

STUDIES ON THE ECOLOGY OF AVIAN MALARIA IN AN ALPINE ECOSYSTEM

by

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To my parents, Guy and Linda Murdock,
for giving me strength of character, a flexible mind, and the desire to realize my story
and to live life fully.

And, to my “academic parents,” Bobbi Low and Carl Simon,
for being my guides through this chapter of my life.

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Abstract

Much of global biodiversity is comprised of parasitic organisms. It is well recognized that the selective pressures imposed by parasites shape host defenses and life-history strategies. Many studies suggest that human changes to the environment facilitate pathogen emergence by disrupting established relationships among parasites, their hosts, and their environment, as is often the case in human-modified landscapes. However, the lack of baseline knowledge of long-term, established host-parasite interactions in wildlife systems challenges our ability to make sound predictions on how future changes to the global environment will affect the health of human, domestic animal, and wildlife populations, as well as global biodiversity. Here we present data from multiple studies on the ecology of avian hemosporidian parasites in a common passerine population, the Mountain White-crowned Sparrow (*Zonotrichia leucophrys oriantha*) breeding in a temperate alpine system. The first study (see Chapter two) is a broad sampling study that describes the avian host, potential biting dipteran vector, and blood parasite communities on our field sites. The second study (Chapter three) is a molecular study describing the *Leucocytozoon* community amplified from ornithophilic black fly species. This study (Chapter four) explores the avian host, dipteran vector, and environmental factors important for transmission of *Leucocytozoon fringillinarum*, the most prevalent parasite in the White-crowned Sparrow population. Finally, the fourth study (Chapter five) examines the effects hemosporidian parasites have on the White-crowned Sparrow population. We determine *Leucocytozoon* spp. were the most prevalent hemosporidian in

the avian community, and that the black fly species *Simulium silvestre* / *S. craigi* had the potential to be a vector for a wide diversity of *Leucocytozoon* spp. in this system. We also established that relapsing infectious adults and young of the year birds are essential for seasonal persistence of *Leucocytozoon* spp. at these sites. Finally, infection with hemosporidians negatively affected bird populations by increasing stress levels and decreasing energy available to allocate towards other energetically expensive activities, such as reproduction.

Chapter I

Introduction

Much of global biodiversity is comprised of parasitic organisms (Toft 1986). It is well recognized that the selective pressures imposed by parasites shape host defenses and life-history strategies (Moller 1997, Schmidt and Roberts 2000). Anti-parasite adaptations such as immunity, preening, and other non-specific responses are energetically costly, and a significant trade-off may occur between the host's energy delegation for current survival and reproduction and its ability to manage parasitic infection (Glick et al., 1981; Glick et al., 1983; Lochmiller and Deerenberg, 2000; Lochmiller et al., 1993; Saino et al., 1997a; Saino et al., 1997b; Sheldon and Verhulst, 1996). Individuals and species vary in their tolerance to parasitism, which results in differential fitness consequences. This in turn can shape interspecific interactions through increases in predation (Schaller 1972, Hudson et al. 1992, Moore 2002) or apparent competition (Greenman and Hudson 2000, Gilbert et al. 2001), which at the population level, can threaten small populations by further decreasing abundance increasing the probability of local extinction (Cunningham and Daszak 1998, Daszak and Cunningham 1999). More recently documented is the role of parasites in shaping ecosystem-level processes. Parasites are no longer perceived as minor actors in ecosystem function. In fact in some ecosystems, parasitic biomass can outweigh the biomass of free living organisms (Kuris et al. 2008). Lost parasite species can have equivalent effects as the loss of keystone or tertiary structure species, triggering

extinction cascades that can alter the composition, diversity, and function of existing ecosystems (Altizer, 2001).

Many studies suggest that human changes to the environment facilitate pathogen emergence. Pathogen emergence is defined as a pathogen that has recently expanded in geographical range, moved from one host species to another, experiences an increase in virulence, or is a newly evolved pathogen (Daszak et al. 2004); emergence is the consequence of disrupting established relationships among parasites, their hosts, and their environment, as is often the case in human-modified landscapes (Harvell et al. 1999, Daszak et al. 2001). Most of our knowledge of wildlife pathogens currently comes from pathogens that infect the human population, our domestic animal populations, or have catastrophic, noticeable effects (Harvell et al. 1999, Dobson and Foufopoulos 2001). However, the lack of baseline knowledge of long-term, established host-parasite interactions in wildlife systems challenges our ability to assess the emergence of pathogens, and to determine whether emergence is due to increased parasite transmission or decreased host resistance (Harvell et al. 1999, Daszak et al. 2004). Further, this scarcity of baseline data hinders our ability to make sound predictions on how future changes to the global environment will affect the health of human, domestic animal, and wildlife populations, as well as global biodiversity (Altizer et al. 2001).

