injection with LPS (Owen-Ashley et al., 2006), thus I expected that any effects on *Zugunruhe intensity* should be apparent in stage 2. I compared support for 165 (329 for white-throated sparrows including the presence versus absence of morph as a main effect) alternative models predicting *Zugunruhe intensity* using the model selection technique described above. Candidate models in each set differed in the presence versus absence of terms for experiment day, stage, LPS, KLH, sex, and interactions of stage × sex, stage × LPS, stage × KLH, sex × LPS, sex × KLH, and LPS × KLH. All models included a random effect of subject to account for individual variation in activity.

2.3 Results

2.3.1 Predictors of body condition

Of the candidate models predicting the percent change in fat mass, LPS and KLH were not included as factors in the top-ranked models the morning after inoculation (Table 2-4a, 5a) or after having one week to recover (Table 2-4c, 5b). By the end of the experiment, control birds gained fat mass (song sparrows: 36.25 %, 0.54 g; white-throated sparrows: 8.65 %, 0.31 g).

Table 2-4 Selection of models predicting song sparrow fat mass the morning after receiving treatment and after one week of recovery.
a. Fat mass morning after: Top-ranked candidate set of linear models (AICc < 2) predicting the percent fat mass change of captive song sparrows the morning after receiving treatment (~ 15 hours after). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Real function parameters of the best-fitting model predicting the percent fat mass change of captive song sparrows the morning after receiving treatment. Conditional averaging results of model averaging are reported. A higher estimate indicates an increase in the percent fat mass change.
c. Fat mass after recovery: Top-ranked candidate set of linear models (AICc < 2) predicting the percent fat mass change of captive song sparrows after recovery.

a. morning after	**K**	**AICc**	**ΔAICc**	**w_i**
~ capture date	3	240.74	0.00	0.19
~ capture date + KLH	4	241.49	0.74	0.13
null	4	242.01	1.27	0.10
~ capture date + LPS	2	242.46	1.72	0.08

b.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	110.43	163.92	-219.4 – 440.3	0.51
capture date	-0.71	0.37	-1.46 – 0.05	0.07

c. after recovery	**K**	**AICc**	**ΔAICc**	**w_i**
null	2	318.73	0.00	0.13
~ tarsus	3	318.97	0.25	0.12
~ KLH	3	319.46	0.76	0.09
~ sex	3	319.82	1.09	0.08
~ capture date	3	320.50	1.77	0.05

Table 2-5 Selection of models predicting white-throated sparrow fat mass the morning after receiving treatment and after one week of recovery.
a. Fat mass morning after: Top-ranked candidate set of linear models (AICc < 2) predicting the percent fat mass change of captive white-throated sparrows the morning after receiving their treatment (~ 15 hours after). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Fat mass after recovery: Top-ranked candidate set of linear models (AICc < 2 and null model) predicting the percent fat mass change of captive white-throated sparrows after recovery.
c. Real function parameters of the best-fitting model predicting the percent fat mass change of captive white-throated sparrows after recovery. Conditional averaging results of model averaging are reported. A higher estimate indicates an increase in the percent fat mass change.

a. morning after	**K**	**AICc**	**ΔAICc**	**w_i**
null	2	216.34	0.00	0.20
~ sex	3	218.00	1.61	0.09
~ KLH	3	218.23	1.89	0.08
~ capture date	3	218.34	1.99	0.07
b. after recovery	**K**	**AICc**	**ΔAICc**	**w_i**
~ tarsus	3	279.45	0.00	0.20
~ tarsus + morph	4	280.61	1.17	0.11
~ tarsus + capture date	4	280.81	1.37	0.10
null	2	284.68	5.23	0.01
c.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	-295.41	477.80	-1226.3 – 681.2	0.55
tarsus	24.28	8.91	5.84 – 42.14	0.010

The percent change in song sparrow lean mass was not predicted by LPS or KLH the morning after inoculation (Table 2-6a) or after having one week to recover (Table 2-6b; Figure 2-2d). The percent change of white-throated sparrow lean mass was not predicted by LPS or KLH the morning after inoculation (Table 2-7a), but LPS was included in the top-ranked model predicting the percent change in lean mass after one week of recovery (Table 2-7b). This model, including capture date as a covariate, received over three times more support than the next-ranked model and revealed white-throated sparrows exposed to LPS had higher lean mass than controls after one week of recovery ($p < 0.018$, Table 2-7c; Figure 2-2c). Lean mass of control birds did not change by the end of the experiment (song sparrows: -0.90 %, -0.14 g; white-throated sparrows: -1.81 %, -0.35 g).

The top-ranked model predicting the change in whole body mass the morning after inoculation included only KLH as a factor for both species (Table 2-8a, 2-9a, respectively). This model received nearly four times the support than the second ranked model in song sparrows and over twice the support in white-throated sparrows. In both cases, whole body mass increased the morning after KLH inoculation (song sparrows $p = 0.049$, Table 2-8b, Figure 2-2b; white-throated sparrows $p = 0.05$, Table 2-9b, Figure 2-2a). This effect was lost after one week of recovery and only tarsus was included in the top-ranked model predicting the percent change in whole mass after one week of recovery for both species (Tables 2-8c, 2-9c). Whole-body mass of controls did not change by the end of the experiment (song sparrows: 0.87 %, 0.18 g; white-throated sparrows: -0.09%, -0.02 g).

Table 2-6 Selection of models predicting song sparrow lean mass the morning after receiving treatment and after one week of recovery.
a. Lean mass morning after: Top-ranked candidate set of linear models (AICc < 2) predicting the percent lean mass change of captive song sparrows the morning after receiving their treatment (~ 15 hours after). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Lean mass after recovery: Top-ranked candidate set of linear models (AICc < 2) predicting the percent lean mass of captive song sparrows after recovery.
c. Real function parameters of the best-fitting model predicting the percent lean mass change of captive song sparrows after recovery. Conditional averaging results of model averaging are reported. A higher estimate indicates an increase in the percent lean mass change.

a. morning after	**K**	**AICc**	**ΔAICc**	**w_i**
null	2	131.89	0.00	0.27
~ capture date	3	133.63	1.74	0.11
~ tarsus	3	133.85	1.95	0.10

b. after recovery	**K**	**AICc**	**ΔAICc**	**w_i**
~ capture date	3	166.41	0.00	0.29
~ capture date + sex	4	167.80	1.39	0.15
null	2	169.29	2.87	0.07

c.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	-47.78	45.00	-138.53 – 47.97	0.30
capture date	0.169	0.127	0.021 – 0.421	0.19

Table 2-7 Selection of models predicting white-throated sparrow lean mass the morning after receiving treatment and after one week of recovery.
a. Lean mass morning after: Top-ranked candidate set of linear models (AICc < 2 and null model) predicting the percent lean mass change of captive white-throated sparrows the morning after receiving their treatment (~ 15 hours after). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Real function parameters of the best-fitting model predicting the percent lean mass change of captive white-throated sparrows the morning after receiving treatment. A higher estimate indicates an increase in the percent lean mass change.
c. Lean mass after recovery: Top-ranked candidate set of linear models (AICc < 2 and null model) predicting the percent lean mass change of captive white-throated sparrows after recovery.
d. Real function parameters of the best-fitting model predicting fat mass of captive white-throated sparrows after recovery.

a. morning after	**K**	**AICc**	**ΔAICc**	**w_i**
~ capture date + sex	4	88.5	0.00	0.45
null	2	93.5	4.99	0.03

b.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	41.10	14.44	11.30 – 70.90	0.009
capture date	-0.14	0.05	-0.24 – -0.04	0.007
sex (female)	-1.09	0.47	-2.05 – -0.12	0.03

c. after recovery	**K**	**AICc**	**ΔAICc**	**w_i**
~ capture date + LPS	4	115.8	0.00	0.46
null	2	130.7	14.9	0.000

d.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	-102.19	22.98	-149.62 – -54.76	< 0.001
capture date	0.33	0.077	0.18 – 0.49	< 0.001
LPS	1.79	0.70	0.33 – 3.24	0.018

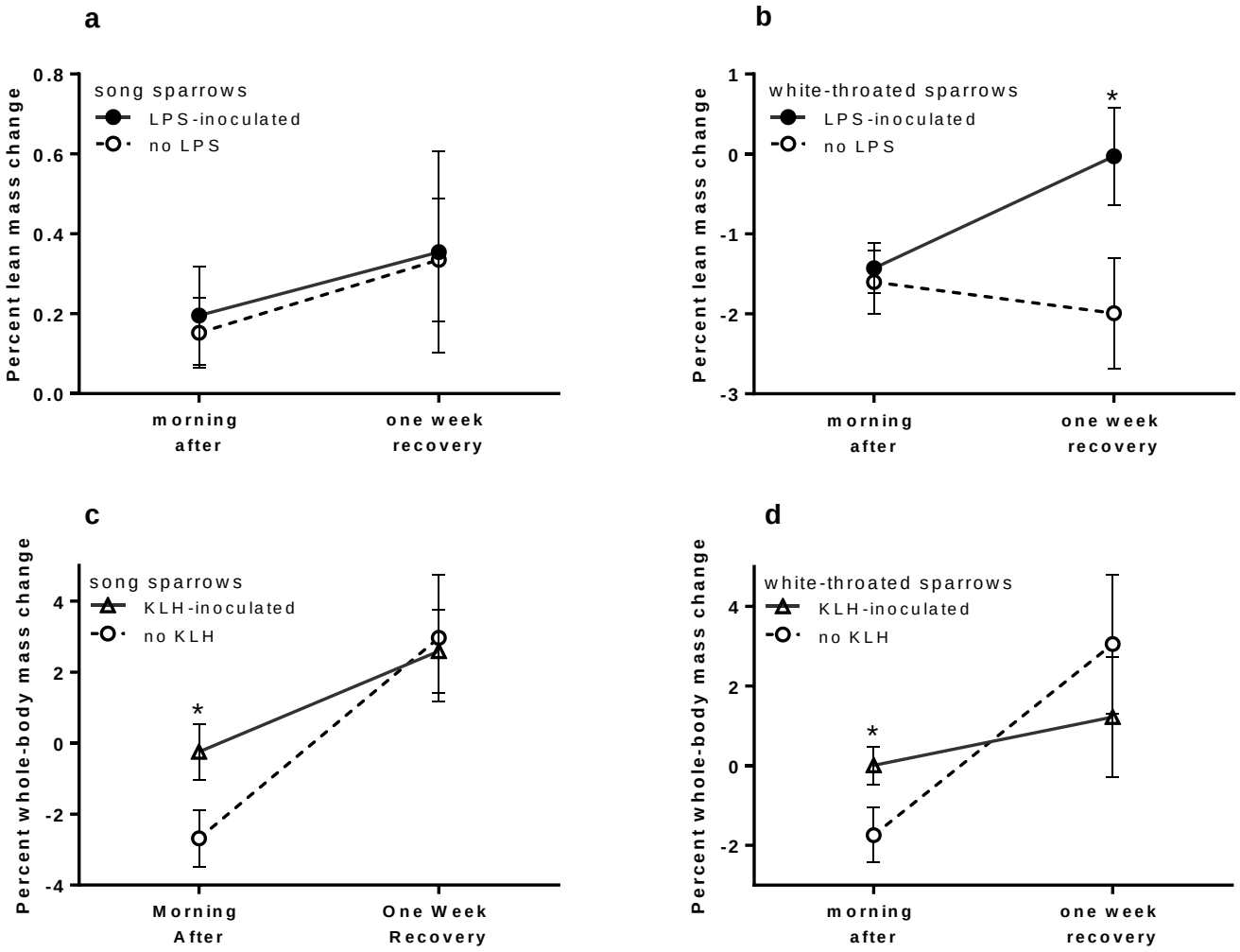
a
song sparrows
LPS-inoculated
no LPS
Percent lean mass change
0.8
0.6
0.4
0.2
0.0
morning after
one week recovery
b
white-throated sparrows
LPS-inoculated
no LPS
*
Percent lean mass change
1
0
-1
-2
-3
morning after
one week recovery
c
song sparrows
KLH-inoculated
no KLH
*
Percent whole-body mass change
4
2
0
-2
-4
Morning After
One Week Recovery
d
white-throated sparrows
KLH-inoculated
no KLH
*
Percent whole-body mass change
4
2
0
-2
morning after
one week recovery

Figure 2-2 The percent change in lean (a, b) and whole-body (c, d) mass of song sparrows (a, c) and white-throated sparrows (b, d) that received an inoculation with LPS (a, b; solid icons) or KLH (c, d; triangles). Empty circles and dashed lines represent individuals that did not receive an inoculation with LPS (a, b) or did not receive and inoculation with KLH (c, d). Asterisk indicates a significant difference between exposed and unexposed birds.

Table 2-8 Selection of models predicting song sparrow whole-body mass the morning after receiving treatment and after one week of recovery.
a. Whole-body mass morning after: Top-ranked candidate set of linear models (AICc < 2 and null model) predicting the percent change in whole-body mass of captive song sparrows the morning after receiving their treatment (~ 15 hours after). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Real function parameters of the best-fitting model predicting the percent change in whole-body mass of captive song sparrows the morning after receiving treatment. Conditional averaging results of model averaging are reported. A higher estimate indicates an increase in the percent whole-body mass change.
c. Whole-body mass after recovery: Top-ranked candidate set of linear models (AICc < 2 and null model) predicting the percent change in whole-body mass of captive song sparrows after recovery.
d. Real function parameters of the best-fitting model predicting the percent change in whole-body mass of captive song sparrows after recovery. Conditional averaging results of model averaging are reported.

a. morning after	**K**	**AICc**	**ΔAICc**	**w_i**
~ KLH	3	144.7	0.00	0.33
null	2	147.3	2.60	0.09

b.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	-2.72	0.76	-4.27 – -1.16	0.001
KLH	2.54	1.11	0.25 – 4.83	0.031

c. after recovery	**K**	**AICc**	**ΔAICc**	**w_i**
~ tarsus	3	175.67	0.00	0.30
~ tarsus + capture date	4	176.22	0.55	0.23
null	2	179.63	3.69	0.04

d.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	60.15	83.60	-107.2 – 227.5	0.481
tarsus	-4.53	1.98	-8.61 – -0.45	0.029

Table 2-9 Selection of models predicting white-throated sparrow whole-body mass the morning after receiving treatment and after one week of recovery.
a. Whole-body mass morning after: Top-ranked candidate set of linear models (AICc < 2) predicting the percent change in whole-body mass of captive white-throated sparrows the morning after receiving their treatment (~ 15 hours after). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Real function parameters of the best-fitting model predicting the percent change in whole-body mass of captive white-throated sparrows the morning after receiving treatment. Conditional averaging results of model averaging are reported. A higher estimate indicates an increase in the percent whole-body mass change.
c. Whole-body mass after recovery: Top-ranked candidate set of linear models (AICc < 2 and null model) predicting the percent change in whole-body mass of captive white-throated sparrows after recovery.
d. Real function parameters of the best-fitting model predicting the percent change in whole-body mass of captive white-throated sparrows after recovery. Conditional averaging results of model averaging are reported.

a. morning after	**K**	**AICc**	**ΔAICc**	**w_i**
~ KLH	3	124.3	0.00	0.24
null	2	126.0	1.72	0.10

b.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	-1.74	0.59	-38.21 – 26.83	0.007
KLH	1.76	0.85	-0.09 – 3.75	0.049

c. after recovery	**K**	**AICc**	**ΔAICc**	**w_i**
~ tarsus	3	171.8	0.00	0.18
~ tarsus + morph	4	172.8	0.99	0.11
null	2	176.6	4.83	0.02

d.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	-67.48	53.40	-175.9 – 40.9	0.22
tarsus	3.01	1.20	0.54 – 5.49	0.02

2.3.2 Predictors of Zugunruhe *intensity*

Neither of the top-ranked models predicting *Zugunruhe intensity* included treatment effects of LPS or KLH. Of the candidate models predicting *Zuguruhe intensity*, models that included terms for experiment day and stage of experiment were ranked higher than models that did not, for both study species (Table 2-10a, 2-11a). Conditional averaging across the full model set determined that activity increased with experiment day for both species but decreased the morning after receiving treatment, regardless of what the treatment was (Table 2-10b, 2-11b; Figure 2-3). Only white-throated sparrows had reduced *Zugunruhe intensity* during the recovery stage compared to the days preceding inoculation but did not interact with treatment (Table 2-11; Figure 2-4). Female white-throated sparrows, but not song sparrows, had lower *Zugunruhe intensity* than males, and tan-stripe morphs had higher *Zugunruhe intensity* than white-stripes (Table 2-11b; Figure 2-5).

Table 2-10 Selection of models predicting song sparrow Zugunruhe intensity.
a. Top-ranked candidate set of linear models (AICc < 2 and null model) predicting Zugunruhe intensity of captive song sparrows. All candidate models include subject as a random variable. Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Real function parameters of the best-fitting model predicting Zugunruhe intensity of captive song sparrows after model averaging. Conditional average results are reported. A higher estimate indicates higher Zugunruhe intensity. Significant terms are italicized.

a.	**K**	**AICc**	**ΔAICc**	**w_i**
~ experiment day + stage + sex	7	721.59	0.00	0.13
~ experiment day + stage + sex + KLH	8	722.42	0.83	0.09
~ experiment day + stage + sex + LPS	8	723.08	1.49	0.06
~ experiment day + stage + sex + LPS + LPS*sex	9	723.32	1.73	0.06
~ experiment day + stage	6	723.50	1.91	0.05
null	3	752.02	30.43	0.00

b.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	-0.36	0.22	-0.79 – 0.07	0.10
experiment day	*0.12*	*0.03*	*0.05 – 0.18*	*<0.001*
stage (morning after)	*-0.49*	*0.22*	*-0.92 – -0.06*	*0.03*
stage (recovery)	-0.17	0.25	-0.67 – 0.32	0.49
sex (female)	-0.31	0.23	-0.76 – 0.13	0.16

Table 2-11 Selection of models predicting white-throated sparrow Zugunruhe intensity. a. Top-ranked candidate set of linear models (AICc < 2) predicting Zugunruhe intensity of captive white-throated sparrows. All candidate models include subject as a random variable. Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Real function parameters of the best-fitting model predicting Zugunruhe intensity of captive white-throated sparrows after model averaging. Conditional average results are reported. A higher estimate indicates higher Zugunruhe intensity. Significant terms are italicized.

a.	**K**	**AICc**	**ΔAICc**	**w_i**
~ experiment day + stage + sex + morph	8	695.69	0.00	0.24
~ experiment day + stage + sex + morph + LPS	9	697.22	1.54	0.11
~ experiment day + stage + sex + morph + KLH	9	697.26	1.57	0.11
null	3	732.02	36.34	0.00

b.	**estimate**	**std. error**	**97.5 % CI**	**p**
(intercept)	*-0.74*	*0.19*	*-1.12 – -0.36*	*<0.001*
experiment day	*0.20*	*0.04*	*0.12 – 0.27*	*<0.001*
stage (morning after)	*-0.58*	*0.22*	*-1.01 – -0.15*	*0.009*
stage (recovery)	*-0.70*	*0.24*	*-1.17 – -0.23*	*0.004*
sex (female)	*-0.57*	*0.23*	*-1.01 – -0.12*	*0.01*
morph (tan)	*0.58*	*0.19*	*0.21 – 0.94*	*0.002*

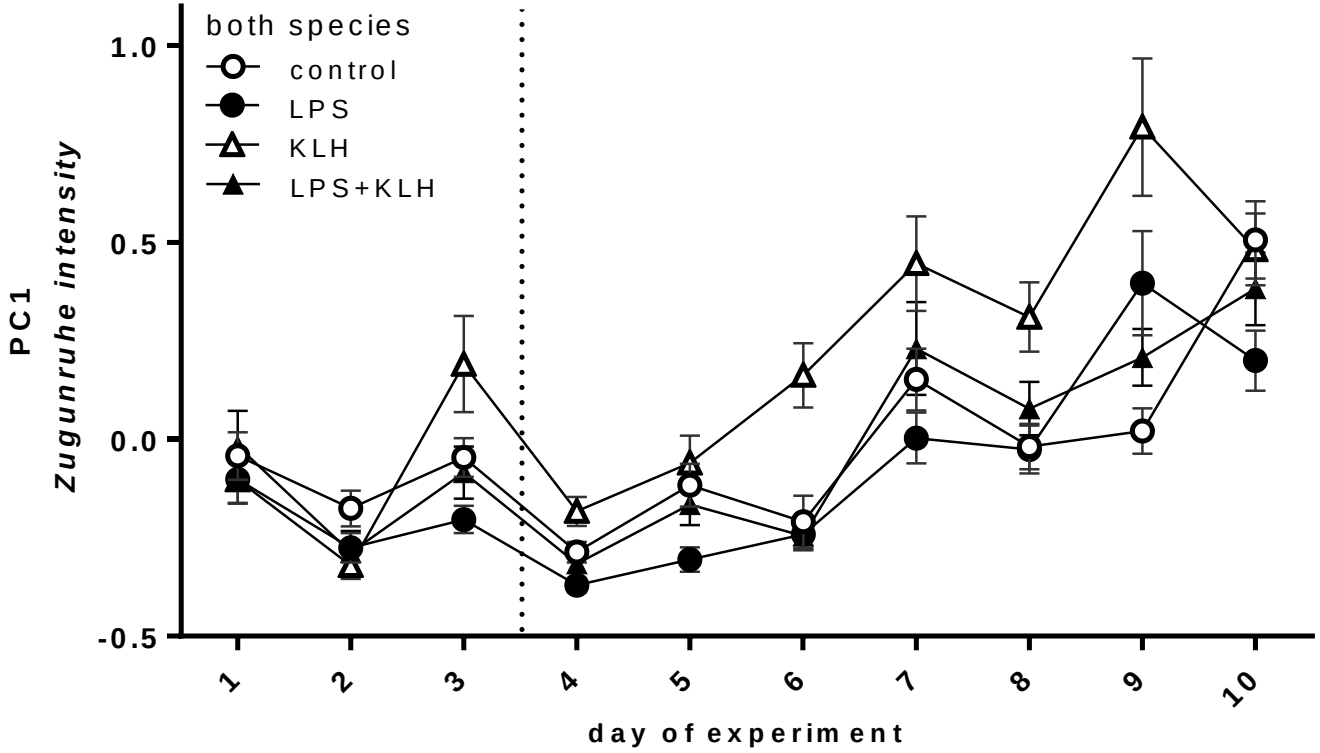

Figure 2-3 *Zugunruhe intensity* (PC1 of nocturnal activity) of song sparrows and white-throated sparrows that received either a saline injection (control; open circle), LPS inoculation (LPS; solid circle), KLH inoculation (KLH; open triangle), or both LPS and KLH (LPS+KLH; solid triangle). The vertical dotted line indicates the evening of inoculation. Error bars represent standard error of the mean.