Avian hemosporidian parasites

Here we present data from multiple studies on the ecology of avian hemosporidian parasites in a common passerine population breeding in a temperate alpine system. Currently there are over 200 morphologically defined species of avian hemosporidian parasites of the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*

(Apicomplexa: Haemosporida), which are vectored by mosquitoes (Diptera: Culicidae), black flies (Diptera: Simuliidae), and biting midges (Diptera: Ceratopogonidae), respectively (Atkinson and van Riper III 1991, Valkiunas 2005a). All genera have broad global distributions (Valkiunas 2005a), however, molecular analyses of parasite mitochondrial DNA suggest that there likely exist many more malarial species than are currently recognized (Ricklefs and Fallon 2002, Bensch et al. 2004, Hellgren et al. 2007a). While much empirical work has been done on the ecology of avian haemosporidian parasites and the effects these parasites have on host fitness (e.g. van Riper et al. 1986, Atkinson and van Riper III 1991, Valkiunas 2005a), our general knowledge across different groups of hemosporidians remains unevenly distributed, with most attention devoted toward human malaria and the genus *Plasmodium* (Valkiunas 2005a).

Avian hemosporidian infections have been characterized by severe pathology in the acute phase (Desser and Ryckman 1976, Atkinson and van Riper III 1991, Atkinson et al. 2001) and high population prevalence, i.e. the proportion of infected individuals in the population (Valkiunas 2005a). Although acutely infected young birds can succumb to hemosporidians, surviving adults typically carry chronic, sublethal infections. However, even birds infected with chronic hemosporidian infections may experience reduced breeding success (Stjernman et al. 2004, Marzal et al. 2005, Tomas et al. 2007), body condition (Bonier et al. 2007), immunity (Millington et al. 2007), and survival (Sol et al. 2003, Marzal et al. 2008).

The density of parasite stages in the blood (parasitemia) changes dynamically throughout the course of an infection (Figure 1). Upon infection, a bird enters the acute

phase of the infection, which is characterized by an initial spike in blood stage parasitemia; the acute phase ranges from one week to several months depending on the parasite species, vertebrate host, and environmental factors. After the acute phase of the infection ends, parasitemia decreases and birds enter the chronic phase of infection which also varies in duration. The chronic phase is characterized by low parasitemia, roughly 1-3 gametocytes per 10,000 red blood cells (Valkiunas 2005a). Upon exiting the chronic phase of infection, the bird then enters a latent stage of infection, when parasites disappear from the peripheral blood and persist in non-circulating tissues, such as the internal organs (Valkiunas 2005a).

Much of what we know about avian hemosporidian parasites comes from the Hawaiian Islands where the recently introduced *P. relictum*, in conjunction with habitat degradation, invasive mosquito vectors, and avian pox, has lead to the endangerment or extinction of many endemic bird species (van Riper et al. 1986, Atkinson et al. 1995, van Riper and Scott 2001, Kilpatrick et al. 2006, Aruch et al. 2007). However, while we have a reasonable understanding of transmission ecology of invasive avian malaria in Hawaii, we know much less about the ecology of endemic hemosporidian infections (especially for the parasite genera *Leucocytozoon* and *Haemoproteus*) in continental bird species. Such mainland systems, characterized by greater parasite diversity and longer evolutionary history of host-parasite interactions, are the rule rather than the exception. Additionally, in light of the abundance of empirical work done on certain hemosporidian systems, there is a surprising lack of theoretical work (either conceptual or analytical) describing transmission dynamics for any of these parasites (exceptions are Beaudoin et al. 1971, Allan and Mahrt 1989).

The study site and focal population

We conducted a series of studies (throughout the summers of 2003-2007) to understand the factors important for transmission of avian hemosporidian parasites within a common passerine species, the Mountain White-crowned Sparrow (*Zonotrichia leucophrys oriantha*), breeding on three field sites located in the vicinity of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Gunnison County, Colorado, U.S.A. Two field sites were located approximately one mile south from RMBL (UTM: N 4312713 E 327700) and one mile north from RMBL (UTM: N 4314126.9 E 327648.3). The other field site was located in the adjacent, Washington Gulch valley (UTM: N 4311531 E 325807). Elevation ranges between 2902 m – 2987 m asl. The field sites are composed mainly of riparian habitat dominated by bog birch (*Betula glandulosa*), mountain alder (*Alnus tenuifolia*), and several species of willow (*Salix* spp.). Adjacent conifer (*Picea engelmannii*) and aspen (*Populus tremuloides*) stands at higher elevations encroach on these riparian and wildflower meadow ecosystems, and provide scattered trees throughout the valley floor. The Mountain White-crowned Sparrow is socially monogamous sparrow that breeds on our sites at the base of willows or dense herbaceous vegetation (Morton 2002). White-crowned Sparrows are an excellent focal species for this research. They have been well-studied, so there is a great deal of baseline knowledge about their habitat preferences, food habits, behavior, breeding, physiology, and predators (for reviews, see Chilton et al. 1995b, Morton 2002).

The mean date of the first White-crowned Sparrow spring arrival to our field sites is May 11 (± 0.87 d SE, $n = 32$, unpublished data, B. Barr). In the early breeding season when sparrows first arrive, food resources are limited and unpredictably available. The

site is snow-covered with little emergent vegetation, arthropod abundance is low, and inclement weather (i.e. rapidly advancing snow storms and freezing rain) occurs throughout early summer (CM, pers. observ.). Around June 11th, vegetation and arthropods begin to emerge, and sparrows initiate nesting. By the end of June sparrows are hatching and feeding young of the year (Johannes Foufopoulos unpublished data), and territories begin to break down in late July with banded birds leaving the field sites by the end of August (CM, pers. observ.). The sparrows breeding on our field sites are also infected with the following four hemosporidian genera: *Leucocytozoon*, *Haemproteus*, *Plasmodium*, and *Trypanosoma*. Thus, the sparrow population must balance multiple life history demands while coping with two natural challenges in this system, early season food scarcity and unpredictable weather, as well as infection with hemosporidian parasites.

Outline of studies on the ecology of avian hemosporidians

To better understand the ecology of White-crowned Sparrow hemosporidian parasites, we conducted a series of studies that are comprised of interdisciplinary approaches that focus on multiple levels (individual, population, and community) within the study system. The first study (see Chapter two) is a broad sampling study that describes the avian host, potential biting dipteran vector, and blood parasite communities on our field sites. In this study we define the species of hemosporidian parasites that infect White-crowned Sparrows, estimate the overall prevalence of hemosporidians in the avian community and sparrow population, and determine whether other bird species were infected with hemosporidian parasites that also infect White-crowned Sparrows. In addition, we describe the avian host and potential dipteran vector communities by

estimating abundances for these species across different habitats and throughout the summer season. Finally, we outline tentative host-parasite-vector associations based on shared habitat preferences and seasonal trends in estimated abundances of avian host and potential dipteran vector species.

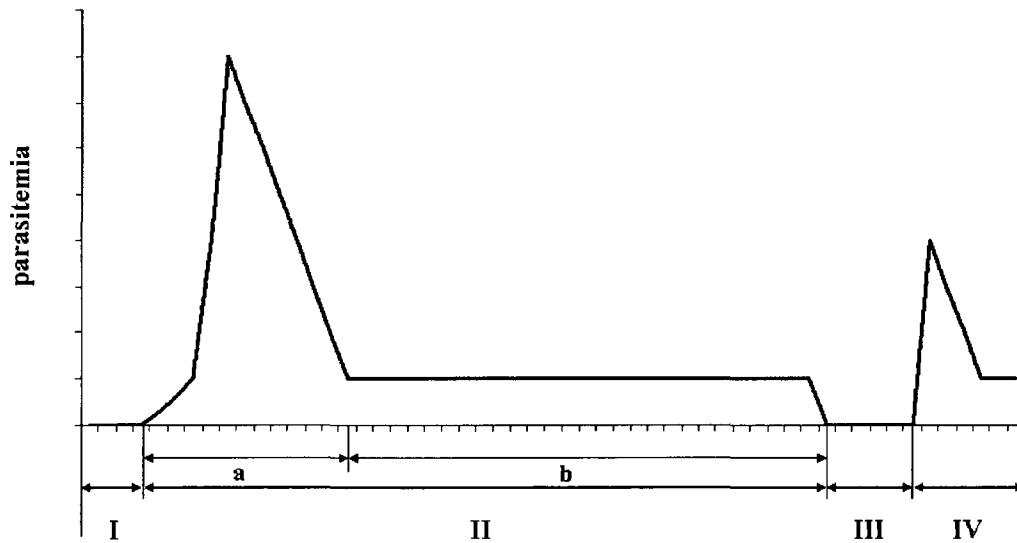
The second study (Chapter three) is a molecular study describing the *Leucocytozoon* community amplified from ornithophilic black fly species. The first study established that *Leucocytozoon* was the most prevalent hemosporidian parasite in the avian community and sparrow population on our field sites, and that *Simulium silvestre* / *S. craigi* might be an important vector in this system. In this study, we amplified *Leucocytozoon* DNA from two common, bird-feeding black fly species, and from avian hosts singly infected with *Leucocytozoon* spp. We then determined whether avian host species, habitat type, or time of season had the largest influence on how *Leucocytozoon* parasites were distributed on our field sites.

In our third study (Chapter four), we present a mathematical model on the ecology of hemosporidian transmission in a temperate, continental system due to the paucity of theoretical studies (analytical or conceptual) on avian hemosporidian parasites and the overemphasis of research on the Hawaiian Island system. This study explores the avian host, dipteran vector, and environmental factors important for transmission of *Leucocytozoon fringillinarum*, the most prevalent parasite in the White-crowned Sparrow population (CM unpublished, see Chapter two). The model is parameterized from field data collected in past studies on the White-crowned Sparrow population (Johannes Foufopoulos unpublished data, see Chapter two), as well as from the *S. silvestre* / *S. craigi* population breeding on these field sites (CM unpublished data, see Chapter two

and three). In this study we examine the relative importance of relapse and young of the year birds for the seasonal persistence of *L. fringillinarum* on these field sites.

Finally, the fourth study (Chapter five) examines the effects hemosporidian parasites have on the White-crowned Sparrow population. We elucidate how parasites affect host fitness by experimentally assessing the potential interactive effects of early season food scarcity and unpredictability and parasitism on stress and immune response, two physiological mediators of survival and reproduction. We examine the effects of food supplementation and antimalarial drug treatment on baseline and stress-induced corticosterone (the primary avian stress hormone), as well as a skin swelling response to an injection with phytohemagglutinin.

Figure 1.1 is a typical representation of how parasitemia of hemosporidian parasites change throughout the course of infection and is adapted from Valkiunas' figure 16 (2005). The phases of infection are the following: prepatent period (**I**); primary parasitemia (**II**) consisting of the acute (**a**) and chronic (**b**) phases; latent phase of infection (**III**); and secondary parasitemia resulting from seasonal relapse (**IV**). The x-axis represents the calendar year and the y-axis is density of blood stages, or parasitemia. Infected birds exhibit the highest parasitemia during the acute phase of the infection, and lowest and intermediate parasitemia during the chronic and relapse phase, respectively. Thus, birds are most infectious during the acute phase of infection and are least infectious during the chronic phase of infection.