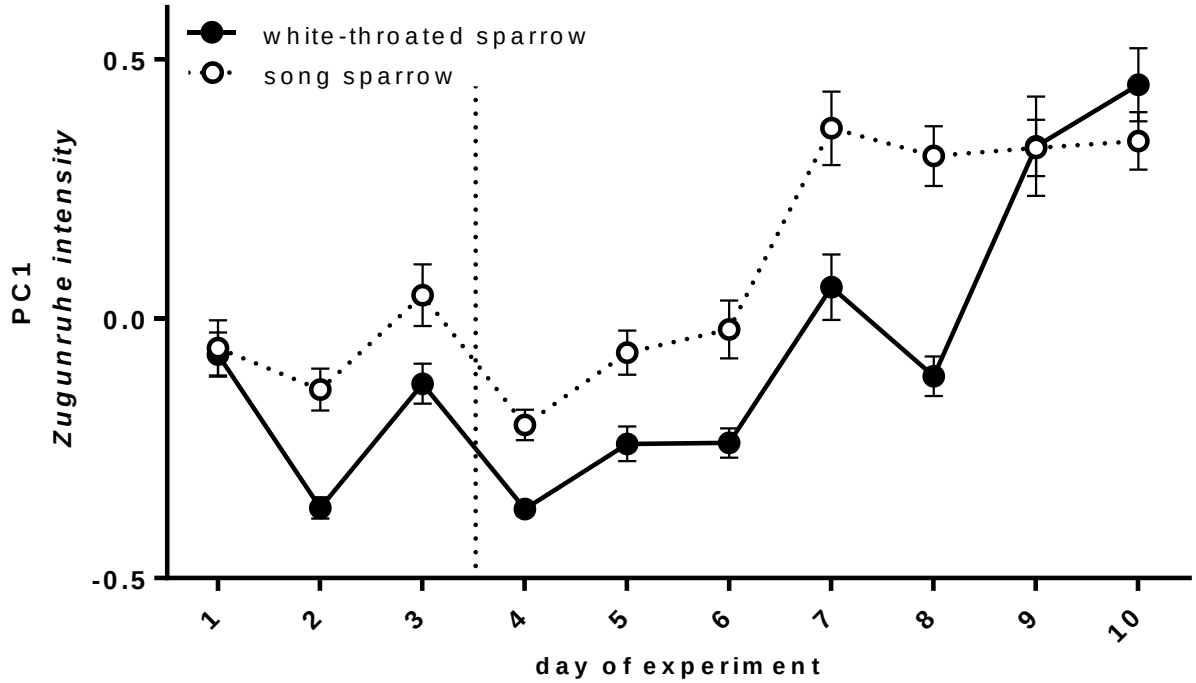

Figure 2-4 *Zugunruhe intensity* (PC1 of nocturnal activity) of white-throated sparrows (black circle, solid line) and song sparrows (open circle, dashed line). The vertical dotted line indicates the evening of inoculation. Error bars represent standard error of the mean.

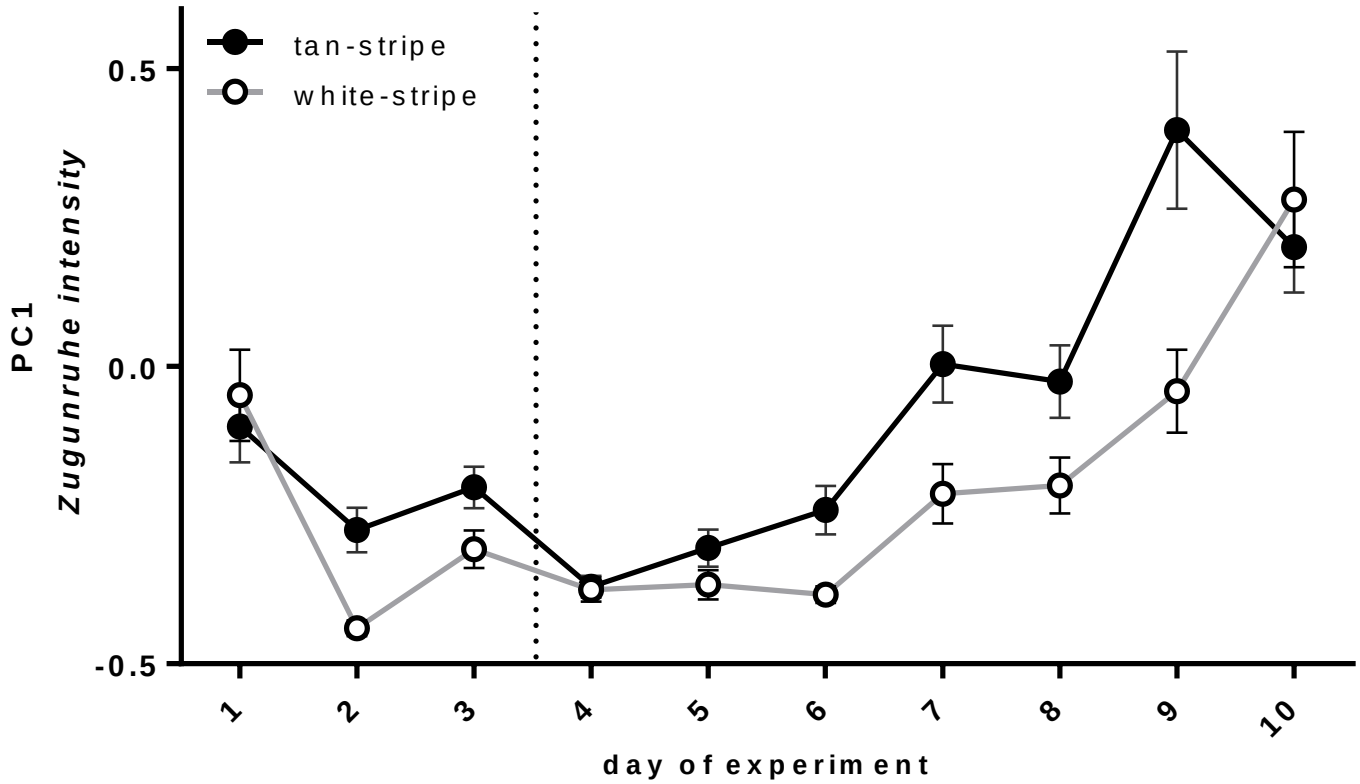

Figure 2-5 *Zugunruhe intensity* (PC1 of nocturnal activity) of white-throated sparrows, separated by tan-stripe morphs (solid circles) and white-stripe morph (open circles). The vertical dotted line represents the evening of inoculation and error bars represent standard error of the mean.

2.4 Discussion

Contrary to predictions, I observed no negative effects of LPS or KLH on body condition or migratory restlessness. Among song sparrows and white-throated sparrows exposed to KLH, whole-body mass increased the morning after inoculation, although this change was not maintained into recovery. In white-throated sparrows, although not in song sparrows, lean mass of individuals exposed to LPS was elevated relative to that of LPS-unexposed birds by one week of recovery. Moreover, mounting an acute-phase immune response to LPS and/or KLH did not affect *Zugunruhe intensity* in either song sparrows or white-throated sparrows. This suggests that observed reductions in *Zugunruhe* following experimental infection with malaria parasites (Chapter 3) are due to the harmful effects of the parasitic infection, not to the costs of upregulating immunity.

This experiment represents the second examination of how non-infectious immune challenges affects behaviour and body composition of birds in migratory condition. Unexpectedly, these challenges appear to have resulted in increases rather than decreases in body condition. Both song sparrows and white-throated sparrows exposed to KLH had higher whole-body mass than KLH-unexposed individuals the morning after exposure (2.54 % and 1.76 %, corresponding to 0.64 g and 0.55 g for song sparrows and white-throated sparrows, respectively). White-throated sparrows exposed to LPS also had increased lean mass (1.79 %, 0.32 g) compared to LPS-unexposed birds after one week of recovery. The injection of LPS and KLH induces a nonspecific acute-phase immune response requiring the secretion of cytokines (Harris & Markl, 1999; Janeway, Travers,

Walport, & Shlomchik, 2001) and inflammatory cells, such as heterophils and macrophages, that are energetically expensive to maintain and deploy (Klasing, 2004). Sparrows in this study had unlimited access to food for the duration of the experiment, so increases in mass may be attributed to bulking in anticipation of maintaining the stimulated immune response during an energetically expensive life-history stage. Captive house sparrows (*Passer domesticus*) exposed to LPS while in breeding condition also increased body mass compared to controls (Bonneaud et al., 2003). By contrast, exposure to LPS reduced body mass of free-living song sparrows during winter, although not during breeding (Owen-Ashley & Wingfield, 2006). In the same study, song sparrows exposed to LPS exhibited greater reductions in aggressive behavior during winter than during the spring breeding season (Owen-Ashley & Wingfield, 2006). Together, these results suggest that the benefits of maintaining critical life-history stage behaviours (e.g., migration, reproduction) may offset the costs of suppressing an acute phase response towards infection during vital times of the year, such as spring migration.

Regardless of treatment, song sparrows and white-throated sparrows reduced *Zugunruhe* the night following injections, likely from handling stress of the inoculation procedure. Hegemann et al. (2018) were the first to inoculate free-living birds with LPS at stopover. While the study reports reduction in movements of LPS-exposed individuals compared to controls, their controls did not experience the inoculation procedure (were not punctured) for fear of this procedure may also reduce activity traits. My results indicate an effect of inoculation procedure and thus it is likely that Hegemann et al.'s (2018) research reports effects of the inoculation procedure rather than effects of mounting an LPS-stimulated acute-phase immune response. I did not find a behavioural

response specific to LPS or KLH injections, suggesting that songbirds can maintain migratory behaviour while mounting an acute phase immune response. Furthermore, captive western sandpipers (*Calidris mauri*; long-distance migrant) exposed to LPS did not differ from controls in voluntary flight speed or duration (Nebel et al., 2013) and reductions in activity of LPS-exposed song sparrows were greatest in non-migratory populations compared to migratory populations (Adelman, Córdoba-Córdoba, Spoelstra, Wikelski, & Hau, 2010). These findings in combination with ours suggest that host-parasite coevolution of migrants has enabled activation of an acute-phase immune response without constraining migratory activity. Previous research has found reductions in activity of birds following exposure to LPS: free-living song sparrows (Adelman et al., 2010; Owen-Ashley & Wingfield, 2006) and white-crowned sparrows (Owen-Ashley et al., 2006) exposed to LPS exhibit reduced aggressive territorial behaviours compared to saline-injected controls; house sparrows (Bonneaud et al., 2003) decreased activity after being exposed to LPS; and captive white-crowned sparrows (Owen-Ashley et al., 2006) had reduced activity compared to saline-injected controls. It is unlikely that I did not administer a sufficient dose of LPS or KLH to observe reductions in activity because I used a higher dose than previous research [4 μg per g body mass versus 1 μg in Bonneaud et al., 2003; Owen-Ashley et al., 2006; Nebel et al., 2013]. It is possible that species-specific effects may be due to song sparrows receiving a consistent dose while white-throated sparrows received mass-specific doses. While the range of white-throated sparrow masses were greater than song sparrows (8 g versus 5 g, respectively), the smallest mass of the range for each species was 74 % of the highest mass, indicating similar variation.

Parasitic infections typically persist longer than 24 hours and prolonged immune upregulation likely incur greater costs than the immune challenge I administered. In a previous experiment, I exposed captive white-throated sparrows to malaria and unsuccessful infection (resistant birds) were slowest to increase *Zugunruhe*, likely due to upregulating immunity to prevent infection (Chapter 3). Additionally, song sparrows that resisted infection by malaria had reduced lean mass compared to controls 14 days after exposure, but did not differ in their timing of departure for fall migration (Kelly, Bonner, MacDougall-Shackleton, & MacDougall-Shackleton, 2018). Thus, there are clear immunological costs to upregulating immunity in response to pathogenic exposure, but those effects appear to be pathogen-specific. Furthermore, in the present study only white-throated sparrows increased lean mass in response to LPS exposure, not song sparrows, but malaria-resistant white-throated sparrows did not reduce body condition (Chapter 3) as did malaria-resistant song sparrows (Kelly et al., 2018), suggesting that responses to pathogen invasion may be species-specific as well.

Knowing the extent that migratory traits are affected in response to successful pathogen invasion are critical to anticipate the spread of disease in our changing climate, but not all experiences with pathogens result in infection. This experiment represents the first step in understanding the extent that migratory traits are affected by costs of upregulating an acute phase immune response. Observational research to date reports later arrival of infected migrants to stopover sites (DeGroote & Rodewald, 2010; López et al., 2013) and to breeding grounds (Møller et al., 2004; Rubaiee, Murayati, & Møller, 2018; Santiago-Alarcon, Mettler, Segelbacher, & Schaefer, 2013) compared to uninfected individuals, but the extent that these delays were due to physical harm from the pathogen or due to

upregulating immunity cannot be discerned. My results support the former since I did not find any negative effects of mounting an acute phase immune response on *Zugunruhe* or body condition. However, experiencing an immune challenge in captivity is difference from field conditions. Sparrows in this experiment had unlimited access to food and water and were restricted to their cage for movement, potentially biasing the results. Additional field-based experiments exposing migrants to LPS and/or KLH in addition to Hegemann et al.'s 2018 study with appropriate control groups are the next step in understanding the balance between immune regulation and migration. These experiments will allow a clear understanding of how pathogen exposure, not successful infection, might influence departure decisions and stopover durations.

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Chapter 3

3 Experimental exposure to malaria affects songbirds' migratory activity, regardless of infection success

3.1 Introduction

Migratory animals move through multiple habitats during migration and stopover, and as a result encounter multiple parasite communities (Kelly et al., 2016; Møller & Erritzøe, 1998). Because migration can increase contact with parasites, often while immune function is compromised due to trade-offs with sustained exercise (Eikenaar & Hegemann, 2016; Nebel et al., 2012), the relationship between migration and disease dynamics is coming under increased scrutiny (Altizer et al., 2011; Fritzsche McKay & Hoye, 2016; Risely, Klaassen, & Hoye, 2018). For example, migratory birds have been implicated in the spread of many diseases, including zoonoses such as West Nile virus (Malkinson et al., 2002), influenza A virus (Kleijn et al., 2010), and Lyme disease (Reed, Meece, Henkel, & Shukla, 2003). However, under some conditions, migration may inhibit disease transmission. Infected individuals may be less likely than uninfected individuals to survive migration (migratory culling (Bradley & Altizer, 2005)); migration may allow hosts to leave habitats infested with parasites (migratory escape (Bartel, Oberhauser, Roode, & Altizer, 2011)); or infected individuals may begin migration, or leave stopover sites, later than uninfected individuals (Latorre-Margalef et al., 2009; van Gils et al., 2007), thus reducing contact between infected and uninfected hosts and minimizing the spread of disease (Galsworthy et al., 2011). Accurately forecasting the effects of migration on the spread of disease thus requires understanding the effects of disease on migration (i.e., on individuals' migratory preparation and timing).

Much of the current understanding of how parasites affect bird migration stems from observational studies on free-living animals during migration and stopover. Such studies generally suggest that parasitic infection may delay migratory departure (reviewed in Risely et al., 2018). However, observational studies are limited in their ability to infer direction of causation. For example, a negative correlation between body condition and infection (DeGroote & Rodewald, 2010; Latorre-Margalef et al., 2009) could suggest that infection worsens condition, or that individuals in poor condition are more susceptible to infection. Furthermore, severely parasitized individuals may disproportionately influence the spread of parasites, but are difficult to observe and sample in the wild (Mukhin et al., 2016; Valkiunas, 2005). Another limitation to observational studies is that susceptibility to parasitism (and potentially to infection-related effects on migratory preparation and timing) may differ between the sexes, age classes or alternative life history strategies (Kelly et al., 2016). Finally, most observational studies are unable to distinguish the subset of individuals that are exposed to parasites but resist infection. In a recent field experiment, song sparrows (*Melospiza melodia*) that were inoculated with malaria (*Plasmodium* sp.) but resisted infection lost more lean mass than either unexposed controls or individuals that became infected (Kelly, Bonner, MacDougall-Shackleton, & MacDougall-Shackleton, 2018). This finding suggests that encountering parasites may be costly even when infection does not occur, and thus that failing to consider exposed-but-resistant hosts can underestimate the effects of disease on migration. Thus, controlled experiments (e.g., Boyd, Kelly, MacDougall-Shackleton, & MacDougall-Shackleton, 2018; Kelly et al., 2018) represent a critical next

step in understanding how infectious disease interacts with animal migration (Mukhin et al., 2016; Risely et al., 2018).

To determine how parasitic infection affects migratory preparation and timing, I experimentally inoculated captive white-throated sparrows (*Zonotrichia albicollis*) with avian malaria parasites (*Plasmodium sp.*) as the birds entered spring migratory condition. I monitored measures of body condition (body composition and hematocrit) and nocturnal migratory restlessness (*Zugunruhe*), which I interpreted as a proxy for migratory behaviour. I compared these measures across individuals that became infected, individuals that were exposed to parasites but did not become infected, and sham-inoculated control individuals that were not exposed to parasites. I predict that infected sparrows will show the greatest reduction in migratory traits compared to controls. I also predict sparrows that resist malarial infection will show reductions in migratory traits, but not as much as infected birds. White-throated sparrows exhibit two distinct plumage and behavioural morphs (Thorneycroft, 1966): tan-striped and white-striped birds differ in traits including aggression (Ficken, Ficken, & Hailman, 1978), refuelling rates (Brown, McCabe, Kennedy, & Guglielmo, 2014), and disease resistance (Boyd et al., 2018). Although this species is widely used to study migration (Brown et al., 2014; Muheim, Phillips, & Deutschlander, 2009; Springer, Price, Thomas, & Guglielmo, 2011), the possibility that morphs differ in their migratory schedules and/or susceptibility to infection-induced delays remains unexplored. Thus, I also compared migratory activity between morphs to determine whether these distinct life history strategies also differ in their response to parasitic infection during spring migration.