Chapter II

Habitat and seasonal distributions of avian and dipteran populations breeding in a temperate, high-elevation ecosystem reveal host, vector, and hemosporidan parasite associations

Introduction

Parasitism is ubiquitous – over half of the world's biodiversity is composed of parasitic organisms (Toft 1986), and the selective pressures imposed by parasites shape host defenses and life-history strategies (Moller 1997, Schmidt and Roberts 2000). At the individual level, parasitic infections typically have negative fitness consequences. Anti-parasite adaptations such as immunity, preening, and other non-specific responses are energetically costly, and a significant trade-off may occur between the host's energy delegation for current survival and reproduction and its ability to manage parasitic infection (Glick et al., 1981; Glick et al., 1983; Lochmiller and Deerenberg, 2000; Lochmiller et al., 1993; Saino et al., 1997a; Saino et al., 1997b; Sheldon and Verhulst, 1996). Because species vary in their tolerance to parasitism, this heterogeneity results in differential consequences to fitness, which may in turn influence interspecific interactions through increased predation (Schaller 1972, Hudson et al. 1992, Moore 2002) or apparent competition (Greenman and Hudson 2000, Gilbert et al. 2001). At the population and ecosystem level, parasitic introductions can threaten small populations by further decreasing abundance, hence increasing the probability of local extinction; if keystone or

tertiary structure species are lost, extinction cascades can completely alter the composition, diversity, and function of existing ecosystems (Altizer, 2001).

Many studies suggest that human changes to the environment facilitate disease emergence in wildlife populations by altering the relationships among parasites, their hosts, and their environment (Harvell et al. 1999, Daszak et al. 2001). Disease emergence in wildlife populations has significant implications for the health of both humans and domestic animals, as well as for the conservation of biological diversity (Altizer et al. 2001). However, the lack of baseline knowledge of long-term, established host-parasite interactions in wildlife systems challenges our ability to assess the emergence of pathogens, and to determine whether emergence is due to increased parasite transmission or decreased host resistance (Harvell et al. 1999, Daszak et al. 2004). Further, this scarcity of baseline data hinders our ability to make sound predictions on how future changes to the global environment will affect the health of wildlife populations.

Here we present data from a study on the ecology of avian hemosporidian parasites in a common passerine population breeding in a temperate alpine system. Currently, there are more than 200 morphologically defined species of avian hemosporidian parasites (Apicomplexa: Haemosporida) of the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* that can have broad global distributions (Valkiunas 2005a). However, molecular analyses of parasite mitochondrial DNA suggest that there likely exist many more malarial species than are currently recognized (Ricklefs and Fallon 2002, Bensch et al. 2004, Hellgren et al. 2007a). While much empirical work has been done on the ecology of specific avian haemosporidian parasites and the effects that

these parasites have on host fitness (e.g. van Riper et al. 1986, Atkinson and van Riper III 1991, Valkiunas 2005a), our knowledge across different groups of hemosporidians remains unevenly distributed, with most attention having been devoted to human malaria and the genus *Plasmodium* (Valkiunas 2005a).

Avian hemosporidian infections have been characterized by severe pathology in the acute phase (Desser and Ryckman 1976, Atkinson and van Riper III 1991, Atkinson et al. 2001) and high prevalence of infection in bird populations (Valkiunas 2005a). Although acutely infected young birds can succumb to hemosporidians, surviving adults typically carry chronic, sublethal infections. However, even birds infected with chronic hemosporidian infections may experience reduced breeding success (Stjernman et al. 2004, Marzal et al. 2005, Tomas et al. 2007), body condition (Bonier et al. 2007), immunity (Millington et al. 2007), and survival (Sol et al. 2003, Marzal et al. 2008).

Much of what we know about avian hemosporidian parasites comes from the Hawaiian Islands where the recently introduced *P. relictum*, in conjunction with habitat degradation, invasive mosquito vectors, and avian pox, has led to the endangerment or extinction of many endemic bird species (van Riper et al. 1986, Atkinson et al. 1995, van Riper and Scott 2001, Kilpatrick et al. 2006, Aruch et al. 2007). However, while we have a reasonable understanding of transmission ecology of invasive avian malaria in Hawaii, we know much less about the ecology of endemic hemosporidian infections in continental bird species (especially for the parasite genera *Leucocytozoon* and *Haemoproteus*). Such mainland systems, characterized by greater parasite diversity and longer evolutionary history of host-parasite interactions, are the rule rather than the exception.

We conducted a broad sampling study as a first step in understanding the factors important for transmission of avian hemosporidian parasites within a common passerine species, the Mountain White-crowned Sparrow (*Zonotrichia leucophrys oriantha*), breeding in the Colorado Rockies. In this study we describe the avian, dipteran, and blood parasite communities in this system, and describe some tentative host-parasite associations. In particular, we had the following objectives. First, we describe the overall prevalence of hemosporidian parasites found in both the avian community and the sparrow population, and determine whether other bird species host hemosporidian parasites that also infect White-crowned Sparrows. Second, we describe the effects of habitat and time of season on bird and dipteran species abundance and determine which species have abundances influenced by similar habitat effects as White-crowned Sparrows. Finally, based on these shared habitat associations and time of season effects, we outline tentative host-vector-parasite relationships.

Methods

Field site description and study design

This study was conducted on two field sites within the vicinity of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Gunnison County, Colorado, U.S.A., during the summers of 2005 and 2007. One field site was located approximately 2 km south from RMBL (UTM: N 4312713 E 327700); the other field site was located in the adjacent, Washington Gulch valley (UTM: N 4311531 E 325807). Elevation ranges between 2,902 m and 2,987 m asl. Both field sites are composed mainly of riparian habitat dominated by bog birch (*Betula glandulosa*), mountain alder (*Alnus tenuifolia*), and several species of willow (*Salix* spp.). Adjacent conifer (*Picea engelmannii*) and

aspen (*Populus tremuloides*) stands at higher elevations encroach on these riparian and wildflower meadow ecosystems, and provide scattered trees throughout the valley floor.

To obtain a representative sample of the bird and biting dipteran communities, we sampled across a variety of common habitats in each site from May 15th – August 12th, in both 2005 and 2007. Each field site is composed of multiple patches of the following broad habitat types: willow, alpine meadow, and forest habitat patches. We stratified our field sites by habitat type, and then took a random sample of two, non-adjacent patches within each habitat type in both field sites.

Blood parasite sampling

During the summer of 2005, (18 June – 16 July, 29 July – 12 August), birds were captured daily between 06:00 and 12:00 in millet-baited, single- or double-cell (18 cm × 18cm × 18 cm per cell), galvanized wire Potter traps or in nylon mist nets (38mm mesh, 6 m wide - Avinet No. BH06, Dryden, NY, USA). Each bird was marked with a uniquely numbered metal U.S. Fish and Wildlife Service band (Bird Banding Laboratory, Patuxent, MD, USA). Upon capture, we sexed each bird, collected standard morphometric measures, and took a blood sample from the brachial vein with a sterile 26-gauge hypodermic needle (Fisher Scientific Precision Glide, No. 14-826-15, Pittsburgh, PA, USA). Blood samples were collected in 70 µL heparinized microcapillary tubes (Fisher Scientific, No. 22-362-566, Pittsburgh, PA, USA) and temporarily stored in a cooler with ice. A small portion of the blood sampled from each bird was placed on a microscope slide (Fisher Scientific, No. 12-542-5, Pittsburgh, PA, USA), air-dried, and stained with a Fisher Hema 3 Stat packTM (Fisher Scientific, No. 22-122911, Pittsburgh, PA, USA). We determined infection status by identifying

hemosporidian blood parasites to the morphological species level (Valkiunas 2005a). Slides were microscopically scanned (10 x and 100 x power) for blood parasites for a total of 15 min. The species-specific infection status, was then used to evaluate community and population level prevalences of hemosporidian infections.

Avian point count methods

During summer 2007, after most birds had established breeding territories, we conducted point counts to assess species-specific abundances in both field sites. Due to the relatively small size of each field site, we did not adopt a typical point count transect design (Ralph et al. 1993). Instead, point count stations were paired to habitat patches selected from the stratified random sample. Each habitat patch had a point count station at its center, and depending on the size of the habitat patch and its proximity to other patches in the sample, had two to four additional point count stations located 250 m away from the central point count station. A distance of 250 m was maintained between point count stations associated with each habitat patch to avoid double counting of birds. At each point count station, birds were recorded by sight and song within a 50 m radius from 05:30 to 10:00 (Ralph et al., 1993). Because we did not have as many point counts per field site as standard point count transects, we recorded birds for 10 min intervals and had two point count periods during 2007 (June 11th to June 16th and July 1st to July 8th) to better detect rare species. To minimize observer biases in detectability, only one of us performed point counts throughout the summer.

Dipteran collection methods

Dipteran sampling was focused on mosquitoes (Culicidae) and black flies (Simuliidae) because species within these families have been shown to be competent

vectors of *Plasmodium* spp. and *Leucocytozoon* and *Trypanosoma* spp., respectively (Valkiunas 2005a). We did not include biting midge (Ceratopogonidae) species, the potential vectors for *Haemoproteus* spp., in this study due to difficulties with identifications of midge species collected at these field sites. Mosquitoes and black flies were collected (Service 1976) from carbon-dioxide baited Center for Disease Control (CDC) miniature light traps (John W. Hock Company, No. 512 fine mesh collection cups, Gainesville, FL, USA) at regular intervals throughout the summer of 2007. CDC traps were reset and checked once every 24 hours. Because of potential trap failure, traps were paired approximately 50 m from each other within each sampled habitat type. We trapped each habitat during consecutive nights over intervals of eight days. Every two days, traps were rotated to the other field site to minimize potential effects of season or weather on trap success. Sampling in 2007 occurred during 19-26 May, 8-17 June, 1-12 July, and 22-31 July.