3.2 Materials and methods

3.2.1 Study animals and housing

I captured 48 white-throated sparrows in mist nets near Long Point, Ontario, Canada (42.554° N, 80.175° W) during fall migration (October 7 – November 2, 2015). Animals were transported to the Advanced Facility for Avian Research, London, Ontario, and housed indoors in individual cages for the duration of the experiment. The animals had *ad libitum* access to water and food (a mixture of parakeet seed and Mazuri Small Bird Maintenance ground bird chow) and were kept on a short-day photoperiod (10 hours light :14 hours dark; 10L:14D) until February 2016. Birds were captured and collected under the required federal permits (Environment Canada Scientific Collecting Permit CA0244; Banding Permit 10691 E) and all animal procedures were approved by the University of Western Ontario's Animal Use Subcommittee (protocol # 2015-047).

3.2.2 Sex and morph determination

Molecular sexing and morph determination using molecular techniques completed as in Chapter 2. To confirm sex, I used primers P2 and P8 (Griffiths, Double, Orr, & Dawson, 1998) to amplify portions of the CHD-W and CHD-Z genes. To confirm morph, I used a PCR-based genotyping assay for a *DraI* restriction fragment length polymorphism (modified from Michopoulos, Maney, Morehouse, & James, 2007).

3.2.3 Characterizing naturally-occurring malaria infections

To identify birds that were already infected with haematozoa (Figure 3-1a), I prepared a thin-film blood smear from each bird using a drop of the blood sample taken upon capture. Smears were air-dried, fixed in 100% methanol, and treated with Wright-Giesma

stain, then examined under a light microscope with 100x objective using oil immersion. I examined 10 000 erythrocytes per bird, noting the presence of any haematozoa and the total number of parasitized cells. See Figure 3-1a for example of malaria-infected blood smear and Figure 3-1b for a depiction of parasite loads over the course of infection.

I extracted DNA from the remainder of the blood sample with an ammonium acetate-based protocol, then a two-stage nested PCR to amplify a portion of the haematozoan mitochondrial cytochrome *b* following Hellgren, Waldenström, & Bensch (2004). The first round of PCR required primers HAEMNFI and HAEMNR3 (Hellgren, Waldenström, & Bensch, 2004) to amplify a 617-bp fragment of cytochrome *b*. The second round used 1 μL of product from the first-round PCR as template, and the internally nested, *Haemoproteus/ Plasmodium*-specific primers HAEMF and HAEMR2 (Hellgren, Waldenström, & Bensch, 2004) to amplify 527 bp of cytochrome *b*. PCR was conducted in 25 μL volumes with conditions described in Hellgren, Waldenström, & Bensch (2004). I ran second-round PCR products at 100 V for 90 minutes on a 2% agarose gel stained with RedSafe™, then visualized under UV light. I excised bands of the expected product size and purified them with a Gel/PCR DNA Extraction Kit (FroggaBio, North York). I sequenced purified PCR products with primer HAEMF, on an ABI 3730 Genetic Analyzer at the London Regional Genomic Center. I then identified the cytochrome b sequences to genus (i.e., *Plasmodium* or *Haemoproteus*) using the BLAST function in GenBank.

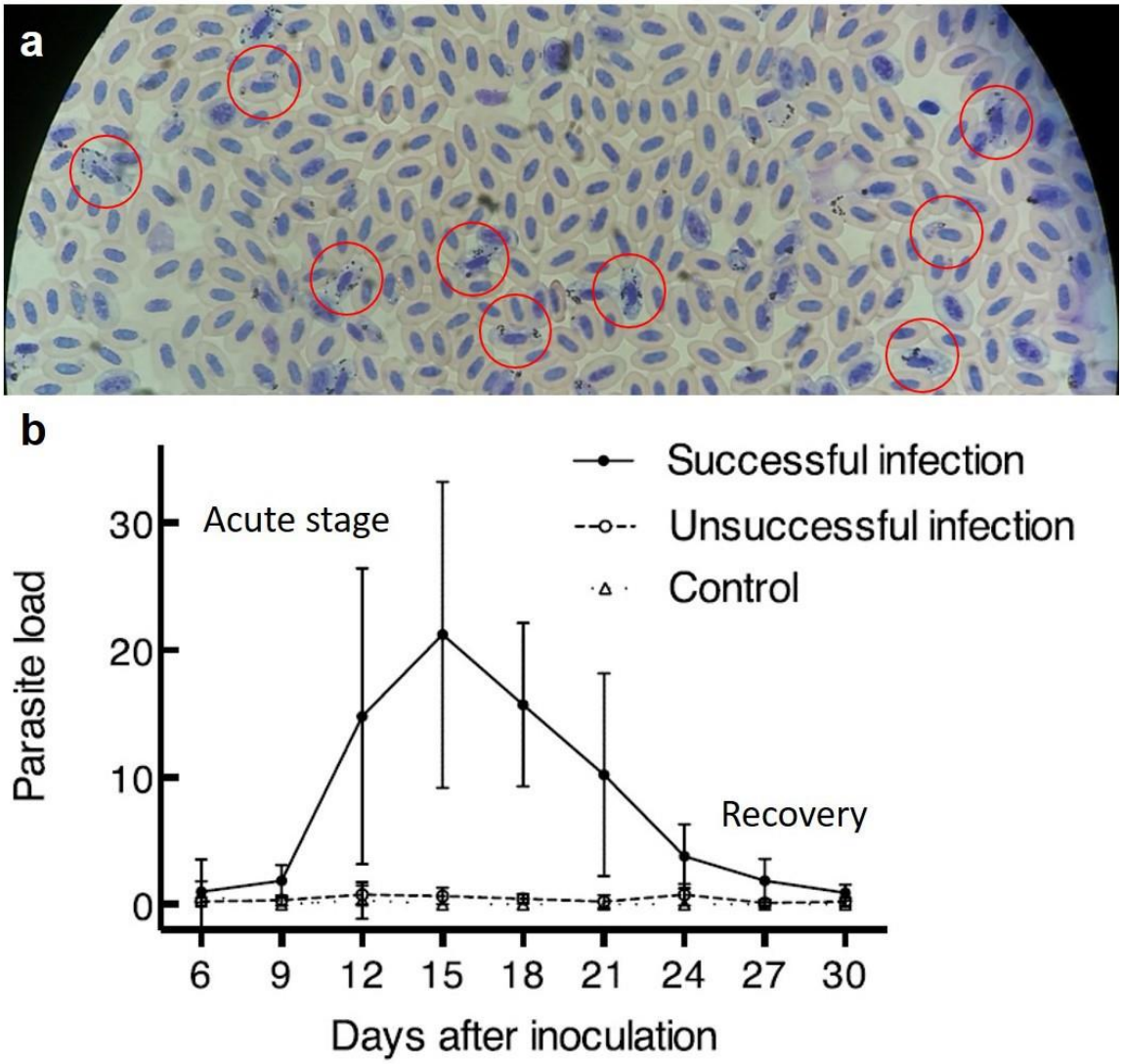

Figure 3-1 a. Blood smear of malaria-infected song sparrow at 100 x magnification. Red circles identify some (but not all) malaria-infected erythrocytes. **b.** Parasite load (± standard error of the mean) of song sparrows exposed to malaria-infected blood (successful infection; unsuccessful infection) or uninfected blood (control) for the duration of the experiment. Figure adapted from Sarquis-Adamson and MacDougall-Shackleton (2016).

3.2.4 Photostimulation and inoculation

On 8 February 2016, I changed the ambient photoperiod from short days (10L:14D) to long days (16L:8D). Captive white-throated sparrows begin showing nocturnal migratory restlessness (*Zugunruhe*) after approximately one week of such photostimulation (Robertson, Hasstedt, Vandermeer, & MacDougall-Shackleton, 2014).

Of 16 birds screened by PCR as potential parasite donors, 9 tested positive for haematozoan infection. All infections were also confirmed by examination of blood smears (range = 1-4 parasitized erythrocytes per 10,000 examined, i.e., 0.01 – 0.04 % parasitaemia). Querying the resultant sequences against BLAST determined that one infection involved *Haemoproteus tartovski* (99% identity to GenBank accession # KY653810), two yielded double peaks characteristic of mixed infections, and the remainder involved *Plasmodium* spp. (96-100% identity to GenBank accession # KY653762, KY653816, LC230052, AF069611 and KT19636, corresponding respectively to morphospecies *circumflexum*, *lutzi*, *relictum*, *elongatum* and *homopolare*). The two individuals with the heaviest naturally-occurring *Plasmodium* infection (0.04% parasitaemia for both) were both infected with lineage TURDUS1 (GenBank accession # KY653762; 97% sequence identity to *P. circumflexum*). These individuals were used as *parasite donors*. Another two individuals, confirmed by PCR and microscopy to be uninfected, were used as *sham donors*. An additional four individuals, confirmed as above to be uninfected, were used as *amplifiers*. The remaining 42 animals (9 tan-striped males, 12 tan-striped females, 14 white-striped males, 7 white-striped females) were used as experimental subjects (Figure 3-2).

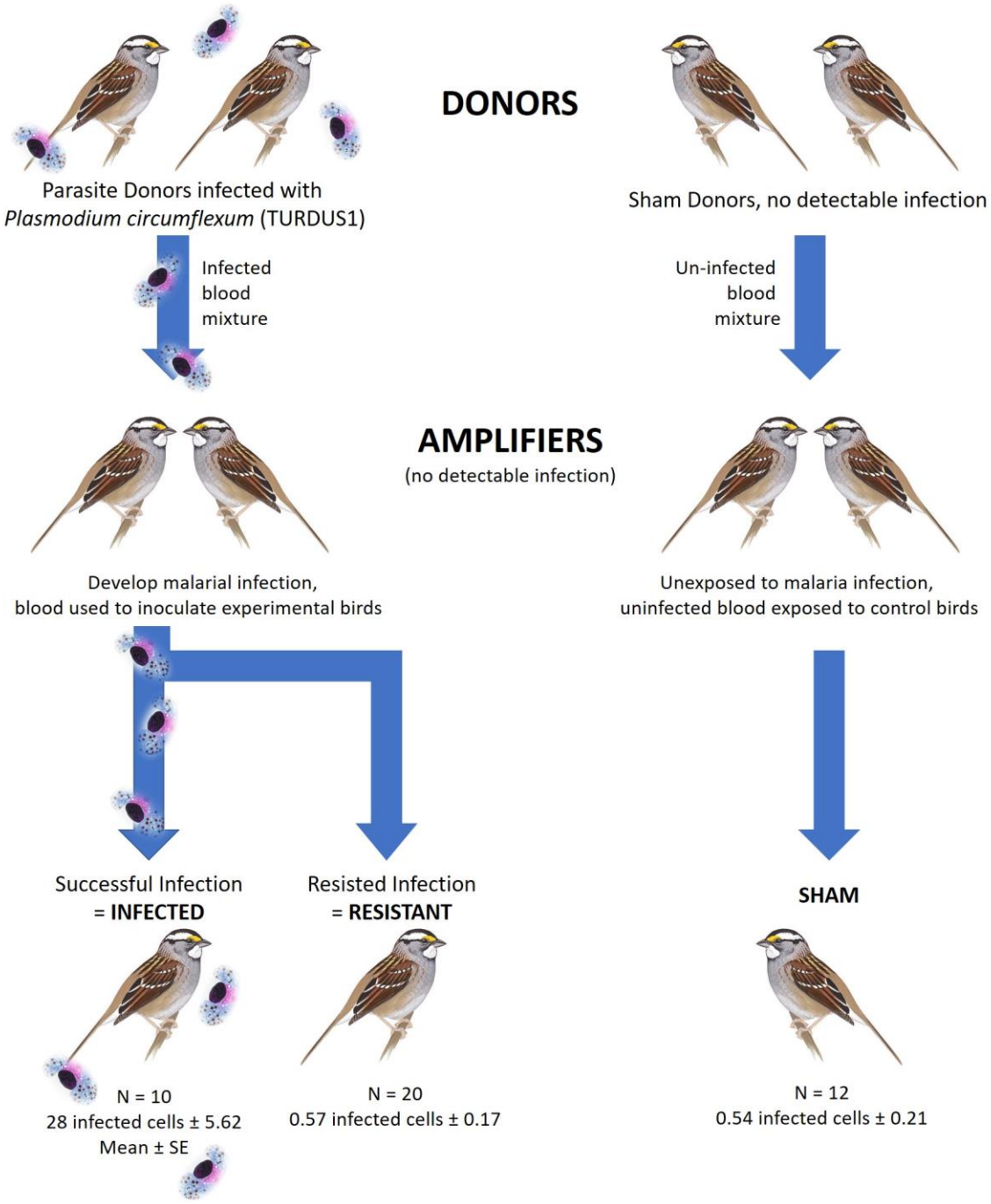

Figure 3-2 The inoculation procedure used to infect white-throated sparrows with *Plasmodium circumflexum* (TURDUS1; GenBank accession # KY653762) and to create the sham group. Malaria paintings were adapted from Karadjian et al. (2013) and David Allen Sibley drew the white-throated sparrow image, adapted from the Audubon Guide to North American Birds.

On 8 February 2016 (i.e., the first day of photostimulation), I collected 200 μL of blood via brachial venipuncture from each of the two naturally-infected *parasite donors* and from each of the two naturally-uninfected *sham donors*. I mixed the blood from both parasite donors, combined it with buffer (see below) and used it to inoculate two previously uninfected *parasite amplifiers*. Similarly, I mixed blood from both sham donors with buffer to inoculate two uninfected *sham amplifiers*. Using a sterile syringe and 26-gauge needle, 200 μL of a mixture containing 80 μL of freshly collected blood, 20 μL of 3.7 % sodium citrate, and 100 μL of 0.9 % saline was injected into the pectoralis muscle of each amplifier. I then monitored the infection status of the amplifiers by collecting small blood samples each week and preparing thin-film blood smears.

By 13 days after inoculation, both parasite amplifiers displayed at least one mature-stage *Plasmodium* parasite per 10,000 erythrocytes, and the sham amplifiers remained uninfected. The following day (February 22, 2016; experimental day 0), all four amplifiers were euthanized by overdose of isofluorane vapours, and blood collected into a syringe via cardiac puncture (1.2 mL from each parasite amplifier, and 400 μL - 800 μL from each sham amplifier). Blood from parasite amplifiers was combined and used to inoculate 30 *experimental* birds, and blood from the sham amplifier was used to inoculate 12 *sham* birds, as described above (Figure 3-2). I inoculated more experimental than sham birds anticipating that about half of experimental birds would resist infection.

On experimental day 14 (i.e., 14 days after inoculating experimental and sham subjects; 28 days after the transition to long days) I collected ~ 20 μL of blood from each subject, for microscopic analysis of haemosporidian parasite loads and infection outcome. I prepared thin-film blood smears and scored them blind regarding experimental

treatment (experimental vs sham). I categorized white-throated sparrows as successfully *infected* if their day 14 parasite load was twice the number of parasites in the most heavily infected *sham* (Boyd et al., 2018; Sarquis-Adamson & MacDougall-Shackleton, 2016). Chronic malaria infections persist for months after exposure and infections can reactivate during migratory periods (Beaudoin, Applegate, Davis, & McLean, 1971) hence the presence of malarial parasites in *sham* birds. Three parasites per 10 000 erythrocytes (0.03 %) was the heaviest parasite load in *shams*, thus I established a threshold for infection success of six or more infected cells per 10 000 (≥ 0.06 %). Malaria-inoculated sparrows below this threshold were classified as *resistant*.

3.2.5 Body condition

I assessed body mass and composition at three timepoints (experimental days -3, 17 and 38). Body mass was measured to the nearest 0.1 g using a spring scale, and lean and fat mass were calculated using quantitative magnetic resonance (QMR). I calibrated the QMR instrument (Echo-MRI-B™, Echo Medical Systems Houston, TX, USA) using standards of canola oil to ensure accurate readings to the nearest 0.001 g (Guglielmo, McGuire, Gerson, & Seewagen, 2011; Seewagen & Guglielmo, 2011). I assessed total mass, fat mass, and lean mass of sparrows at three time points of the infection: 3 days before inoculation, 17 days after inoculation (peak parasitemia [Sarquis-Adamson & MacDougall-Shackleton, 2016]) and 38 days post-inoculation (recovery). I averaged two replicate scans that used four primary accumulations while gently immobilizing the bird in a ventilated holding tube (4.5 cm diameter).

I assessed hematocrit at three timepoints (experimental days -5, 14, and 35) by collecting approximately 140 μL of blood from each subject by brachial venipuncture.

One end of each capillary tube (2 tubes per subject) was capped with clay and samples were spun for five minutes in a micro-hematocrit centrifuge at 13 000 g. I recorded the percentage by volume of packed cells, averaged across both tubes, as hematocrit.

3.2.6 Behavioural analysis

Behavioural analysis of activity completed as in Chapter 2.

3.2.7 Statistical analysis

I used Pearson's correlation to characterize relationships among measures of migratory restlessness (*Zugunruhe*) obtained from EthoVision XT (distance moved [cm], time moved [s], time mobile [s]). Because measures were significantly correlated, I entered them into a principal component analysis (PCA), using the *prcomp* command in base R version 3.4.1 (R Core Team, 2017). I constructed 95% confidence intervals for eigenvalues, retained the component with an eigenvalue greater than 1 for further analysis, and rotated components using varimax rotation. I term this PC1 factor *Zugunruhe intensity*.

To determine whether malaria infection and/or resistance affected *Zugunruhe intensity*, I constructed a global linear mixed model using lme4 (Bates, Maechler, Bolker, & Walker, 2015). I used the PC scores of the first principal component (*Zugunruhe intensity*) as the dependent variable. An information theoretic approach (Anderson & Burnham, 2002) and Akaike's information criterion corrected for small sample sizes (AICc) was used to compare support for alternative models predicting *Zugunruhe intensity*, automated by the *dredge* command in MuMIn (Bartoń, 2018). To avoid over-fitting the models, I included only the main effects and the interaction terms for which

there were clear a prioi predictions. Candidate models differed in the presence and absence of terms for the global model: ~ day of experiment (day) + phase + treatment + morph + day × phase + phase × treatment + day × phase × treatment and included random intercept and slope terms for the effect of each individual over experimental days to account for repeated measures. Preliminary results indicated that sex contributed minimally to the model and was thus excluded from the analysis. The choice of a random component was based on (AIC) comparisons using maximum likelihood estimation to fit the fixed components of the global model with two alternative random components: random intercept terms for each day or for each individual. Phase represents differences in the development and number of malaria parasites (Figure 3-1b) and, as such, I expect migratory responses to differ in these phases: phase one (acute phase) is represented by days 1 through 17 of the experiment and phase two (recovery phase) by days 17 through 38 (Yorinks & Atkinson, 2000). To determine if reductions in *Zugunruhe intensity* had similar effects on displacement between *infected*, *resistant*, and *sham* birds I calculated the cumulative distance moved (cm; center-point detection) for the duration of the experiment for each individual and compared treatments using a one-way ANOVA.

To determine whether malaria infection and/or resistance affected body composition, I constructed a global linear mixed model for each physiological parameter: whole-body mass, fat mass, lean mass, and hematocrit. I used the percent change in mass (percentage in the case of hematocrit) as the dependent variable, calculated using the following formula:

$$\%\, change = \left(\frac{(mass\ at\ time\ 2 - mass\ at\ time\ 1)}{mass\ at\ time\ 1} \right) \times\ 100\%.$$

The percent change was calculated for the acute phase (time 1 = day -3, time 2 = day 17) and recovery phase (time 1 = day 17, time 2 = day 38). An information theoretic approach (Anderson & Burnham, 2002) and Akaike's information criterion corrected for small sample sizes (AICc) was used to compare support for alternative models predicting each physiological parameter, automated by the dredge function in MuMIn (Bartoń, 2018). Candidate models differed in the presence and absence of terms for the global model: ~ phase + treatment + sex + phase × treatment + phase × sex + treatment × sex and included a random effect of subject. Preliminary results indicated that morph contributed minimally to the model and was thus excluded from this analysis. If the top two models fell within two AICc units of each other, I compiled model-averaged parameter estimates from the full set of AICc-ranked candidate models using the conditional averaging method (Anderson & Burnham, 2002) implemented in *model.avg* in the R package MuMIn (Bartoń, 2018).

3.3 Results

3.3.1 Infection success

Of the 30 white-throated sparrows inoculated with *P. circumflexum*, 10 became successfully infected by my threshold of 0.06% parasitemia 14 days after inoculation (Table 3-1). I categorized the remaining 20 birds as *resistant*. Mean (± SEM) parasitaemia for the *infected* group was 28.00 ± 5.62 (n = 10) infected cells per 10 000, as compared to 0.54 ± 0.21 for *shams* (n = 14) and 0.53 ± 0.17 (n = 20) for the *resistant* birds.