Within two hours of removal from traps, all biting dipterans were placed for five minutes into a garbage bag and exposed with triethylamine-soaked cotton (Fisher Scientific, Amber Glass, No. BP616-500, Pittsburgh, PA, USA). Once immobilized, all mosquitoes were immediately identified to species level (Darsie Jr. and Ward 2005, Harmston and Lawson 1967, Smith 1966) and then stored in a -80°C freezer, while black flies were placed immediately in 95% ethanol for later identification. Female black flies were identified to species or species complex based on structural characters presented in the keys and illustrations of Adler et al. (2004). Identification was facilitated by genital preparations of selected specimens. Representative specimens have been deposited in the Clemson University Arthropod Collection, Clemson, South Carolina.

Statistical analyses

Parasite Prevalences

To estimate parasite prevalence from presence / absence data, we determined the proportion of birds infected with *Leucocytozoon*, *Haemoproteus*, *Plasmodium*, and *Trypanosoma* spp. in the avian community consisting of all species sampled except White-crowned Sparrows. To determine prevalence of hemosporidian parasites in White-crowned Sparrows, we pooled parasite data with similar data from White-crowned Sparrows collected in 2005 from sites 2 km north of RMBL (Foufopoulos unpublished). This increased the sample size to a total of 222 sparrows, thereby reducing the chance of misrepresenting prevalence patterns through small sample sizes. From the pooled data, we calculated the prevalence of *Leucocytozoon fringillinarum*, *L. majoris*, *Haemoproteus coatneyi*, *Plasmodium relictum*, *P. vaughani*, and *Trypanosoma avium*. A series of X^2 one-sample tests for goodness of fit was used to determine if prevalence of hemosporidian parasites was higher or lower than expected if parasites were equally distributed in the avian community and the White-crowned Sparrow population. We then used a X^2 test of independence to compare the prevalence of parasites in the White-crowned Sparrow population to the rest of the avian community.

Bird abundance estimates

The abundance of bird species in our avian community was estimated using a model-based approach, because detectability of birds can be biased by differences in site, habitat, and distance from observer (Thompson and La Sorte 2008). Analysis using Generalized Linear Model in SAS (version 9.1.2) was restricted to the common bird species (≥ 10 counts) present at each field site, because transmission of hemosporidians is

likely not to be affected by rarer host species. When possible, models were run with the Poisson distribution (Link and Sauer 1998). However, for bird species that had smaller raw abundances and were not evenly distributed across site and habitat types, estimated abundance used models that assumed a normal distribution (Table 1). We treated counts of species at a point as the index of abundance (the response variable) and included site (*RMBL* or *Washington Gulch*), habitat type (*Forest*, *Willow*, or *Meadow*), and distance (*0-25 m*, *25-50 m*, or *>50 m*) as categorical fixed effects. The effects of the potential interaction between site and habitat type were also incorporated. Point also was included as a repeated random effect with a first-order autoregressive correlation structure to account for any covariance among counts at the same points. We used Bonferonni-adjusted post hoc tests to determine significant differences among the means of estimated abundances (Table 1).

We also used GLM analysis to estimate the abundance of potential mosquito and black fly vector species in this dipteran community. The number of species captured in each habitat type was considered abundance index, and included the following categorical, fixed effects: site (*RMBL* or *Washington Gulch*), habitat type (*Forest*, *Willow*, or *Meadow*), and sampling session as a measure of season (*18 May - 4 June*, *8 June - 17 Jun*, *25 June - 14 July*, or *15 July - 31 July*). The effects of potential interactions were included in each model: site by session, site by habitat type, and session by habitat type. Again Bonferonni-adjusted post hoc tests were used to determine significant differences among the means of estimated abundances. We focused analysis on the black fly species that had avian feeding preferences. Even though *Leucocytozoon* spp. have successfully completed development in a range of mammalophilic black fly

species (Desser and Yang 1973), these species most likely have low vectorial capacity for avian blood parasites in this system, due to their preference for mammalian hosts.

Results

Prevalence of hemosporidian infections

During summer 2005, we captured, took blood samples from, and determined the infection status of a total 130 birds from 23 species, including 28 White-crowned Sparrows (Table 1). *Leucocytozoon* spp. (42%) infections had the highest prevalence among all bird species sampled, while *Haemoproteus* (17%), *Plasmodium* (8%), and *Trypanosoma* (24%) spp. had significantly lower prevalences ($\chi^2_{1,3} = 27.37$, $n = 102$, $p < 0.05$, Figure 1). Infected White-crowned Sparrows harbored the following hemosporidian species: *Leucocytozoon fringillinarum*, *L. majoris*, *Haemoproteus coatneyi*, *Plasmodium relictum*, *P. vauhani*, and *Trypanosoma avium*. Reflecting patterns in the broader avian host community, infections with *Leucocytozoon* spp. were the most prevalent; White-crowned Sparrows, however, had an even higher prevalence of *Leucocytozoon* spp. (52%) than the rest of the bird community. In contrast, prevalences of *Haemoproteus* (8%) and *Trypanosoma* (4%) spp. (Figure 1) were lower in sparrows than among the rest of the avian community ($\chi^2_{1,3} = 19.86$, $n = 304$, $p < 0.05$).

Of the 23 bird species sampled for hemosporidian parasites, 17 were infected with the same hemosporidian species that also infect White-crowned Sparrows (Table 1). *L. fringillinarum* or *L. majoris* were identified from 11 other bird species, *H. coatneyi* and *P. relictum* were present in two other bird species, *P. vauhani* was identified in five other bird species, and all but two of the 17 species had individuals infected with *T. avium*. Yellow Warblers (*Dendroica petechia*) and Lincoln's Sparrows (*Melospiza*

lincolni) had the highest degree of overlap in their blood parasite fauna with White-crowned Sparrows. Captured Yellow Warblers and Lincoln's Sparrows were infected with five out of the six possible parasite species known to infect White-crowned Sparrows; we failed to observe infections with *P. relictum* in birds of these two species captured on our field sites.

Avian abundance estimates

We estimated the population abundance of 26 common bird species belonging to 13 families observed in 2007 (for a complete list of avian species observed, see Appendix A). The abundances of very few species were not affected by site, habitat type, or distance from observer in our model analysis (Table 2); only Tree Swallows (*Tachycineta bicolor*) and Red-winged Blackbirds (*Agelaius phoeniceus*) had abundances that were unaffected by the categorical fixed effects. Distance from the observer had significant, but variable effects on the detectability of almost all species, with only Tree Swallows, Red-winged Blackbirds, and Hairy Woodpeckers (*Picoides villosus*) as the exceptions (Table 2). Nine bird species had estimated abundances affected by site: Fox Sparrows (*Passerella iliaca*), Green-tailed Towhees (*Pipilo chlorurus*), Lazuli Buntings (*Passerina amoena*), MacGillivray's Warblers (*Oporornis tolmiei*), Yellow Warblers (*Dendroica petechia*), and American Robins (*Turdus migratorius*) had higher abundances on the RMBL site, while Lincoln Sparrows, Mountain White-crowned Sparrows, and Hairy Woodpeckers had higher abundances on the Washington Gulch site (Table 2).

Habitat also had significant impacts on estimated avian abundances. The Gray-headed Junco (*Junco hyemalis*), Mountain Chickadee (*Poecile gambeli*), Audubon's Warbler (*Dendroica coronata auduboni*), House Wren (*Troglodytes aedon*), and the

Western Wood-pewee (*Contopus sordidulus*) had higher estimated abundances in forested habitat types (Table 3). The only bird species with higher estimated abundance in meadow habitat was the Green-tailed Towhee, while Wilson's Warblers (*Wilsonia pusilla*) and Yellow Warblers had higher estimated abundances in willow habitat types (Table 3). Lastly, Fox Sparrows, Mountain White-crowned Sparrows (Figure 2), Brown-headed Cowbirds, and Broad-tailed Hummingbirds (*Selasphorus platycercus*) had equally high abundances in both meadow and willow habitat types, and estimated abundances of the remaining bird species were unaffected by habitat type (Table 3). There were five species of which estimated abundances were affected by a site and habitat type interaction: the Green-tailed Towhee, Lazuli Bunting, Mountain Chickadee, Yellow Warbler, and the Warbling Vireo (Table 3).

Biting dipteran abundance estimates

During summer 2007 we captured a total of 4,755 mosquitoes of 17 different species. In addition, 2,951 black flies belonging to 18 different species were captured, of which 10 feed predominantly on birds (for a complete list of dipteran species captured, see Appendix B). We estimated abundance by site, time of season, and habitat type for all but one of the ornithophilic black fly species and nine mosquito species (Table 4). GLM analyses were not run to estimate abundances on one species of black fly (*Simulium decorum*) and eight species of mosquitoes (*Culiseta inornata*, *Cs. incidens*, *Ochlerotatus dorsalis*, *O. fitchii*, *O. impiger*, *O. melanimon*, *O. pionips*, and *O. schizopinax*) due to low capture sample sizes ($n \leq 5$).

Not surprisingly, season (sampling session) had the strongest effect on abundance estimates of the majority of mosquito and black fly species (Table 4); only the estimated

abundance of the mosquito species *O. punctor* was unaffected by site, time of season, or habitat type. The mosquitoes *Cs. alaskaensis* and *Cs. impatiens* reached their peak estimated abundances in the first sampling session (18 May – 4 June), suggesting that *Culiseta* spp. emerges and is most abundant early in the summer season (Table 5). The mosquitoes *O. cataphylla* and *O. hexodontus*, and the black fly *Greineira denaria*, experienced their highest abundances during the second sampling session (8-17 June). However, the majority of dipterans were most abundant during the third sampling session (25 June – 14 July); these included the mosquitoes *O. communis*, *O. intrudens*, and *O. pullatus*, and black flies *Helodon onchodactylus*, *Metacnephia jeanae*, *S. arcticum* complex, *S. silvestre* / *S. craigi*, and *S. vittitatum* (Table 5).

Some species did not show clear abundance patterns across multiple sampling sessions. *O. implicatus* was most abundant from 8 June - 14 July (second and third sampling session), while *S. exulatum* / *S. pilosum* and *S. irritatum* / *S. venustum* were highly abundant during 25 June – 31 July (third and fourth sampling sessions). Only one species of black fly, *S. canonicolum*, was equally abundant across three sampling sessions (8 June – 31 July). The most common, significant interaction among the fixed effects in our model (site, habitat, and sampling session), was the interaction between site and sampling session (time of season).