3.3.2 Zugunruhe intensity

All variables quantified by the Noldus Ethovision software were significantly correlated with one another (Table 3-2a). Only the first principal component had an eigenvalue greater than 1 (Table 3-2b) and this component explained 93 % of the overall variance in activity. All activity variables loaded positively in the first principal component (Table 3-2c). I retained only the first component and hereafter refer to this component as *Zugunruhe intensity*.

Table 3-1 Experimental design. The number of individuals assigned to each treatment group, broken down by sex, morph, previous infection status, and, for the experimental groups, whether or not the inoculation resulted in infection by the fourteenth day of infection (success = *infected*); failure = *resistant*).

sex:	**male**				**female**			
morph:	**white**		**tan**		**white**		**tan**	
previous infection:	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**N**
treatment success (*infected*)	0	4	0	1	1	0	1	3
treatment failure (*resistant*)	2	4	1	3	0	4	3	3
sham	1	3	2	2	1	1	0	2

Table 3-2 Creating principle components of measures of nocturnal activity.
a. Correlation matrix of variables produced by Noldus. Pearson's r (r), sample size (n; average of activity per night), and significance values (p) are reported.
b. Summary statistics for the principal component analysis of activity variables.
c. Factor loadings for each principal component.

a.	**distance moved (cm)**	**time moving (s)**	**time mobile (s)**
time moving (s)	r = 0.91 *n* = 1597 *p* < 0.0001		
time mobile (s)	r = 0.81 *n* = 1597 *p* < 0.0001	r = 0.96 *n* = 1597 *p* < 0.0001	

b.	**PC1**	**PC2**	**PC3**
eigenvalue	2.79	0.19	0.02
proportion of variance	0.929	0.065	0.006
cumulative proportion	0.929	0.994	1.00

c.	**PC1**	**PC2**	**PC3**
distance moved (cm)	0.563	0.770	-0.300
time moving (s)	0.594	-0.125	0.794
time mobile (s)	0.574	-0.625	-0.528

Of the candidate models predicting *Zugunruhe intensity*, the best-supported model included all variables except morph (Table 3-3a). The second-ranked model differed from the first by including morph effects. Combined, the first and second-ranked models receive all of the support (weight = 0.57 and 0.43, respectively; Table 3-3a) and were the only models to include day x phase x treatment, indicating the importance of this three-way interaction. *Zugunruhe intensity* increased throughout the experiment (day) and was higher in the recovery phase than the acute phase (Table 3-3b; Figure 3-3). *Resistant* birds during the acute phase tended toward having the slowest increase in *Zugunruhe intensity*, resulting in *resistant* birds having lower *Zugunruhe intensity* than *shams* in the recovery phase (Table 3-3; Figure 3-3). *Infected* birds tended toward lower *Zugunruhe intensity* than *shams* during the recovery phase, attributed to a gradual in *Zugunruhe intensity* during the recovery phase (Table 3-3b; Figure 3-3). I found similar displacements for *infected* (distance = 9532 m) and *resistant* (9 395 m) birds compared to *shams* (10 987 m), but there was no statistical difference between treatments (one-way ANOVA: $F_{2,39} = 0.391$, $p = 0.68$).

3.3.3 Body composition

There was no effect of malaria exposure on any measures of body composition, but fat mass and hematocrit decreased during the recovery phase (Figure 3-4; Tables 3-4 – 3-7).

Table 3-3 Results of model selection predicting *Zugunruhe intensity.*

a. Top-ranked candidate linear models (AICc < 2 and null model) predicting *Zugunruhe intensity* of white-throated sparrows exposed to *P. circumflexum* strain TURDUS1 infected blood (infected and resistant groups) or uninfected blood (shams). Reported in the table are second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), number of parameters (K) and proportional weight of each model (w_i).

b. Real-function parameters of the best-fitting model (~ day of experiment (day) + phase + treatment + day × phase + phase × treatment + day × phase × treatment) predicting white-throated sparrow Zugunruhe intensity. Phase effects are estimated with reference to the acute phase, and treatment with reference to the sham group. A higher estimate indicates higher *Zugunruhe intensity*. Significant effects are italicized.

a. ranked candidate models	**K**	**AICc**	**ΔAICc**	**w_i**
day + phase + treatment + day × phase + phase × treatment + day × phase × treatment	16	2655.65	0.00	0.57
day + phase + treatment + morph + day × phase + phase × treatment + day × phase × treatment	17	2656.25	0.59	0.43
null	5	2929.39	273.73	0.00

b. parameters of top model	**estimate**	**std. error**	**97.5% CI**	***p***
intercept	*-1.17*	*0.12*	*-1.41 – -0.94*	*< 0.001*
day	*0.08*	*0.01*	*0.06 – 0.11*	*<0.001*
phase (recovery)	*1.56*	*0.17*	*1.23 – 1.89*	*<0.001*
Treatment (infected)	-0.07	0.17	-0.40 – 0.26	0.663
Treatment (resistant)	0.12	0.15	-0.17 – 0.40	0.417
day × phase (recovery)	*-0.07*	*0.01*	*-0.08 – -0.05*	*<0.001*

phase (recovery) × treatment (infected)	*0.45*	*0.25*	*-0.03 – 0.94*	*0.067*
phase: recovery × treatment (resistant)	*-0.77*	*0.21*	*-1.19 – -0.35*	*<0.001*
day × phase (acute) × treatment (infected)	0.003	0.02	-0.03 – 0.04	0.833
day × p*hase (recovery) × treatment (infected)*	*-0.04*	*0.01*	*-0.07 – -0.007*	*0.017*
day × p*hase (acute) × treatment (resistant)*	*-0.03*	*0.014*	*-0.05 – 0.002*	*0.066*
day × phase (recovery) × treatment (resistant)	-0.002	0.01	-0.03 – 0.02	0.83

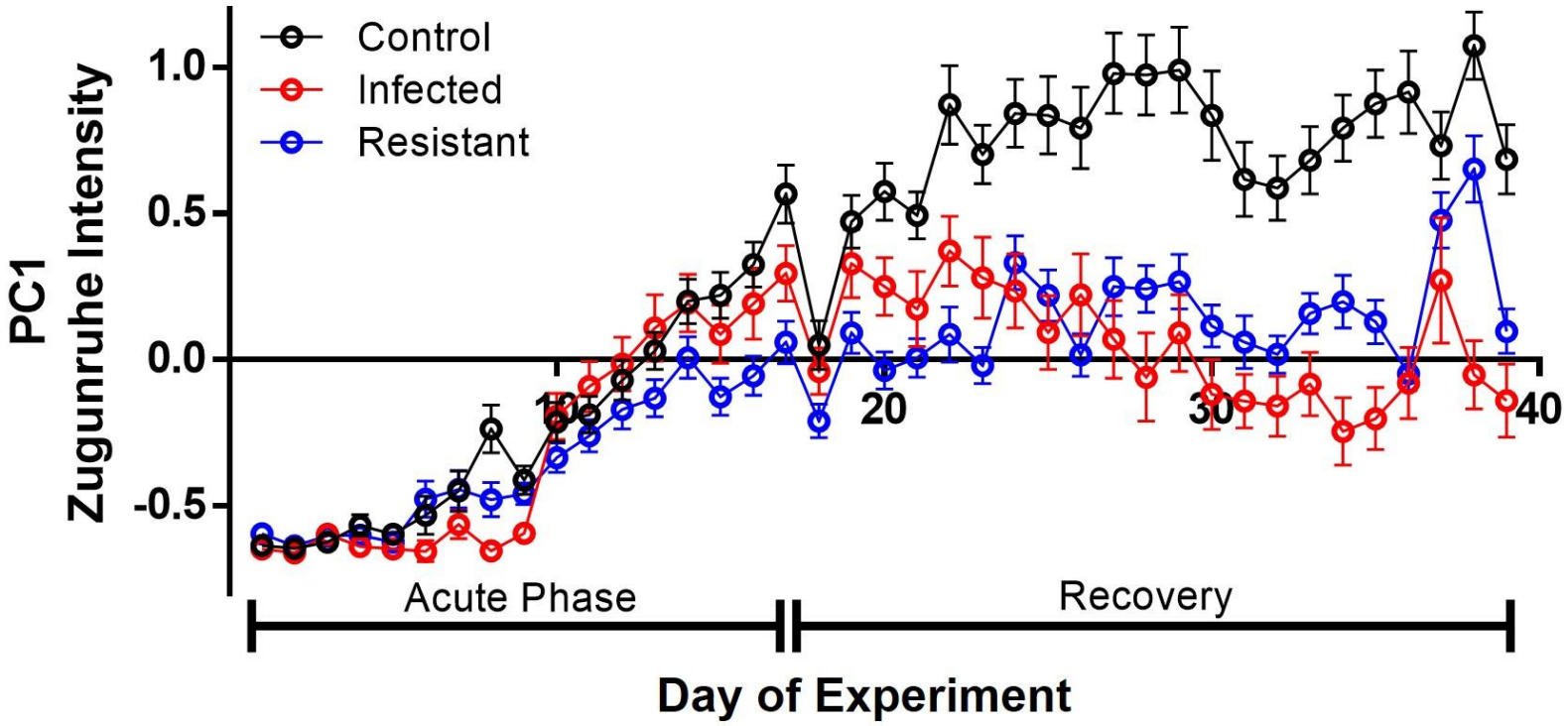

Figure 3-3 *Zugunruhe intensity* of white- and tan-striped white-throated sparrow after exposure to uninfected blood (*sham*) or blood containing *P. circumflexum* strain TURDUS1 (*infected* and *resistant* groups) for the experiment duration. During the acute phase resistant birds had reduced *Zugunruhe intensity*, but during the recovery phase, both infected and resistant birds had reduced *Zugunruhe intensity*. Error bars represent standard error of the mean.

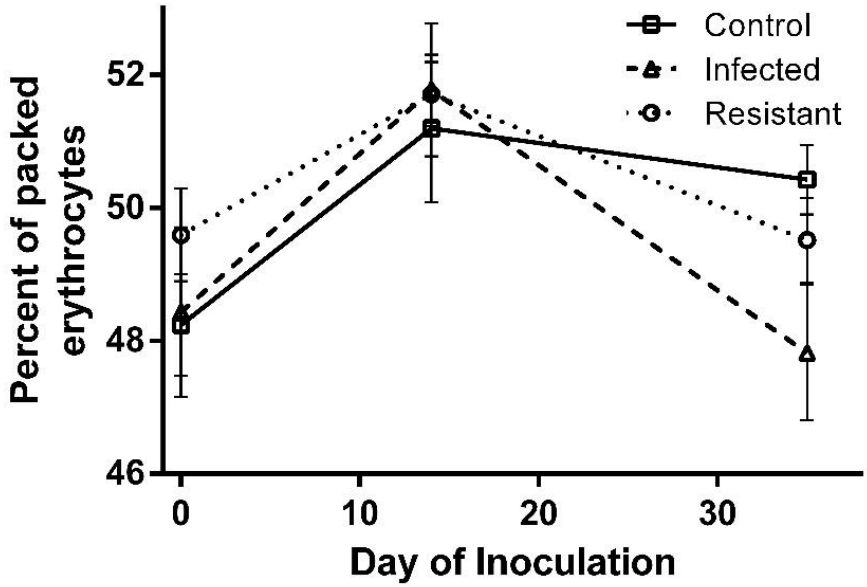

Figure 3-4 Percent of packed erythrocytes for white-throated sparrows exposed to uninfected blood (controls) or blood containing *P. circumflexum* strain TURDUS1 (infected and resistant groups) on the day of inoculation, 14 days and 35 days after inoculation. Error bars represent the standard error of the mean.

Table 3-4 Selection of models predicting captive white-throated sparrow fat mass.
a. Top-ranked candidate linear models (AICc < 2 and null model) predicting the percent change in fat mass (g) of captive white-throated sparrows after exposure to *P. circumflexum* strain TURDUS1 infected blood (infected and resistant groups) or uninfected blood (controls). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Real function parameters of the best-fitting model predicting the percent change in fat mass of captive white-throated sparrows. A higher estimate indicates higher fat mass. Significant variables are italicized.

a. ranked candidate models	**K**	**AICc**	**ΔAICc**	**w_i**
phase + treatment + sex + phase × treatment + phase × sex + treatment × sex	11	961.2	0.00	0.982
null	3	1094.1	132.86	0.00

b. parameters of top model	**estimate**	**std. Error**	**97.5 % CI**	***p***
intercept	420.19	138.07	161.84 – 678.55	0.002
phase (recovery)	*-483.48*	*192.66*	*-843.99 – -122.98*	*0.012*
treatment (infected)	-53.90	141.45	-318.58 – 210.77	0.703
treatment (resistant)	-156.01	134.93	-408.50 – 96.47	0.247
sex (female)	-80.87	47.21	-169.21 – -7.47	0.087
phase (recovery) × treatment (infected)	108.38	192.76	-252.30 – 469.07	0.574
phase (recovery) × treatment (resistant)	181.72	190.00	-173.79 – 537.23	0.339
phase (recovery) × sex (female)	*115.64*	*58.73*	*5.74 – 225.54*	*0.049*
treatment (control) × sex (female)	-61.97	60.87	-175.86 – 51.92	0.309

Table 3-5 Selection of models predicting captive white-throated sparrow lean mass.
a. Top-ranked candidate linear models (AICc < 2 and null model) predicting the percent change in lean mass (g) of captive white-throated sparrows after exposure to *P. circumflexum* strain TURDUS1 infected blood (infected and resistant groups) or uninfected blood (controls). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Real function parameters of the best-fitting model predicting the percent change in lean mass of captive white-throated sparrows. Conditional averaging results are reported. A higher estimate indicates higher lean mass.

a. ranked candidate models	**K**	**AICc**	**ΔAICc**	**w_i**
phase + treatment + phase × treatment	8	385.2	0.00	0.283
phase + sex + treatment + phase × treatment + phase × sex	10	385.9	0.67	0.203
phase + sex + treatment + phase × treatment + phase × sex + treatment × sex	11	386.3	1.06	0.166
phase + sex + treatment + phase × treatment	9	386.5	1.26	0.151
phase + sex + treatment + phase × treatment + sex × treatment	10	386.8	1.58	0.129
null	3	467.86	82.77	0.00

b. parameters of top model	**estimate**	**std. error**	**97.5 % CI**	***p***
(intercept)	0.43	2.57	-4.69 – 5.55	0.87
phase (recovery)	5.94	3.56	-1.14 – 13.03	0.10
treatment (infected)	-3.22	2.61	-8.47 – 2.02	0.23
treatment (resistant)	-0.79	2.55	-5.89 – 4.29	0.76
phase (recovery) × treatment (infected)	1.97	3.78	-5.43 – 9.37	0.60
phase (recovery) × treatment (resistant)	-0.30	3.72	-7.60 – 7.00	0.94

Table 3-6 Selection of models predicting captive white-throated sparrow whole-body mass.
a. Top-ranked candidate linear models (AICc < 2 and null model) predicting the percent change in whole-body mass (g) of captive white-throated sparrows after exposure to *P. circumflexum* strain TURDUS1 infected blood (infected and resistant groups) or uninfected blood (controls). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i).
b. Real function parameters of the best-fitting model predicting the percent change in whole-body mass of captive white-throated sparrows. Conditional averaging results are reported. A higher estimate indicates higher whole-body mass.

a. ranked candidate models	**K**	**AICc**	**ΔAICc**	**w_i**
phase + treatment + sex + phase × treatment + phase × sex + treatment × sex	11	523.4	0.00	0.47
phase + treatment + sex + phase × treatment + treatment × sex	10	524.9	1.41	0.23
null	3	634.1	110.69	0.00

b. parameters of top model	**estimate**	**std. error**	**97.5 % CI**	***p***
intercept	9.68	6.78	-3.83 – 3.20	0.16
phase	-10.91	9.40	-29.63 – 7.81	0.25
treatment (infected)	6.44	6.97	-7.43 – 4.42	0.36
treatment (resistant)	5.79	6.68	-7.53 – 19.11	0.39
sex (female)	0.04	2.20	-4.33 – 4.41	0.98
phase (recovery) × treatment (infected)	-7.68	9.53	-26.68 – 11.32	0.43
phase (recovery) × treatment (resistant)	-9.39	9.40	-28.13 – 9.35	0.33
phase (recovery) × sex (female)	0.95	2.93	-4.88 – 6.79	0.75
sex (female) × treatment (infected)	-3.12	3.03	-9.16 – 2.92	0.31

Table 3-7 Selection of models predicting captive white-throated sparrow hematocrit. a. Top-ranked candidate linear models (AICc < 2 and null model) predicting the percent change in hematocrit of captive white-throated sparrows after exposure to *P. circumflexum* strain TURDUS1 infected blood (infected and resistant groups) or uninfected blood (controls). Reported in the table are the number of parameters (K), second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), and the proportional weight of each model (w_i). b. Real function parameters of the best-fitting model predicting the percent change in hematocrit of captive white-throated sparrows. Conditional averaging results are reported. A higher estimate indicates higher hematocrit.

a. ranked candidate models	**K**	**AICc**	**ΔAICc**	**w_i**
phase + treatment + sex + phase × treatment + phase × sex + treatment × sex	11	523.2	0.00	0.536
phase + treatment + sex + phase × treatment + phase × sex	10	524.8	1.56	0.245
null	3	582.0	58.77	0.00

b. parameters of top model	**estimate**	**std. error**	**97.5 % CI**	***p***
intercept	12.01	6.88	-1.70 – 25.71	0.086
phase	*-23.07*	*9.72*	*-42.42 – -3.71*	*0.019*
treatment (infected)	-3.76	6.98	-17.67 – 10.16	0.600
treatment (resistant)	-6.73	6.74	-20.15 – 6.70	0.326
sex (female)	-1.47	2.33	-6.10 – 3.16	0.533
phase (recovery) × treatment (infected)	11.49	9.61	-7.66 – 30.64	0.240
phase (recovery) × treatment (resistant)	12.17	9.47	-6.70 – 31.05	0.206
phase (recovery) × sex (female)	4.17	2.92	-1.65 – 9.99	0.161
sex (female) × treatment (infected)	-1.30	3.03	-7.35 – 4.75	0.673

3.4 Discussion

This study is the first to demonstrate that mere exposure to malaria can affect migratory behaviour of songbirds. *Zugunruhe intensity* of *resistant* white-throated sparrows was lower than *shams* in the recovery phase of infection, due to their slower increase in activity during the acute phase of infection. In the recovery phase, *resistant* birds continued to increase *Zugunruhe intensity* while that of *infected* birds began to decrease. *Infected* and *resistant* birds had similar final displacements by the end of the experiment compared to *shams*. Thus, while infected and *resistant* birds differ in the time which *Zugunruhe intensity* is affected, the overall effect on movement was similar. White- and tan-stripe birds did not differ in *Zugunruhe intensity* until the recovery phase when tan-stripe birds were consistently more active. I found no effect of malaria exposure on any physiological parameter examined.

While I did not examine immunological measures in this experiment, the slow increase in *Zugunruhe intensity* of *resistant* birds in the acute phase may be due to a trade-off with immune defence. The acute phase first stimulates an inflammatory response with the release of cytotoxic compounds that orchestrate fever, anorexia, and recruitment of acute phase proteins from the liver (Klasing, 2004). Recruiting the liver away from its normal functions in secreting nutrients to support growth or activity is the single most expensive component of immunity during an acute phase response (Klasing, 2004) and may contribute to the reduced activity in *resistant* birds after exposure to malaria. In the next few days, B- and T-lymphocytes that recognize antigenic determinants of malaria begin to proliferate as part of the acquired response and antibody production begins after ~ one week (Klasing, 2004), demanding additional energetic

resources. Decreases in activity of *infected* birds during the recovery phase may thus be attributed to the acute immune response, but also to acquired immune responses. Indeed, circulating levels of IgY are greater in wild lesser black-backed gulls (*Larus fuscus*) infected with *Haemoproteus* than uninfected birds (Arriero, Müller, Juvaste, Martínez, & Bertolero, 2015). In addition to upregulating immunity, costs of erythrocyte replacement may further contribute to energetic and nutritional deficits otherwise directed to migratory activity. However, there is no evidence that reductions in hematocrit affect exercise capacity and thus migratory behaviour; experimentally-infected great reed warblers (*Acrocephalus arundinaceus*) did not differ from uninfected birds in any measures of metabolic rate, endurance capacity, or haemoglobin concentration, indicative of oxygen supply (Hahn et al., 2018). Thus, there is a need for investigations of mechanisms besides aerobic capacity that mediate malaria-induced delays such as impacts on hosts' internal organs that parasites reside in, as well as a controlled investigation of immunological changes of migrant birds during malarial invasion.

Despite differential effects of malaria exposure on *Zugunruhe intensity*, fat mass, lean mass, whole-body mass, and hematocrit were unaffected by malaria exposure. Captive great reed warblers exposed to malaria did not exhibit changes in metabolic rates (resting or maximal) or haemoglobin (Hahn et al., 2018), and my finding no effect of malaria exposure on hematocrit corroborate with these results, further support that malaria does not appear to reduce aerobic capacity of migratory birds, but captive conditions and unlimited access to food in both of these experiments may confound the results. Null effects of malaria exposure on fat and lean mass in this captive experiment corroborate with null effects on body composition of captive and free-living great reed

warblers with malaria infections during the fall and winter, respectively (Hahn et al., 2018; Sorensen et al., 2016). However, I inoculated song sparrows with malaria in a previous experiment and found that *resistant* birds had lower lean mass than *shams* 14 days after exposure relative to their susceptible counterparts (Kelly et al., 2018). Furthermore, (Risely et al., 2018) found infected individuals to have lower body stores. Null effects on body composition in this study may be because the white-throated sparrows were in captivity for six months leading up to the experiment and had unlimited access to food and water, allowing birds to have the body stores large enough to negate the physical effects of malarial infection. In contrast, song sparrows (Kelly et al., 2018) had spent only two months in captivity prior to inoculation, providing less time to increase body stores. Alternatively, the effects of malaria inoculation may be specific to the interactions of the parasite lineage and host as a result of host-parasite coevolution (Isaksson, Sepil, Baramidze, & Sheldon, 2013).

An important finding of this study is white- and tan-stripes differ in *Zugunruhe intensity*. White-throated sparrows have been used as a model species to investigate migratory traits (exercise physiology [Springer et al., 2011] , navigation [Muheim et al., 2009] , and stopover refuelling [Brown et al., 2014]) yet this is the first time, to my knowledge, that differences have been recorded in migratory activity. Unexpectedly, tan-stripes were more active than white-stripes during the recovery phase of activity, despite tan-stripes having a greater peak parasite load than white-stripes (Boyd et al., 2018). This may suggest white- and tan-stripes have different migratory strategies but data comparing field-observed white- and tan-stripe migratory timing is required to confirm such

conclusions. Regardless, future migration research should control for morph-specific effects when using white-throated sparrows as their study species.

Not all parasitic encounters end in successful infection. *Resistant* birds in this experiment represent a group of individuals exposed to potential infection that did not succumb to it. This self-selected group has been overlooked in observational research since it is difficult to determine what pathogens a bird has been exposed to if they are not currently infected. The slow increase in *Zugunruhe intensity* of *resistant* birds during the acute phase of infection suggests separation of healthy and exposed individuals during migration, supporting the migratory escape hypothesis (Bartel et al., 2011). Additional support for this hypothesis, presuming malaria-exposed birds migrate successfully (otherwise support the migratory culling hypothesis (Bradley & Altizer, 2005)), is in the reduced activity of *infected* birds during the recovery phase of infection. The observed reductions in response to this experimental malaria exposure, whether successfully infected or not, are critical to modelling the spread of disease. Further experimental research is required using alternative hosts and pathogens for a complete understanding of how disease affects animal migration in order to predict the how animal migration contributes to the spread of disease.

3.5 References

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Chapter 4

4 Exposing migratory sparrows to *Plasmodium* suggests costs of resistance, not necessarily of infection itself [3]

4.1 Introduction

Each year, billions of animals migrate between breeding and wintering grounds, often covering huge distances and crossing obstacles such as mountain ranges and oceans (Dingle, 2014). Migrants move through diverse habitats during migration and stopover, and as a result, encounter multiple parasite communities (Figuerola & Green, 2000; Møller & Erritzøe, 1998; Møller & Szép, 2010). The relationship between animal migration and disease dynamics is thus coming under increased scrutiny (Altizer, Bartel, & Han, 2011; Fritzsche McKay & Hoye, 2016). Because migration can increase rates of contact between hosts and parasites, often while immune function is compromised due to trade-offs with sustained exercise (Dolan et al., 2016; Eikenaar & Hegemann, 2016; Nebel et al., 2012; Owen & Moore, 2008; van Dijk & Matson, 2016), it is reasonable to expect that migration enhances the spread of infectious disease. However, in some systems migration may inhibit disease transmission, for example if infected hosts are unable to migrate successfully (migratory culling; Bradley & Altizer, 2005) or if migration allows hosts to escape from infected habitats (migratory escape; Bartel, Oberhauser, Roode, & Altizer, 2011). Even in systems where parasitized hosts are

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capable of migrating successfully, such individuals may delay departure from the breeding grounds or stopover sites (Latorre-Margalef et al., 2009; van Gils et al., 2007). Models of disease transmission predict that these infection-induced delays in migration should decrease infection rates, by reducing contact between infected and uninfected hosts (Galsworthy et al., 2011).

Field studies on free-living animals provide some evidence that parasitic infection may affect migratory timing, potentially mediated through effects on body condition and stores. Risely, Klaassen, & Hoye (2018) compared 41 studies (85 observations across vertebrate and invertebrate species) to examine how infection can affect changes in body stores, refueling rates, movement capacity, phenology, and survival across taxa. Overall, host infection status was weakly associated with reduced body stores, delayed migration, and lower survival. Following this comprehensive analysis, Risely et al. (2018) noted there remains little understanding of how infections affect hosts during the migratory period, which is critical to understand pathogen transmission. Studies relating naturally-occurring variation in parasite load and prevalence to variation in body condition and migratory timing provide an important foundation to understanding interactions between parasites and migration. However, observational studies, such as those included in the above meta-analysis (Risely, Klaassen, & Hoye 2018) are limited in their ability to infer direction of causation. Naturally-infected individuals may suffer reduced body condition or migratory delays due to the cost of parasitic infection, but an alternative explanation is that poor body condition or late departure timing makes some individuals susceptible to infection. Moreover, observational field studies of naturally-occurring variation in infection status or parasite load are generally unable to detect individuals that do not

survive infection, and individuals that successfully clear infection. Such studies may thus underestimate effects of parasites on condition and migratory performance. Experimentally manipulating the infection status of migratory animals represents a key next step to understand how migration and infectious disease interact.

Migratory birds have been implicated in the spread of many diseases, including zoonoses such as West Nile virus (Malkinson et al., 2002), influenza A virus (Kleijn et al., 2010), and Lyme disease (Reed & Medical, 2003). Although birds encounter many types of parasites, much recent attention has focused on interactions with haemosporidia (family Apicomplexa), bloodborne protozoans that are transmitted between vertebrate hosts by insect vectors. Collectively, these parasites infect nearly 70% of bird species, occur on every continent save Antarctica, and are expanding their range as well as the latitudes at which transmission can occur (Atkinson & Van Riper, 1991; Garamszegi, 2011; Loiseau et al., 2012; Zamora-Vilchis, Williams, & Johnson, 2012). Haemosporidians of genera *Plasmodium* and *Haemoproteus*, associated with avian malaria, have been implicated in extinctions and severe population declines in many bird species (Warner, 1968; Van Riper et al., 1986). Such infections can induce muscle wasting, anemia, fever, organ damage and inflammation in their avian hosts (Booth & Elliott, 2002; de Macchi et al., 2013), particularly during the first few weeks of infection corresponding to the acute, or primary, phase. In extreme cases, these infections can result in the death of the host individual (de Macchi et al., 2013; Ilgūnas et al., 2016), but otherwise subside to chronic-phase infections associated with lower parasite burdens that may persist for months or years following initial infection (Asghar et al., 2012). Haematozoa of genus *Plasmodium* have received particular scrutiny. This is due partly to

their broad distribution, high prevalence and harmful effects on host fitness, but also because *Plasmodium* is capable of proliferating in the peripheral blood of their vertebrate hosts (Atkinson & van Riper, 1991). This trait makes *Plasmodium* highly suitable for experimental inoculations (Marzal, Bensch, Reviriego, Balbontin, & De Lope, 2008), allowing infections to be transferred directly between host individuals in a controlled setting (Dimitrov et al., 2015; Sarquis-Adamson & MacDougall-Shackleton, 2016). Thus, behavioural and physiological effects of *Plasmodium* infection can be assessed experimentally without the confound of pre-existing variation in host condition.

In this study, my primary objective was to assess the effect of *Plasmodium* infection on the timing of fall migration in free-living songbirds. I hypothesized that individuals with acute-phase *Plasmodium* infections would depart later from the breeding grounds, relative to individuals not exposed to *Plasmodium*, in order to repair tissue damage and recover energy stores needed for successful migration. Importantly, not all host individuals exposed to parasites will become successfully infected: some individuals mount immune defences that prevent parasites from establishing an acute-phase infection. However, such defences can be costly to deploy (Klasing, 2004; Lee, 2006), for example incurring energetic or collateral-damage costs resulting from inflammation (Martin et al., 2017). As a result, avoiding or eradicating parasitic infection may not necessarily be the optimal strategy (Raberg, Graham, & Read, 2009). This demonstrates that observational studies necessarily overlook individuals that are exposed to parasites but remain uninfected. Thus, encountering parasites is likely to be costly not only to individuals that become infected but also to those that successfully resist or clear infection.

I experimentally inoculated song sparrows (*Melospiza melodia*) with *Plasmodium* parasites in late summer, monitored infection success and body composition, then released birds and monitored the timing of fall migration using radiotelemetry. By experimentally manipulating migratory birds' exposure to parasites, I can compare the costs of resisting versus tolerating parasitic infection, and to assess how these challenges affect condition and migratory timing in free-living animals.

4.2 Materials and methods

4.2.1 Study animals and housing

Study subjects were 38 adult (after-hatch year) song sparrows (*Melospiza melodia melodia*) captured on their breeding grounds in southern Ontario, Canada. Previous research on nearby populations of song sparrows suggests that individuals breeding in southern Ontario vary substantially in their overwinter latitude, ranging from as far south as Florida to as far north as New York (Kelly et al., 2016). I captured sparrows on their breeding territories between 5 July and 24 August 2016, using mist nets and playback of conspecific song. Birds were captured at two field sites: Elginfield Observatory (43.191 N, 81.315 W; 9 males, 3 females;) and the University of Western Ontario campus (43.009 N, 81.282 W; 20 males, 6 females).

After capturing each bird, I determined sex based on the presence (male) or absence (female) of a cloacal protuberance, supplemented by measuring unflattened wing length to the nearest 0.1 mm with dial calipers. I weighed each individual to the nearest 0.1 g using a spring scale at the time of capture and collected a small (~ 25 μL) blood sample by brachial venipuncture to assess haematozoan infection status as described below. I

transported birds to the Advanced Facility for Avian Research at the University of Western Ontario, and housed them indoors in vector-free rooms maintained between 20 and 22 °C. Birds were kept in individual cages (39 × 34 × 42 cm) under a light schedule mimicking the natural photoperiod (ranging from 15 h light:9 h dark [15L:9D] on 5 July 2016 to 12L:12D on 29 September 2016) and had *ad libitum* access to water and food (parakeet seed supplemented with Mazuri Small Bird Maintenance chow). Birds were captured under a Scientific Collecting Permit from the Canadian Wildlife Service (CA 0244; Banding Permit 10691 E). All animal procedures were approved by Western University's Animal Use Subcommittee (protocol # 2016-017).

4.2.2 Characterizing naturally occurring infections

To identify birds that were already infected with haematozoa, I prepared a thin-film blood smear from each bird using a drop of the blood sample taken upon capture. Smears were air-dried, fixed in 100% methanol, and treated with Wright-Giesma stain, then examined under a light microscope with 100x objective using oil immersion. I examined 10 000 erythrocytes per bird, noting the presence of any haematozoa and the total number of parasitized cells.

To identify potential parasite donors, I supplemented microscopic analysis with genetic screening for *Plasmodium* spp. I extracted DNA from the remainder of the blood sample with an ammonium acetate-based protocol, then a two-stage nested PCR to amplify a portion of the haematozoan mitochondrial cytochrome *b* following Hellgren, Waldenström, & Bensch (2004). The first round of PCR required primers HAEMNFI and HAEMNR3 (Hellgren, Waldenström, & Bensch, 2004) to amplify a 617-bp fragment of cytochrome *b*. The second round used 1 μL of product from the first-round PCR as

template, and the internally nested, *Haemoproteus/ Plasmodium*-specific primers HAEMF and HAEMR2 (Hellgren, Waldenström, & Bensch, 2004) to amplify 527 bp of cytochrome *b*. PCR was conducted in 25 μL volumes with conditions described in Hellgren, Waldenström, & Bensch (2004). I ran second-round PCR products at 100 V for 90 minutes on a 2% agarose gel stained with RedSafe™, then visualized under UV light. I excised bands of the expected product size and purified them with a Gel/PCR DNA Extraction Kit (FroggaBio, North York). I sequenced purified PCR products with primer HAEMF, on an ABI 3730 Genetic Analyzer at the London Regional Genomic Center. I then identified the cytochrome b sequences to genus (i.e., *Plasmodium* or *Haemoproteus*) using the BLAST function in GenBank.

Following Sarquis-Adamson & MacDougall-Shackleton (2016), I used previously-uninfected individuals as *amplifiers*: i.e., individuals inoculated with infected blood, allowed to develop an acute infection, then euthanized and their blood used to inoculate experimental subjects. Two *parasite amplifiers* received blood from a *parasite donor* (inoculation details below), and a third *control amplifier* received unparasitized blood from a *clean donor* confirmed by microscopy and PCR to have no haematozoan infection. The remaining 35 song sparrows, including the original parasite donor and clean donor, were assigned to experimental and control treatments (i.e., inoculated with parasitized and unparasitized blood, respectively; details below) in a block-randomized design. Groups were balanced with respect to capture site, previous infection status, and sex, as specified in Table 1. To account for imperfect infection success, I assigned more birds to the experimental treatment (n = 22) than to the control treatment (n = 13).

Table 4-1 Experimental design. The table indicates the number of individuals assigned to each treatment group, broken down by sex; capture site; previous infection status; and for the experimental group, whether or not the inoculation resulted in infection (success = infected; failure = resistant; n = 35).

sex:	**male (n = 25)**				**female (n = 10)**			
site:	**Elginfield**		**campus**		**Elginfield**		**campus**	
previous infection:	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**N**
treatment – infected	2	3	1	1	1	0	0	1
treatment – resistant	0	0	3	5	0	0	3	2
control	1	3	2	4	1	1	0	1

4.2.3 Inoculation procedures

On 31 August 2016, I collected 200 μL of blood from the naturally-infected parasite donor via brachial venipuncture and used this blood to inoculate the two parasite amplifiers. Using a sterile, single-use syringe and 26-gauge needle, I slowly (i.e., over 10-15 s) injected 80 μL of fresh collected blood (i.e., collected within 5 min), mixed with 20 μL of 3.7% sodium citrate and 100 μL of 0.9% saline, into the pectoralis muscle of each amplifier. I repeated this procedure to inoculate one control amplifier with uninfected blood from a clean donor.

Fourteen days later, when parasitemia was expected to be near peak (Sarquis-Adamson & MacDougall-Shackleton, 2016), I assessed the infection status of the three amplifiers by collecting 20 μL blood samples and preparing thin-film blood smears. Parasite amplifiers showed one and two infected cells, respectively, in a scan of 10 000 erythrocytes, while the control amplifier had no detectable parasites. I euthanized all three amplifiers by overdose of isofluorane vapors, and immediately collected 600 μL of blood from each into a syringe through cardiac puncture. I combined blood from the two parasite amplifiers, then mixed amplifier blood with the saline/sodium citrate buffer as described above. I injected each of the 22 experimental birds with 200 μL of the infected blood mixture, and each of the 13 control birds with 200 μL of the uninfected blood mixture, as described above.

4.2.4 Assessing infection success

Twelve days after inoculating experimental and control birds with infected or uninfected blood, respectively, I collected 20 μL of blood from each individual via brachial venipuncture. I prepared and scanned thin-film blood smears as described above, except

that smears were examined blind as regards experimental treatment. Parasite loads of controls ranged from 0-2 infected cells per 10 000 screened (mean ± SE = 0.46 ± 0.22). Based on these values, which presumably reflect chronic rather than acute-phase infections, I established an arbitrary threshold for infection success of twice the maximum observed chronic-phase parasitaemia (Sarquis-Adamson & MacDougall-Shackleton, 2016). Thus, birds in the experimental treatment with at least 4 infected cells per 10 000 were considered to have been successfully infected and exhibiting an acute phase of infection. Birds in the experimental treatment with 3 or fewer infected cells per 10 000 were considered to have resisted infection.

4.2.5 Body composition, release procedure, and monitoring departure

After collecting blood samples on day 12 post-inoculation, I measured each bird's total body mass and measured lean and fat mass using quantitative magnetic resonance (QMR). The QMR instrument (Echo-MRI-B™, Echo Medical Systems, Houston, TX, USA) was calibrated using standards of canola oil to ensure accurate readings to the nearest 0.001 g (Guglielmo, McGuire, Gerson, & Seewagen, 2011; Seewagen & Guglielmo, 2011). I averaged two replicate scans for each individual, using four primary accumulations and gently immobilizing the bird in a ventilated holding tube (4.5 cm diameter). Following QMR (total duration = 220 s) I outfitted each individual with a radio tag (Lotek; NTQB-2; 0.35 g) glued to a figure-eight backpack-style harness (Rappole & Tipton, 1991). Each loop of the harness consisted of 38 mm of elastic thread, slipped over the bird's legs so that the transmitter rested securely over the synsacrum. Birds were kept in their home cages overnight to habituate to the harness and to confirm

fit. The next morning (i.e., 29 September 2016) I released all birds at their site of capture. Of the 35 birds inoculated with parasitized or unparasitized blood, all survived to release.

To monitor migratory departure, I visited each capture site every second day (weather permitting), beginning the day after release (30 September 2016) until seven weeks later (18 November 2016) after which time the battery life of radio tags was no longer guaranteed. This period corresponds to the typical timing of fall migration for song sparrows in southwestern Ontario. In Long Point, Ontario (a major stopover site 100 km south-east of London), peak numbers of song sparrows occur during mid-October (Bird Studies Canada, 2017).

I used a hand-held Lotek Biotracker receiver (SRX 600) and Yagi antenna to scan for the presence or absence of each individual's radio tag. I searched for each tag until its signal was detected or for a maximum of 15 minutes per individual, unless two individuals shared territories (mated pairs) in which case the site was searched for 15 minutes or until both birds were detected. Searching included hiking around in areas where the individual was captured and previously detected. The antenna was primarily held at shoulder height but was also angled down at high points of elevation. After detecting a tag, I confirmed that it remained affixed to a live (moving) bird, by holding the antenna still and observing variation in signal strength (indicative of movement). If signal strength remained constant, I made a loud noise to startle the subject and confirmed that signal strength decreased (indicative of the animal moving away). In all cases where tags were detected, I confirmed that they remained on live (moving) birds.

4.2.6 Data analysis

To determine whether infection and/or resistance affected body composition, I constructed two sets of linear models: one with lean mass as the dependent variable, and another with fat mass as the dependent variable. Lean and fat mass were considered separately because migrating birds invest differentially in these tissue types (Battley & Piersma, 1997; McWilliams, Guglielmo, Pierce, & Klaassen, 2004). Candidate models in each set differed in the presence versus absence of terms for sex and treatment (i.e., infected/ resistant/ control), such that I constructed four candidate models per set: sex + treatment; sex; treatment; and a null model. Model selection and inference were conducted using second-order Akaike's Information Criterion (AICc; Anderson, Burnham, & White, 1994). I conducted analyses using the lm() command in the *stats* package in R Studio version 3.4.1. Unless otherwise noted, values are presented as means ± SEM.

I removed song sparrows that were naturally infected with haematozoa at the time of capture (N = 8) from the analyses reported below, to avoid bias resulting from immune priming. Results including these birds are reported in Appendix A. To determine whether infection and/or resistance affected the timing of migratory departure, I analyzed resighting (i.e., radio-tracking) data using Program MARK Version 8.1 (White & Burnham, 1999). I fit extensions of the Cormack-Jolly-Seber (CJS) model to estimate weekly survival rates (φ_w) (i.e., the proportion of birds remaining on the breeding grounds each week) and resighting probabilities (*p*) (see Lebreton, Burnham, Clobert, & Anderson, 1992 for general details on the CJS model). The size of this dataset did not allow me to fit general models, and so I focused on changes in the survival rate. Survival

rates were permitted to vary across weeks, treatments, and sexes, whereas resighting probability was assumed to remain constant across weeks, treatments, sexes and sites. I compared models in which weekly survival rates varied between treatments and/or between the sexes, to models in which weekly survival rates did not vary between groups. As above, model selection to compare alternative hypotheses regarding the survival rate was based on second-order Akaike information criterion (AICc). As parasite load has been related to decreases in animal movements (Risely, Klaassen, & Hoye, 2018), I repeated this analysis replacing treatment groups with parasite load (Appendix B).

4.3 Results

Eight song sparrows tested positive for haematozoan infection on the date of initial capture as assessed by PCR. Querying the resultant sequences against BLAST confirmed that all eight infections were *Plasmodium* spp. (88-100% sequence identity when compared to other published *Plasmodium* sequences) and I observed no double peaks indicative of mixed infections. Infections were also detectable by microscopy (1-4 infected cells detected in the screen of 10 000 erythrocytes). I selected the individual with the heaviest parasite burden as assessed by microscopy (i.e., 4 infected cells per 10 000) as the parasite donor. The lineage amplified from this individual showed 99% sequence identity to lineage P-SOSP 2 previously described for the study population (Sarquis-Adamson & MacDougall-Shackleton, 2016; GenBank accession # KT193628), and 96% sequence identity to *P. circumflexum* strain TURDUS1 (GenBank accession # KM361492).

4.3.1 Infection success

Six of the 20 experimental birds (i.e., individuals inoculated with P-SOSP2) became successfully infected as assessed by my threshold of 0.04% parasitemia (i.e., four or more infected cells per 10 000 scanned) twelve days after inoculation. Mean (± SEM) parasitemia for the *infected* group was 252.17 ± 243.78 infected cells per 10 000, much larger than mean parasitemia for controls (0.14 ± 0.5) and *resistant* birds (0.86 ± 0.64). Mean parasitemia within the infected group was heavily influenced by one individual with an unusually high parasite load (1471 infected cells per 10 000). Excluding this individual, parasitemia was 8.4 ± 3.2 infected cells per 10 000 cells. Infection success did not differ between sexes (logistic regression, ß = 0.37, SE = 1.39, Wald = 0.07, p = 0.79).

4.3.2 Body composition

Of the candidate models predicting lean mass twelve days after exposure to parasites or to uninfected blood, the best-supported model included only the main effect of treatment (Table 4-2). This model received four times more support (as measured by the AICc weights) than the next most competitive model, which included main effects of sex and treatment. Parameter estimates derived from the top model (treatment) are reported in Table 4-3; lean mass prior to release was higher in the control and infected groups than in birds that resisted infection (Figure 4-1). Of the candidate models predicting fat mass twelve days after exposure to parasites or uninfected blood, the null model received 2.3 – 8.1 times more support than any of the more complex models (Table 4-4), suggesting that neither sex nor treatment contributed significantly to fat mass.

Table 4-2 Ranked candidate set of linear models predicting lean mass of 27 song sparrows (infected at capture removed), twelve days after exposure to Plasmodium lineage PSOSP-2 (infected and resistant groups) or to uninfected blood (control group). Reported in the table are second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), number of parameters (K) and proportional weight of each model (w_i).

model	AICc	ΔAICc	K	w_i
~treatment	85.8	0.00	4	0.804
~sex + treatment	88.8	2.93	5	0.186
~sex	95.8	10.00	2	0.005
null (~1)	96.1	10.30	3	0.005

Table 4-3 Predictors of lean mass in 27 song sparrows (infected at capture removed), twelve days after exposure to Plasmodium lineage PSOSP-2 (infected and resistant groups) or to uninfected blood (control group). Estimates are derived from the top-ranked model (lean mass ~ treatment). Treatment effects are estimated with reference to the control group.

predictor	β	p	95% confidence interval
treatment (infected)	-0.75	0.211	-1.95 – 0.45
treatment (resistant)	-2.00	<0.001	-3.00 – -0.99

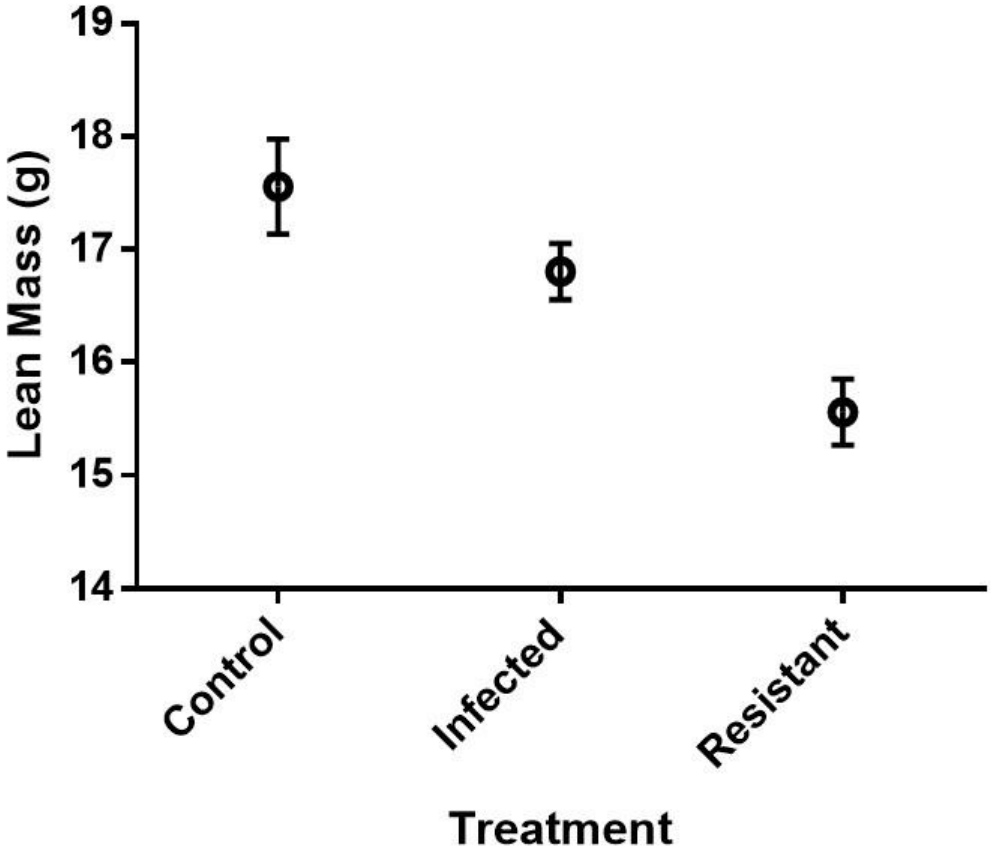

Figure 4-1 Mean (± SEM) lean mass of 35 song sparrows, twelve days after exposure to uninfected blood (control, n = 13) or to *Plasmodium* lineage PSOSP-2 (infected, n = 6; resistant, n = 14).

Table 4-4 Ranked candidate set of linear models predicting fat mass of 27 song sparrows (infected at capture removed), twelve days after exposure to Plasmodium lineage PSOSP-2 (infected and resistant treatments) or to uninfected blood (control treatment). Reported in the table are second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), number of parameters (K) and proportional weight of each model (w_i).

model	AICc	ΔAICc	K	w_i
null (~1)	32.1	0.00	2	0.545
~treatment	32.9	1.7	4	0.233
~sex	33.8	2.51	3	0.155
~sex + treatment	35.4	4.19	5	0.067

4.3.3 Migratory timing

Overall, I detected no significant effects of infection or resistance on the timing of migration departure. Figure 4-2 shows Kaplan-Meier survivorship (i.e., detection) curves for the infected, resistant, and control groups of animals. These curves appear to indicate that individuals categorized as infected tended to remain at the release site for longer than did controls or individuals that resisted infection (Figure 4-2). Importantly, though, the curves in this figure ignore the issue of detectability (i.e., the figure shows the time until individuals were last detected and not the time that they were last at the site, which cannot be observed directly). By contrast, AICc ranking of CJS models indicated that the best-supported model was the simplest model tested: this model included week-specific, but not sex- or treatment-specific, probabilities of "survivorship" (i.e., remaining at the site; Table 4-5). Real-function parameter estimates of this best-supported model are shown in Table 4-6 and Figure 4-3.

Weekly survival rates were lower in the last two weeks of radiotracking (November 6-19) than in the first five weeks (Table 4-6; Figure 4-3), indicating that birds were more likely to leave the study sites during these two weeks. Four individuals (three controls, one resistant) were still detectable at the release site by the end of radiotracking.

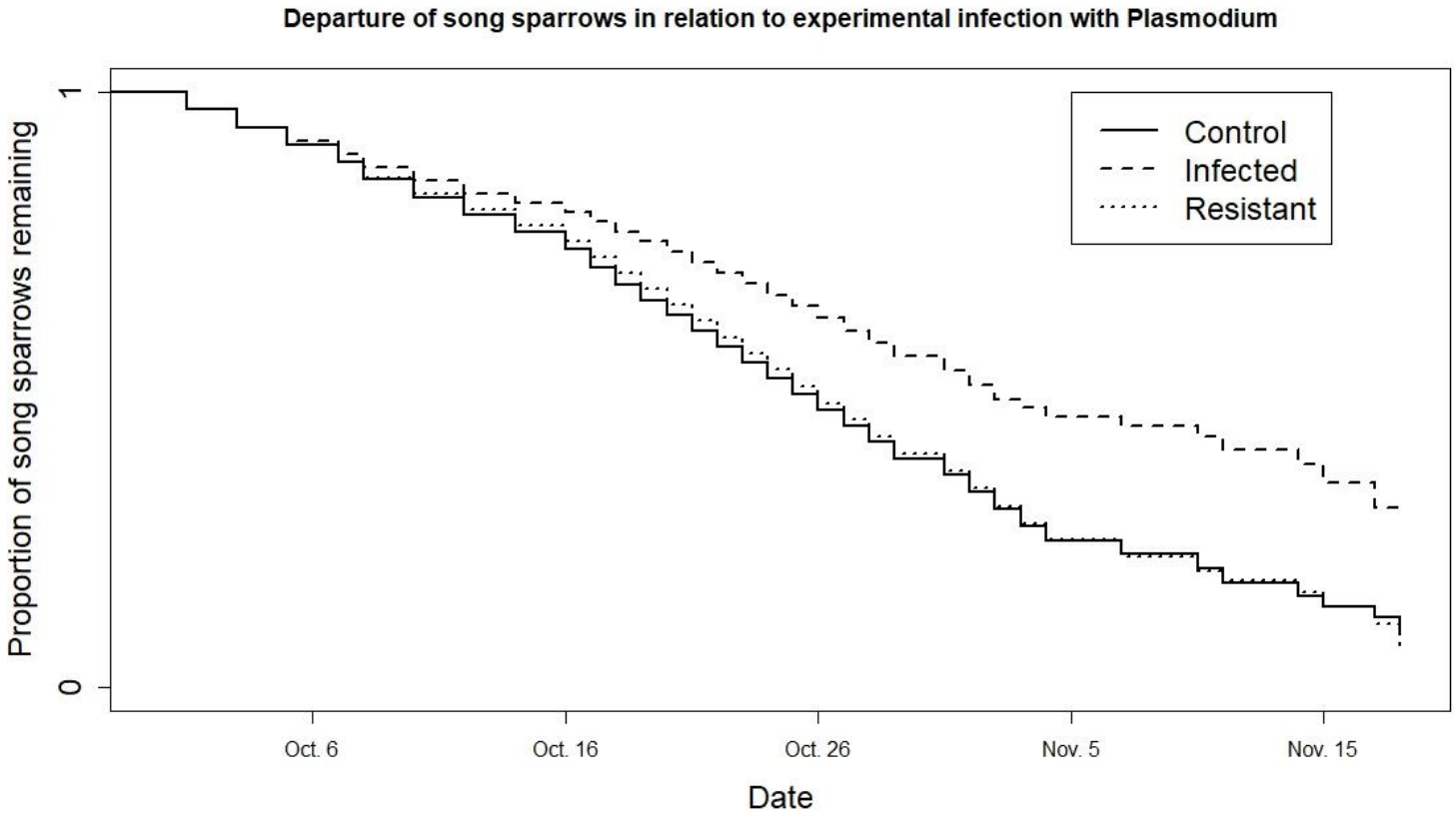

Figure 4-2 Kaplan-Meier survival curves for 35 song sparrows (control, n = 13; infected, n = 9; resistant, n = 13), showing the proportion of birds detected at the release site between 30 September – 18 November, 2016. Departure dates were inferred as the last day the individual's frequency was detected

Table 4-5 Ranked candidate set of models predicting apparent probability of remaining at release site on breeding grounds, for 27 song sparrows (infected at capture removed). Survival probability ϕ_w (probability of remaining at the site for a given week) varied weekly, and in some models, varied between sexes and/or treatments. All models included a constant probability of being resighted, if actually present, p(.). Reported in the table are second-order Akaike information criterion (AIC_c), the difference in AICc between candidate models (ΔAIC_c), number of parameters (K) and proportional weight of each model (w_i).

model	AICc	ΔAICc	K	w_i
time only [$\phi_{w+}p(.)$]	629.79	0.00	8	0.794
time and sex [ϕ_w(sex) $+ p(.)$]	639.76	9.96	15	0.005
time and treatment [ϕ_w(treatment) $+ p(.)$]	644.83	15.04	22	<0.001
time, sex and treatment [ϕ_w(sex+treatment) $+ p(.)$]	669.61	38.82	36	0.000

Table 4-6 Real function parameters of the best-fitting model (~ week) of song sparrow survival events. A lower estimate is related to an increase in departure events.

		95% confidence	
parameter	**estimate**	**lower**	**upper**
week 1: ϕ_w	0.96	0.91	0.98
week 2: ϕ_w	0.99	0.93	1.0
week 3: ϕ_w	0.99	0.97	1.0
week 4: ϕ_w	0.99	0.93	1.0
week 5: ϕ_w	0.99	0.84	1.0
week 6: ϕ_w	0.83	0.69	0.91
week 7: ϕ_w	0.80	0.51	0.94
resighting probability (p)	0.67	0.63	0.71

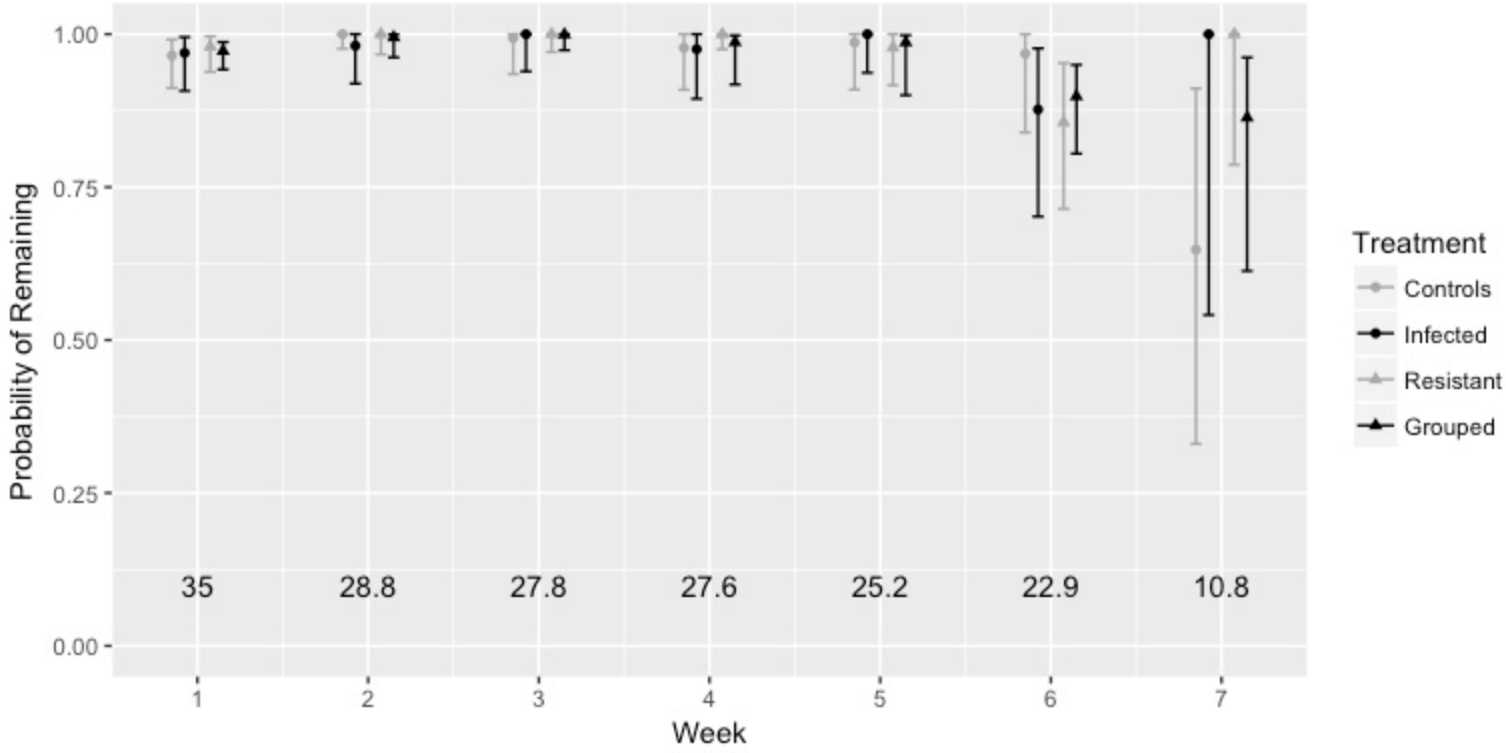

Figure 4-3 Plot comparing the estimated weekly probabilities of song sparrows (n = 35) remaining at the breeding grounds. The black triangles represent the estimates from the best-fitting model (weekly effects only). The grey circles (control), black circles (infected), and grey triangles (resistant) points represent the estimates for each treatment group from the second model (weekly effects and differences between treatment groups). Error bards represent 95 % confidence intervals. Numbers represent the number of song sparrows remaining at the sites at the start of each week as estimated by the best fitting model.

4.4 Discussion

Birds preparing for fall migration face several concurrent challenges: the need to amass body stores to sustain long-distance flight often overlaps with moult, juvenile growth and dispersal, or the provision of parental care (Newton, 2008). Exposure to parasites represents an additional challenge at this key stage in the annual cycle. Individuals that become infected experience direct physiological costs: for example, haematozoa damage blood cells and other tissues (Booth & Elliott, 2002; de Macchi et al., 2013). However, even individuals that successfully resist infection may incur energetic and inflammatory costs when mounting an immune response (Klasing, 2004; Lochmiller & Deerenberg, 2000). Thus, even among individuals that do not become infected, exposure to parasites may have far-reaching effects on host body condition, migratory timing, and ultimately migration success.

I inoculated song sparrows with malarial parasites (*Plasmodium* spp.) to assess the relative costs of resistance and infection with respect to body composition and fall migratory timing. Birds that resisted infection had lower lean mass following inoculation than controls or birds that became infected. This finding is consistent with trade-offs between body stores and immunity. To the extent that body composition predicts migration success, this finding also suggests that tolerating rather than resisting parasitic infection (Kutzer & Armitage, 2016) may facilitate preparation for migration. By contrast, I did not detect significant differences between infected, resistant and control animals in the timing of migratory departure. This and future work should inform models

of how animal migration affects the spread of infectious disease, because such models depend critically on the ability of infected individuals to migrate, and the degree to which infection induces migratory delays (Altizer et al., 2011; McKay & Hoye, 2016).

4.4.1 Body composition and resistance

Individuals that were exposed to *Plasmodium* but resisted infection had lower lean mass twelve days post-exposure, relative to controls inoculated with uninfected blood and birds that became infected (Table 4-3; Figure 4-1). This suggests that mounting an immune response while in migratory condition trades off against building or maintaining body stores, particularly lean mass. Observational studies that found reduced body condition of haematozoan-infected migrants in comparison to their uninfected counterparts cannot reliably conclude that the infection was responsible for the reduction. My results partly support this direction of causation as I found that exposure to malaria can reduce lean mass of birds in migratory condition during the acute phase of malarial infection. It remains possible that the negative effects of successful malaria infection are not apparent until after the acute phase (not examined in this study). Further experimental research is required to determine if malaria can affect body condition of migrants during the chronic/recovery phase of infection.

Experimentally manipulating exposure to parasites, as in this study, represents a significant advance over observational studies on free-living animals that correlate natural variation in infection status to condition or migratory timing. First, manipulating parasitic exposure allows individuals to be assigned randomly to exposure or non-exposure treatments, minimizing the potentially confounding effects of individual variation in quality or condition. Second, monitoring individuals from initial exposure

through peak infection avoids the problem of failing to sample individuals that do not survive parasitic infection. However, experimental infection studies cannot randomize the outcome (i.e., infection versus resistance) of exposure to parasites. As a result, I cannot conclusively determine whether group differences in lean mass following inoculation reflect the costs of mounting a successful immune defence (Klasing, 2004; Lochmiller & Deerenberg, 2000) and/or heavier individuals being more susceptible to infection. Importantly, however, both these possible explanations are consistent with trade-offs between body composition and immune defence. Furthermore, because all birds in this study survived past 12 days after inoculation, I can exclude differential mortality as a source of group differences in body composition.

Birds in this study had free access to food during the twelve-day post-inoculation period, which may help to explain why I did not observe group differences in fat mass (Table 4-4). Unrestricted access to food, as in this and many captive studies, may obscure the effects of immune response and/or parasitic infection on body composition. In free-living animals, with unpredictable access to food, mounting an immune response could potentially reduce fat stores as well as lean mass. Conversely, parasitic infection might reduce fat and/or lean mass stores in free-living animals but this effect may be masked under captive conditions with unrestricted access to food. Recovery and deposition of protein stores in lean tissue is slow relative to fat deposition (McWilliams & Karasov, 2001), suggesting that the lower lean mass observed for resistant individuals likely persisted for some weeks after release. Given that migratory birds require increased muscle mass to meet the physiological demands of long-distance flight (Barboutis, Mylonas, & Fransson, 2011), my findings suggest that resisting parasitic infection,

particularly when exposure occurs shortly before migration, imposes costs to body composition that could reduce the likelihood of migrating successfully. Whereas models of migratory culling (Bradley & Altizer, 2005) posit that infected individuals are less likely than their uninfected counterparts to migrate successfully, my results suggest that encountering parasites but resisting infection may incur a previously unappreciated cost of body stores.

4.5 References

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Chapter 5

5 General Discussion

The predictable migrations of animals create ecological connections between otherwise isolated sites and may enable pathogen transport along the way, thus having important ecological, biogeographical, and evolutionary consequences (Bowlin et al., 2010; Viana, Santamaría & Figuerola 2016). Birds have been implicated in the spread of diseases (Peterson, Vieglais & Andreasen 2003; Ip et al., 2008; Ogden et al., 2008), but we still lack a fundamental understanding of when and how birds contribute to such spread. Prior to my thesis research, most studies relied upon observational studies that are unable to discern cause from effect (Risely, Klaassen & Hoye 2018), and the costs of upregulating immunity from those of pathogenic infection. Such observational studies also overlook the consequences of being exposed to pathogens without subsequent infection. Experimental research is critical to discern the causal effects of pathogen infection, consequences of upregulating immunity during migration, and the costs of pathogen exposure in order to anticipate range expansions of pathogens in our changing climate (Garamszegi 2011; Fuller et al., 2012; Risely et al., 2018).

Using captive and field-based experiments for my doctoral research, I answered three primary research questions: (1; Chapter 2) are nocturnal migratory restlessness (*Zugunruhe*) and body condition affected by mounting an acute phase immune response during migration; (2; Chapter 3) what are the impacts of parasitic infection on *Zugunruhe* and body condition compared to those of upregulating immunity; and (3; Chapter 4) are the observed consequences of successful parasite infection in captive conditions also realised in nature?

5.1 Upregulating immunity during migration

In Chapter 2, I administered injections of lipopolysaccharide (LPS) and/or keyhole limpet hemocyanin (KLH) to song sparrows (*Melospiza melodia*) and white-throated sparrows (*Zonotrichia albicollis*) captured during stopover and brought into captivity. LPS and KLH nonspecifically activate a wide range of immune cells and induce the secretion of cytokines responsible for the inflammatory response (Harris & Markl 1999; Janeway et al., 2001) without inducing parasitic infection. Thus any behavioural or physiological changes observed after inoculation can be attributed to upregulating the acute phase immune response (Kent et al., 1992; Harris & Markl 1999; Demas et al., 2011).

Observational research has reported worse body condition (lower fat levels or reduced body mass) of infected relative to uninfected migrants at stopover (Møller, De Lope & Saino 2004; Latorre-Margalef et al., 2009; DeGroote & Rodewald 2010; Shurulinkov, Chakarov & Daskalova 2012; Shutler, Alisauskas & McLaughlin 2012; Hahn et al., 2018), but whether these reductions are caused by the infectious agent as opposed to upregulating immunity had been difficult to disentangle. My experiment represents the first captive examination of LPS and KLH exposure while the subject was in migratory disposition and, as such, is an important advance in our understanding the costs of upregulating immunity versus the harmful effects of infection during migration. Field-based experimentation has demonstrated longer stopover durations in European songbirds following exposure to LPS, compared to unmanipulated controls (Hegemann et al., 2018), but I did not observe reductions in *Zugunruhe* intensity in captivity. However, Hegemann et al.'s (2018) study design cannot distinguish between the effect of LPS exposure and the inoculation procedure since controls were not injected with vehicle.

Also contrary to expectations, in my study KLH-treated sparrows increased whole-body mass the morning after inoculation, and LPS-exposed white-throated sparrows had higher lean mass compared to controls one week after recovery. These findings suggest that the aforementioned poor condition of parasite-infected migrants observed at stopover are likely caused by the infectious agent (parasite) itself, rather than the direct costs of upregulating immunity in response to infection.

Inflammatory cells of the acute phase response, such as heterophils and macrophages, are nutritionally expensive to maintain, particularly during an immune response (Klasing 2004). Sparrows in my study had ad libitum access to food for the duration of the experiment: thus, increases in mass may be attributed to bulking in anticipation of maintaining an upregulated immune response during an energetically expensive life-history stage. Indeed, parasitic infections typically persist longer than 24 hours, and thus likely incur greater energetic costs than the immune challenge I administered. Considering this, I experimentally exposed sparrows to malaria (*Plasmodium* sp.) in the following two data chapters of my thesis (Chapters 3 & 4). Captive white-throated sparrows that resisted infection (did not become infected) were slowest to increase *Zugunruhe* intensity for two weeks following inoculation, likely attributed to upregulating immunity to prevent infection. Additionally, song sparrows that resisted infection by malaria had reduced lean mass compared to controls 14 days after exposure. Thus, there are clear immunological costs to upregulating immunity in response to pathogenic exposure, but such responses are likely specific to the pathogenic threat. In addition, I observed species-specific effects to the same pathogen: only white-throated sparrows, not song sparrows, increased lean mass in response to LPS exposure, and

malaria-resistant white-throated sparrows did not reduce body condition (Chapter 3) while malaria-resistant song sparrows had reduced lean mass comparted to controls (Chapter 4). These host-pathogen specific findings highlight the importance of investigating dynamics of infection using a variety of pathogens and hosts. As outlined in Chapter 1, research investigating relationships between pathogens and their host during migration is dominated by studies of blood parasites (> 50 % of studies reviewed) and avian influenza virus (> 25 %), leaving < 25 % of studies on all other pathogens (e.g., helminths, mites, West Nile Virus, fungal and bacterial infections). Thus, future research should address the effects of overlooked pathogens on migratory traits to identify which pathogens are most at risk to spread in our changing climate.

5.2 Experimental malaria infection during migration

In Chapters 3 and 4, I experimentally infected sparrows with malaria to quantify the impacts of malaria infection on *Zugunruhe*, migratory timing, and body condition: I examined these effects in both captive (Chapter 3) and field-based (Chapter 4) contexts. Previous observational studies have reported later arrival of infected, compared to uninfected, migrants to stopover (Boone, Rodewald & DeGroote 2010; López et al., 2013; Santiago-Alarcon et al., 2013) and breeding sites (Møller et al., 2004; Rubaiee, Murayati & Møller 2018). However, whether these delays were induced by infection -- or conversely, whether migratory delay made individuals vulnerable to infection -- cannot easily be discerned without experimental manipulation of infection status. I found that experimentally induced infection caused decreases in *Zugunruhe* intensity in captive white-throated sparrows, beginning 14 days post-inoculation (Chapter 3). This finding clarifies that it is likely the harmful effects of the pathogen that is cause for the previously

observed migratory delays. Furthermore, experimental infection may delay migratory departures in song sparrows, though I did not have enough statistical power to thoroughly test this hypothesis (Chapter 4). These experiments are the first to examine the consequences of controlled, experimental malaria infections on migratory activity: they indicate that infection-induced delays in migration occur two weeks after exposure to the malarial parasite. Thus, the migration schedules of infected versus uninfected individuals might not begin diverging until weeks after exposure. Substantial overlap before this two-week period may thus allow infected individuals to transport malaria at a smaller geographic scale before their migratory behaviour is impacted. Within 36 – 48 hours after an infectious mosquito bite, immature sporozoites from mosquito saliva mature into merozoites, are released into the bloodstream, transported to macrophages in the brain, liver, spleen, kidney, and lung where they reproduce asexually. This new generation of merozoites infect red blood cells, reproduce, and burst the host red blood cells open releasing new, infectious, merozoites (Valkiunas 2005). While there is yet to be empirical studies examining the precise timing that a bird is infectious after exposure, mosquitos become infected by acquiring gametocytes from a blood meal and, thus, *Plasmodium* transmission is likely during the two-week period that infected individuals do not exhibit migratory delays.

Poor condition of infected compared to uninfected migrants is often observed at stopover sites (Møller et al., 2004; Latorre-Margalef et al., 2009; DeGroote & Rodewald 2010; Shurulinkov et al., 2012; Shutler et al., 2012; Hahn et al., 2018), but again, whether the infection caused poor body condition, or the poor condition caused susceptibility to infection is unknown. I found that neither whole body mass, lean mass, or fat mass were

affected by experimentally induced malarial infection in captivity, for either white-throated sparrows (Chapter 3) or song sparrows (Chapter 4). These findings are consistent with previous reports that experimentally infecting immunologically naïve, juvenile great reed warblers (*Acrocephalus arundinaceus*) preparing for spring migration has no effect on fat mass (Zehtindjiev et al., 2008). Together, these findings imply that the poor body condition of migrants at stopover, perhaps due to energetic costs of sustained flight (Wikelski et al., 2003; McWilliams et al., 2004), made migrants susceptible to infection, rather than the infection causing reduced body condition. Future research should examine the likelihood of infection in individuals exerting high levels of exercise versus those untrained, or individuals in poor versus healthy body conditions.

Experimental inoculations with malaria outside of the migratory period have mostly been conducted in native Hawaiian forest birds, such as i'iwi (*Vestiaraia coccinea*), nutmeg manakins (*Lonchura punctulate*), omao (*Myadestes obscurus*), and apapane (*Himatoine sanguinea*), and Hawaii amakihi (*Hernignathus virens*). Infection in these species resulted in decreases in body mass for all species (Atkinson et al., 1995, 2000, 2001b; Atkinson, Dusek & Lease 2001a; Yorinks & Atkinson 2000), decreases in subcutaneous fat in apapane (Yorinks & Atkinson 2000), and severe anemia in i'iwi and nutmeg manakin (Atkinson et al., 1995). However, the susceptibility of Hawaiian avifauna to malarial parasites may reflect their lack of evolutionary history with these parasites. Experimental infection with malaria does not induce loss of body mass in many species that have historically been exposed to these parasites, such as greenfinches (*Carduelis chloris*) (Palinauskas et al., 2009), chaffinch (*Fringeilla coelebs*), siskin (*Spinus spinus*), common crossbill (*Loxia curvirostra*), house sparrow (*Passer*

domesticus), and starlings (*Sturnus vulagris*) (Palinauskas et al., 2008, 2011), and consistent with my findings with white-throated sparrows and song sparrows (Chapter 3 and 4, respectively). Thus, selection for disease tolerance imposed by additional parasite exposure during migration likely contribute to the hardiness of white-throated sparrows and song sparrows in response to malarial infection, although experimental evidence using the same pathogen are necessary to draw such conclusions. However, whereas I observed no effect of infection on hematocrit, reductions in hematocrit have been observed in greenfinches, chaffinch, siskin, and common (Palinauskas et al., 2008, 2011), suggesting responses to malaria infection may vary between host species, between different parasite lineages, and/or with different host-parasite combinations.

5.3 Contrasting captive- and field-based experiments

Observational studies, generally conducted in the field, lack control over a multitude of potential confounding variables, but captive lab conditions that can control for such variables differ vastly from natural conditions. Thus, while cause and effect are clearly distinguished in lab experiments it may be difficult to determine whether patterns demonstrated in captivity also apply in the field. The acute-phase immune response I elicited using LPS and KLH in song sparrows and white-throated sparrows captured at stopover did not have any negative effects on *Zugunruhe* in captivity. However, several free-living European songbird species exposed to LPS in the field exhibited longer stopover durations, regardless of being short- or long-distance migrants (Hegemann et al., 2018). While these findings suggest upregulating immunity may impede migration timing, this study did not track individuals across multiple stopover sites and the observed reductions in movement were related to foraging, not active migratory flights.

Thus, the different parameters evaluated in these studies make it difficult to compare results.

An alternative cause for differences between my findings and those of Hegemann et al. (2018) is that I injected my control birds with saline; conversely, Hegemann et al. (2018) left controls unmanipulated on the rationale that even saline injections might cause inflammation and elicit an immune response. Indeed, I found reductions in *Zugunruhe* intensity for both song sparrows and white-throated sparrows the night following inoculation, regardless of treatment, indicating an effect of the inoculation procedure. Thus, it is possible that the differences in movement that Hegamann et al. (2018) observed between LPS-injected birds and controls were because of injection, not due to mounting an acute phase immune response. It is therefore difficult to directly contrast these findings and highlights the necessity for consistency in methodological approaches when contrasting captive- and field- based experiments.

To consider the harmful effects of parasites on movement and body condition, I exposed migratory sparrows to malaria in both captive- (Chapter 3) and field-based (Chapter 4) experiments to determine whether effects observed in captivity can be extrapolated to draw conclusions about natural settings. In captivity, infected white-throated sparrows reduced *Zugunruhe* two weeks after exposure (Chapter 3). This timeframe aligns well with the field-based experiment on song sparrows (Chapter 4), in which I released birds and began tracking migratory departure two weeks after exposure. Thus, assuming similar time courses of malarial infection and infection-induced delays between the two species, any infection-induced delays of migratory behaviour should have been evident in the departure of song sparrows for migration. However, I did not find statistically

significant differences between infected and control song sparrows in the timing of departure (Chapter 4). One interpretation is that encountering malaria parasites does not appreciably delay migratory departure and, thus, the observed reductions of *Zugunruhe* in captivity do not translate to significant delays of migratory behaviour in the wild. However, the sample size for infected song sparrows was low (n = 9), meaning the effect size would have to be very large to be detected and there may be underlying between-group differences muddied by large within-group variability. Furthermore, the autumn that I conducted the research reported in Chapter 4 was much warmer than typical regional temperatures (5 °C warmer; Environment and Climate Change Canada, 2016) and I observed low departure probabilities until early November, substantially later than the historical timing for song sparrows in southwestern Ontario (Bird Studies Canada, 2017). Unseasonably warm weather may have obscured potential effects of infection on migratory timing by allowing infected birds time to repair damage to blood cells and tissues. Consequently, infection-induced delays to migration might have been masked by unusually warm weather and might have been observable with a higher sample size. Thus, it remains possible that lab- (Chapter 3) and field-based experiments (Chapter 4) would find similar effects of malaria inoculation, provided adequate sample size and consistent experimental design, and depending on environmental conditions.

5.4 Effects of pathogenic exposure

The experimental approaches of Chapters 3 & 4 allowed the first controlled evaluation of how exposure to malaria, without subsequent infection, might impose physiological demands on the host. Observational studies provide a snapshot in time for a captured individual and cannot determine whether that individual was previously exposed to a

pathogen, nor the timeframe of when it would have occurred. This self-selected group has inherently been overlooked in observational research to date, and experimental studies inoculating birds with malaria either do not report on birds that resist infection (Palinauskas et al., 2008, 2011; Asghar et al., 2012; Dimitrov et al., 2015) or all exposed individuals became infected (Atkinson et al., 2000; Yorinks & Atkinson 2000; Palinauskas et al., 2008; Zehtindjiev et al., 2008; Hahn et al., 2018). Thus, Chapters 3 & 4 represent the first account of the costs incurred from resisting malarial infection during migration.

The acute phase immune response is the first response to a foreign pathogen and stimulates an inflammatory response with the release of cytotoxic compounds that orchestrate fever, anorexia, and recruitment of acute phase proteins from the liver (Klasing 2004). Activating components of the immune response entail high metabolic costs of production and maintenance (Klasing 2004; Millet et al., 2007), but recruiting the liver away from its normal functions in secreting nutrients to support growth or activity represents the single most expensive component of immunity during the acute phase response (Klasing 2004). In this light, it is not surprising that I found behavioural and physiological differences between birds that resisted malarial infection and unexposed birds. Resistant captive white-throated sparrows were the slowest to increase *Zugunruhe* intensity the first two weeks after exposure to malaria compared to controls and successfully infected individuals. Costs of immune upregulation in response to malaria exposure appear to be mitigated after the initial two-week period after exposure as *Zugunruhe* intensity of resistant birds continued to increase compared to controls that held a steady intensity of *Zugunruhe* and infected individuals that began to exhibit

reductions in *Zugunruhe* intensity. There are therefore different cost schedules to resisting versus succumbing to malarial infection, the former having immediate energetic costs. It is reasonable, then, that resistant song sparrows in Chapter 4 did not give any indication that malaria exposure affects departure timing several weeks after exposure.

Chapter 3 & 4 provide mixed evidence on the effects of malaria resistance on physiological traits. Captive, resistant white-throated sparrows did not exhibit any reductions in body condition (Chapter 3) but song sparrows had reduced lean mass compared to controls two weeks after malaria exposure prior to release (Chapter 4). The difference between experiments may be due to the duration subjects were kept in captivity prior to inoculation with malaria. White-throated sparrows were held captive for up to five months prior to inoculation, while song sparrows were held for no longer than two months, with some individuals having less than a month to adjust to captivity. It is possible, then, that the additional three months of captivity and access to unlimited food and water mitigated the costs of upregulating immunity in resistant white-throated sparrows. To mitigate the effects of captivity, future research should restrict food access during experimental inoculations with malaria to better mimic unpredictable access to food that is experienced by free-living migratory songbirds. This would also increase our understanding of how migratory preparation (increasing energy stores) contributes to disease resistance.

5.5 Temporal effects on host-parasite interactions

The response of individuals to malaria exposure depends on more than whether the infection was successful, including the local abiotic conditions. Chapter 4 was unexpectedly impacted by warmer-than-average temperatures that appear to have delayed

the departure for fall migration in song sparrows. Temperature is the climatic variable most frequently related to avian phenology (reviewed by Gordo 2007). I was cautious to time the release of malaria-inoculated song sparrows to coincide with the historical peak of fall migration for song sparrows in southwestern Ontario (mid-October), but my departure probabilities remained low until early November. Unseasonably warm weather may have obscured effects of malaria inoculation on migratory timing by allowing birds time to repair damage to blood cells and tissues caused by immunopathology and the invading parasite. Furthermore, the extended activity of insect vectors may increase the proportion of hosts infected and infections may be more intimately timed with fall departure. Warming fall climates may thus obscure infection-induced migratory delays, ultimately increasing temporal overlap between infected and uninfected individuals along migratory routes. Thus, the timing of infection relative to normal migratory chronology is likely to influence the degree that migration is delayed, and differences are expected between migrants preparing for migration (Chapters 3 & 4) versus migrants that have already initiated migration (Chapter 2). To further explore these temporal dynamics, future research should consider tracking the migration of individuals experimentally inoculated with malaria along stopover sites.

Immunity is modulated throughout the annual cycle (Hegemann et al., 2012a; b; Kelly et al., 2017) but is thought to reflect fluctuating environmental conditions rather than being a pre-programmed phenomenon related to predictable ecological changes (Hegemann et al., 2012a). Thus, while warming temperatures allow for predictions in infection-induced migratory delays, predictions for immunity are likely context specific according to the pathogen at-hand, previous encounters with the pathogen, and

availability of resources (Martinez & Merino 2011). Song sparrows and white-throated sparrows did not reduce *Zugunruhe* intensity when challenged with LPS and/or KLH to mount an acute phase immune response, and exhibited increases, not reductions, in body mass as a response (Chapter 2). Seasonal-specific responses to LPS challenges are reported where reductions in activity are greater in the winter than during breeding (Owen-Ashley & Wingfield 2006; Owen-Ashley et al., 2006) and individuals in breeding condition either increased body mass (Bonneaud et al., 2003) or remained unaffected compared to controls whilst exhibiting reductions in mass in the winter (Owen-Ashley & Wingfield 2006). In combination, these results suggest that the benefits of successfully executing critical life-history stages (e.g., breeding and migration) may offset the costs of suppressing an acute phase response towards infection. Thus, extended preparatory periods for fall migration due to increasing temperatures should increase resistance to pathogens prior to fall migration by allowing more time to bulk and prepare for migration.

5.6 Concluding remarks

Over the past decade, our warming climate has caused profound changes in the prevalence and severity of some infectious diseases (Harvell et al., 2002; Garamszegi 2011). Thus, our ability to predict the spread of infectious pathogens in our changing climate is critical for public health and conservation of not-yet-exposed and/or threatened species (Fuller et al., 2012). To date, research examining the relationships between pathogens, immunity, body condition and migration relied upon observational studies. While informative, such studies are limited in their ability to discern causal relationships and conclusions remain speculative, making it difficult to anticipate how birds contribute

to the spread of pathogens. The lab- and field-based experiments conducted for this thesis are among the first controlled manipulations of malaria infection and immune challenges in migratory songbirds and provide a first step towards clarify the causal relationships suggested by findings from observational field research. Future work must continue to examine the complex relationships between pathogens and their migratory hosts, using controlled experiments to identify opportunities to mitigate the impacts of emerging threats to wildlife health caused by, and affecting, migratory animals.

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Appendices

Appendix A: Chapter 4 body composition supplementary analysis

Here I report the effects of malarial infection on fat and lean mass of song sparrows, including the birds that were infected at the time of capture. These birds were excluded from the original analysis for cautionary effects of immune priming due to previous malarial exposure. The results do not change the significant treatment effects.

Table AA-1 Ranked candidate set of linear models predicting lean mass of 35 song sparrows (including birds infected at capture), twelve days after exposure to Plasmodium lineage PSOSP-2 (infected and resistant groups) or to uninfected blood (control group). Reported in the table are second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), number of parameters (K) and proportional weight of each model (w_i).

model	AICc	ΔAICc	K	w_i
~sex + treatment	121.8	0.00	5	0.609
~treatment	123.8	2.06	4	0.217
~sex	124.7	2.91	3	0.143
null (~1)	127.7	5.96	2	0.031

Table AA-2 Predictors of lean mass in 35 song sparrows (including birds infected at capture), twelve days after exposure to *Plasmodium* lineage PSOSP-2 (infected and resistant groups) or to uninfected blood (control group). Estimates are derived from the top-ranked model (lean mass ~ sex + treatment). Treatment effects are estimated with reference to the control group.

predictor	β	p	95 % confidence interval
sex (female)	-0.99	0.41	-1.94 – -0.04
treatment (infected)	-0.75	0.211	-1.95 – 0.45
treatment (resistant)	-2.00	<0.001	-3.00 – -0.99

Table AA-3 Ranked candidate set of linear models predicting fat mass of 35 song sparrows (including birds infected at capture), twelve days after exposure to *Plasmodium* lineage PSOSP-2 (infected and resistant treatments) or to uninfected blood (control treatment). Reported in the table are second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), number of parameters (K) and proportional weight of each model (w_i).

model	AICc	ΔAICc	K	w_i
null (~1)	35.1	0.00	2	0.649
~sex	37.3	2.17	3	0.219
~treatment	38.8	3.70	4	0.102
~sex + treatment	41.3	6.13	5	0.030

Appendix B: Chapter 4 migratory timing supplementary analysis

Here I include the ranked candidate set of models predicting departure of song sparrows from the breeding grounds, but substitute treatment groups for parasite load. This analysis was completed in recognition of Risely et al.'s (2018) meta-analysis that found support for parasite load affecting host movements. Substituting parasite load for infection status did not change the top-ranked model (~ week; Table AB-1), and estimates produced from this top model remain the same (Table AB-2).

Table AB-1 Ranked candidate set of models predicting apparent probability of remaining at release site on breeding grounds, for 27 song sparrows (infected at capture removed). These models include parasite load as a continuous variable instead of treatment groups. We removed an individual with an uncommonly high parasite load (1471 infected cells per 10 000 red blood cells) to avoid any bias this outlier could cause. All models included a constant probability of being resighted if actually present, p(.). Survival probability ϕ_w (probability of remaining at the site for a given week) varied weekly, and in some models, varied between sexes and/or treatments. Reported in the table are second-order Akaike information criterion (AIC_c), the difference in AICc between candidate models (ΔAIC_c), number of parameters (K) and proportional weight of each model (w_i). One individual was removed from the analysis as its inclusion drove a positive relationship between parasite load and departure date.

model	AICc	ΔAICc	K	w_i
week only [ϕ_w + p(.)]	623.47	0.00	8	0.32
week and parasite load [ϕ_w(load) + p(.)]	624.33	0.86	9	0.21
week and sex [ϕ_w(sex) + p(.)]	625.59	2.11	9	0.11
week, sex and parasite load [ϕ_w(sex+load) + p(.)]	626.47	3.00	10	0.07

Table AB-2 Real function parameters of the best-fitting model of song sparrow survival events using parasite load as a continuous variable instead of treatment groups (see SM-8). A lower estimate is related to an increase in departure events.

		95% confidence	
parameter	**estimate**	**lower**	**upper**
week 1: ϕ_w	0.96	0.92	0.98
week 2: ϕ_w	0.99	0.93	1.00
week 3: ϕ_w	0.99	0.97	1.00
week 4: ϕ_w	0.98	0.93	1.00
week 5: ϕ_w	0.99	0.84	1.00
week 6: ϕ_w	0.83	0.69	0.91
week 7: ϕ_w	0.80	0.51	0.94
resighting probability (p)	0.67	0.63	0.71

Appendix C: Permission to reproduce published material

The content contained within Chapter 4 has been published in the *Journal of Experimental Zoology* (2018). Permission to reproduce the material was granted:

"… provided that the re-used material constitutes less than half of the new publication and that any changes are noted."

-Y. V. Srinivas
Production Editor on behalf of Wiley
August 13, 2018

Curriculum Vitae

Tosha Ruth Kelly

EDUCATION

Doctor of Philosophy in Biology (2014 - present)
Western University, London, Ontario, Canada.
Supervisors: Dr. Beth MacDougall-Shackleton, Dr. Scott MacDougall-Shackleton

Master of Science in Biology (2012-2014)
Western University, London, Ontario, Canada.
Supervisors: Dr. Beth MacDougall-Shackleton, Dr. Scott MacDougall-Shackleton

Bachelor of Science (Honours) in Biology (2008-2012)
Trent University, Peterborough, Ontario, Canada.

REFEREED PUBLICATIONS

10) *Vojtěch, B.,* J. Koleček, M. Burgess, S. Hahn, M. Krist, J. Ouwehand, E.L. Weiser, P. Adamík, J.A. Alves, D. Arlt, S. Barišić, D. Becker, E.J. Belda, V. Beran, C. Both, S.P. Bravo, M. Briedis, B. Chutný, D. Ćiković, N. Cooper, J.S. Costa, V.R. Cueto, T. Emmenegger, K. Fraser, O. Gilg, M. Guerrero, M.T. Hallworth, C. Hewson, D. Humple, F. Jiguet, J. Johnson, **T.R. Kelly**, D. Kishkinev, M. Leconte, T. Lislevand, S. Lisovski, C. López, K. MacFarland, P.P. Marra, S.M. Matsuoka, P. Matyjasiak, C.M. Meier, B. Metzger, J.S. Monrós, R. Neumann, A. Newman, R. Norris, T. Pärt, V. Pavel, N. Perlut, M. Piha, J. Reneerkens, C. Rimmer, A. Roberto-Charron, C. Scandolara, N. Sokolova, M. Takenaka, D. Tolkmitt, H. van Oosten, A. Wellbrock, H. Wheeler, J. van der Winden, K. Witte, B. Woodworth, P. Procházka. 2018. Weak effects of geolocators on small birds: a meta-analysis controlled for phylogeny and publication bias. Submitted to the Journal of Animal Ecology September 19, 2018.

9) **Kelly, T.R.**, B.D. Rubin, S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. 2018. Experimental exposure to malaria affects songbirds' migratory activity, regardless of infection success. Submitted to Physiological and Biochemical Zoology September 20, 2018 (PBZ-18117).

8) **Kelly, T.R.**, K.A. Hobson, S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. 2018. Long-term winter-site fidelity in Song Sparrows *Melospiza melodia*. Submitted to The Auk July 12, 2018 (AUK-18-111). Preparing for resubmission.

7) Boyd, R.J., **T.R. Kelly**, S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. 2018. Alternative reproductive strategies in white-throated sparrows are associated with differences in malarial parasite load following experimental infection. Biology Letters. 14(7): 10.1098/rsbl.2018.0194.

6) Grieves, L.A., **T.R. Kelly**, M.A. Bernards & E.A. MacDougall-Shackleton. 2018. Chemical composition of preen oil does not signal malarial infection in songbirds: results from an experimental study. The Auk: Ornithological Advances. 135(3): 767-776.

5) **Kelly, T.R.**, S.J. Bonner, S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. 2018. Exposing migratory sparrows to *Plasmodium* suggests costs of resistance, not necessarily of infection itself. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology. 329(1): 5-14.

4) **Kelly, T.R.**, H.L. MacGillivray, K.A. Hobson, S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. 2017. Immune profiles vary seasonally, but are not significantly related to migration distance of natal dispersal, in a migratory songbird. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology. 327(5): 284-292.

3) Slade, J.W.G., M.J. Watson, **T.R. Kelly**, G.B. Gloor, M.A. Bernards & E.A. MacDougall-Shackleton. 2016. Chemical composition of preen wax reflects MHC similarity in breeding songbirds. Proceedings of the Royal Society B. 283: 20161966.

2) Lymburner, A.H., **T.R. Kelly**, K. Hobson, E.A. MacDougall-Shackleton & S.A. MacDougall-Shackleton. 2016. Testosterone, migration distance, and migratory timing in song sparrows *Melospiza melodia*. Hormones and Behavior. 85: 102-107.

1) **Kelly, T.R.**, H.L. MacGillivray, Y. Sarquis-Adamson, M.J. Watson, K.A. Hobson & E.A. MacDougall-Shackleton. 2016. Seasonal migration distance varies with natal dispersal and predicts parasitic infection in song sparrows. Behavioral Ecology and Sociobiology. 70: 1857-1866.

[*in prep*] **Kelly, T.R.**, A.M. Boyer, E.A. MacDougall-Shackleton & S.A. MacDougall-Shackleton. 2018. No effect of mounting an acute immune response on migratory restlessness or body condition in captive sparrows. Preparing for Animal Migration.

CONFERENCE AND INVITED PRESENTATIONS

Invited Presentations

7) ***Kelly, T.R.***, S.A. MacDougall-Shackleton, S.J. Bonner, B.D. Rubin & E.A. MacDougall-Shackleton. "Hitchhikers guide to migration: dynamics of malaria infection in migratory sparrows." International Ornithological Conference, Vancouver, British Columbia, Canada August 2018. [Talk]

6) "Life on the move: migration as a behavioural adaptation", Western University, Animal Behaviour (BIOL3436), October 2017. [Lecture]

5) "Life on the move: migration as a behavioural adaptation", Western University, Animal Behaviour (BIOL3436), December 2016. [Lecture]

4) "A day in the life of a field researcher: how we collect data and what we learn from it." Queen's University Biology Station, Elgin, Ontario, Canada, May 2015. [Talk]

3) "Life on the move: migration as a behavioural adaptation", Western University, Animal Behaviour (BIOL3436), December 2015. [Lecture]

2) "A day in the life of a field researcher: how we collect data and what we learn from it." Port Burwell Provincial Park, Port Burwell, Ontario, Canada, August 2014. [Talk]

1) "A day in the life of a field researcher." Queen's University Biology Station, Elgin, Ontario, Canada, May 2014. [Talk]

Contributed Presentations

My name is bolded, presenting author is italicized.

17) ***Kelly, T.R.***, K.A. Hobson, S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. "Track me if you can: long-term site fidelity in song sparrows" Ontario Ecology, Ethology and Evolution Colloquium, Western University, London, Ontario, Canada, May 2018. [Talk]

16) ***Kelly, T.R.***, S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. "Effects of experimental *Plasmodium* infection on spring migratory behaviour and body condition in white-throated sparrows (*Zonotrichia albicollis*)." Society for Integrative and Comparative Biology, San Francisco, California, USA, January 2018. [Talk]
**Selected as Best Student Presentation in Division of Ecoimmunology and Disease Ecology.*

15) **Kelly, T.R.**, S.J. Bonner, S.A. MacDougall-Shackleton & *E.A. MacDougall-Shackleton*. "Exposing migratory songbirds to malarial parasites suggests costs of

resistance, not of infection." Society for Integrative and Comparative Biology, San Francisco, California, USA, January 2018. [Talk]

14) ***Kelly, T.R.***, S.A. MacDougall-Shackleton, S.J. Bonner & E.A. MacDougall-Shackleton. "Costs of malaria infection and resistance in migratory birds: is the cure worse than the disease?" Biology Graduate Research Forum, Western University, London, Ontario, Canada, October 2017. [Talk]
* *Selected as Best Ecology and Evolution Standard Talk.*

13) ***Kelly, T.R.***, S.A. MacDougall-Shackleton, S.J. Bonner & E.A. MacDougall-Shackleton. "Costs of malaria infection and resistance in migratory birds: is the cure worse than the disease?" Animal Behavior Society, University of Toronto, Scarborough, Ontario, Canada, June 2017. [Talk]

12) ***Kelly, T.R.***, A.H. Lymburner, E.A. MacDougall-Shackleton, K.A. Hobson & S.A. MacDougall-Shackleton. "Testosterone, migration distance, and migratory timing in song sparrows *Melospiza melodia*." Canadian Society of Zoologists, Western University, London, Ontario, Canada, May 2016. [Talk]

11) **Kelly, T.R.**, J.W.G. Slade, J. Ho, K.A. Hobson, S.A. MacDougall-Shackleton & *E.A. MacDougall-Shackleton*. "Migration, disease, and birdsong: bridging two worlds?" Winter Annual Behaviour Conference, Steamboat Springs, Colarado, USA, January 2016. [Talk]

10) **Kelly, T.R.**, H.L. MacGillivray, M.J. Watson, Y. Sarquis-Adamson, K.A. Hobson, S.A. MacDougall-Shackleton & *E.A. MacDougall-Shackleton*. "Seasonal migration distance varies with natal dispersal and predicts parasitic infection in song sparrows (*Melospiza melodia*)." The Society for Integrative and Comparative Biology, Portland, Oregon, USA, January 2016. [Talk]

9) ***Kelly, T.R.***, A. H. Lymburner, K.A. Hobson, E.A. MacDougall-Shackleton & S.A. MacDougall-Shackleton. "Testosterone as a potential mediator of migration distance and migratory timing in song sparrows, *Meospiza melodia*." The Society for Integrative and Comparative Biology, Portland, Oregon, USA, January 2016. [Talk] **Selected to compete in best student oral presentation.*

8) **Kelly, T.R**. H.L. MacGillivray, M.J. Watson, Y. Sarquis-Adamson, K.A. Hobson, S.A. MacDougall-Shackleton & *E.A. MacDougall-Shackleton*. "Seasonal migration distance varies with natal dispersal and predicts parasitic infection in song sparrows (*Melospiza melodia*)." The Society for Integrative and Comparative Biology, Portland, Oregon, USA, January 2016. [Talk]

7) **Kelly, T.R**., H.L. MacGillivray & *E.A. MacDougall-Shackleton*. "Birds on the move: migration, dispersal, and immunity." Winter Animal Behaviour Conference, Steamboat Springs, Colorado, USA, January 2015. [Talk]

6) ***Kelly, T.R***., S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. "Infection status and immune function is related to migration distance in song

sparrows (*Melospiza melodia*). Biology Graduate Research Forum, Western University, London, Ontario, Canada, October 2014. [Talk]

5) ***Kelly, T.R***., S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. "Infection status and immune function is related to migration distance in song sparrows (*Melospiza melodia*). Animal Behaviour Conference, Princeton University, Princeton, New Jersey, USA, August 2014. [Talk]

4) ***Kelly, T.R***., S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. "Song sparrows *Melospiza melodia* allocate immune function based on migratory distance and life-history stage". Western Research Forum, Western University, London, Ontario, Canada, March 2014. [Poster]

3) ***Kelly, T.R***., S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. "Song sparrows *Melospiza melodia* allocate immune function based on migratory distance and life-history stage". Sustainability and Environment Research Showcase, Western University, London, Ontario, Canada, February 2014. [Poster] **Honourable mention for best student poster.*

2) ***Kelly, T.R***., S.A. MacDougall-Shackleton & E.A. MacDougall-Shackleton. "Stable isotope analysis of migratory distance and its relationship to immune allocation in song sparrows Melospiza melodia". Biology Graduate Research Forum, Western University, London, Ontario, Canada, October 2013. [Poster]

1) ***Kelly, T.R***. & G. Burness. "Experimental manipulation of prenatal environment in Japanese Quail and effects on offspring phenotype". Ontario Biology Day, Laurentian University, Sudbury, Ontario, Canada, March 2012. [Talk]

PUBLISHED PHOTOGRAPHY

1) Cover image for the Journal of Experimental Zoology: A, Ecological and Integrative Physiology. Volume 329, Issue 1, January 2018. Features a singing, colour-banded song sparrow.

STUDENT MENTORING/SUPERVISION

2017 – 2018 Alexandra Cannon, Honour's Thesis

2017 – 2018 Emma Churchman, Honour's Thesis

2016 – 2017 Rachel Boyd, Independent Study (refereed publication 7).

2015 – 2016 Alannah Lymburner, Honour's Thesis (refereed publication 2).

2015 – 2016 Jennifer Ho, Independent Study

GRANTS

Student Research Grant, Animal Behavior Society, 2016, $1000.

Taverner Award, Society of Canadian Ornithologists / Société des ornithologistes du Canada, 2015, $2000.

AWARDS AND SCHOLARSHIPS

Biology Graduate Student Travel Award, Western University, 2018, $285.

Ontario Graduate Scholarship (OGS), Western University, 2017-2018, $15000.

Biology Graduate Student Travel Award, Western University, 2017, $50.

Robert & Ruth Lumsden Graduate Awards in Science, 2017, $1500.

Biology Graduate Student Travel Award, Western University, 2015, $300.

Dean's Honour Roll, Trent University, September 2008 - April 2009, $1000.

Dean's Honour Roll, Trent University, September 2010 - April 2011, $1000.

Dean's Honour Roll, Trent University, September 2011 - April 2012, $1000.

President's Honour Roll, Trent University.

LEADERSHIP

2018	Ontario Ethology, Ecology and Evolution Colloquium – Submission & Program
2017	Biology Graduate Research Forum – Abstract and Submission Committee
2015 – current	Western University Biology Department Field Safety Committee – Graduate Student Representative
2015 – 2016	SOBGS PSAC Local 610 – Steward Representative
2014 – 2015	Western University's Society of Biology Graduate Students (SOBGS) –Undergraduate Education Committee
2012 – 2013	PSAC Local 610 Biology Steward
2009 – 2012	Trent University's Pre-Veterinary and Animal Science President (2010 – 2012) and Vice President (2009 – 2010)

TEACHING APPOINTMENTS

Fall 2018	Instructor, Conservation Biology (BIOL 3442), Western University.
Winter 2018	Teaching assistant, Wildlife Ecology and Management (BIOL 3446), Western University.
Fall 2017	Teaching assistant, Conservation Biology (BIOL 3442), Western University.
Winter 2017	Teaching assistant, Biology for Science II (BIOL 1002), Western University.
Fall 2016	Teaching assistant, Conservation Biology (BIOL 3442), Western University.
Fall 2016	Teaching assistant, Scientific Methods in Biology (BIOL 2290), Western University.
Winter 2016	Teaching assistant, Wildlife Ecology and Management (BIOL 3446), Western University.
Fall 2015	Teaching assistant, Conservation Biology (BIOL 3442), Western University.
Fall 2015	Teaching assistant, Scientific Methods in Biology (BIOL 2290), Western University (half TAship).
Winter 2015	Teaching assistant, Biology for Science II (BIOL1002), Western University.
Fall 2014	Teaching assistant, Biology for Science I (BIOL 1001), Western University.
Winter 2014	Teaching assistant, Biology for Science II (BIOL 1002), Western University.
Fall 2013	Teaching assistant, Biology for Science I (BIOL 1001), Western University.
Winter 2013	Teaching assistant, Biology for Science II (BIOL 1002), Western University.
Fall 2012	Teaching assistant, Statistics for Science (BIOL 2442), Western University.