Abundances of most dipteran species did not differ between the two field sites, except for *Cs. alaskaensis*, which was more abundant on the RMBL field site than the Washington Gulch site (Table 4). Habitat type influenced estimated abundances of some dipteran species, although black fly abundances were relatively unaffected. Three black fly species (*S. irritatum* / *S. venustum*, *S. exulatum* / *S. pilosum*, and *S. vittitatum*) were

most abundant in forest (Bonferroni Post-Hoc Test: $\chi^2_{1,1} = 6.09$, $n = 61$, $p = 0.0136$), meadow (Bonferroni Post-Hoc Test: $X^2_{1,1} = 6.68$, $n = 84$, $p = 0.0097$), and willow (Bonferroni Post-Hoc Test: $\chi^2_{1,1} = 15.48$, $n = 51$, $p < 0.0001$) habitat types, respectively. In contrast, most mosquito species exhibited higher estimated abundances in forest habitat types; however, *O. hexodontus* was more abundant in meadow while *O. implicatus* and *O. punctor* appeared to be equally abundant across all habitat types (Table 6).

Discussion

The most prevalent infections in the community of birds sampled on these field sites were with *Leucocytozoon* and *Trypanosoma* spp. This is not surprising because species of both genera are transmitted among avian hosts by black flies (Adler et al. 2004, Valkiunas 2005a). *Leucocytozoon* spp. can successfully complete development across a wide-range of black fly species (Desser and Yang 1973, Valkiunas 2005a); for example, 90-100% of onitophilic black fly species in Algonquin, Ontario had individuals with *Leucocytozoon* sporozoites present in their salivary glands (Adler et al. 2004). Additionally, the most common bird feeding black fly species (*S. silvestre* / *S. craigi* and *S. arcticum* complex) are present throughout most of the bird breeding season, reaching peak abundances around the time most avian species are hatching and feeding susceptible young (CM per obs.). Furthermore, our results showed that these black flies are more or less abundant in all habitats

In contrast, the low prevalence of *Plasmodium* spp. in the avian community reflects the weak avian feeding preferences of all mosquito species present on our field sites. All common boreal *Culiseta* and *Ochlerotatus* species captured in this study have

mammalian feeding preferences (Downe 1960, Mezenov 1976), or are linked to transmission of encephalitis viruses in mammalian foci (Brummerkorvenkontio and Saikku 1975, Campbell et al. 1991, Andreadis et al. 2008). Mosquitoes captured in our study that are competent vectors of *Plasmodium relictum* include *Cs. inornata*, *Culex tarsalis*, and *O. dorsalis* (Reeves et al. 1954, Huff 1965). *O. communis* has also been linked to the transmission of an unidentified *Plasmodium* spp. (Huff 1965), and may be capable of transmitting *Plasmodium* spp. on our field sites. However, because *Cs. inornata*, *Cx. tarsalis*, and *O. dorsalis* are present in low abundances throughout the summer season, and most mosquito species (including *O. communis*) appear to favor forest habitat types, transmission of *Plasmodium* spp. is most likely infrequent, explaining the overall low prevalence of *Plasmodium* spp. in the avian community that we studied.

Unexpectedly, 17 of the 23 bird species sampled were infected with hemosporidian species that also infect White-crowned Sparrows. The majority of avian species shared infections of *Leucocytozoon* spp. with White-crowned Sparrows, which is not surprising considering the overall high prevalence of *Leucocytozoon* in the avian community. Five of six avian species (Fox Sparrows, Green-tailed Towhees, Lincoln's Sparrows, Wilson's Warblers and Yellow Warblers) that had similar meadow and willow habitat associations as White-crowned Sparrows also were infected with the same hemosporidian species that infect White-crowned Sparrows (Table 1), suggesting that locally active transmission of these hemosporidians may occur in these particular habitat types. Specifically, due to the large overlap in parasite communities among Yellow Warblers, Lincoln's Sparrows, and White-crowned Sparrows, they may be important

reservoir hosts of these shared hemosporidian parasites due to their large abundances on these field sites their mutual habitat associations. Studies using molecular identifications of hemosporidians suggest there are more parasite species that exist than are currently recognized (e.g. Ricklefs and Fallon 2002, Bensch et al. 2004, Krizanaskiene et al. 2006); thus, these conclusions are tentative and need to be confirmed with molecular identifications. If cryptic species (species that share similar morphology) do exist, our study may have overestimated the true host range of hemosporidians infecting White-crowned Sparrows.

Based on dipteran species abundance patterns, which varied by site, time of season, and habitat type, various dipteran species may be vectors for hemosporidian parasites in this system. *Cx. tarsalis*, *Cs. inornata*, *O. dorsalis*, and *O. communis* are competent vectors for *Plasmodium* spp. (Reeves et al. 1954). Even though three of these mosquito species were recovered in low abundances on both field sites, together they may be important for *Plasmodium* spp. transmission in forested habitats. The sheer numbers of dipteran individuals captured during trapping suggest that *O. cataphylla* and *O. implicatus* may contribute to transmission of *Plasmodium* in the avian community. Both species exhibit strong, feeding preferences for large mammals (CM unpublished data, Downe 1960), but opportunistic feeding on encountered birds may still occur. In addition, *O. implicatus* abundance is not affected by habitat, which would facilitate transmission of *Plasmodium* spp. throughout the bird community. Finally, *O. communis*, *O. cataphylla*, and *O. implicatus* reach peak abundances during the second and third sampling sessions (8 June – 14 July), a time when most bird species are hatching susceptible young (CM per sobs). Because most nestlings in this avian community are

altricial, lack feathers for an interval of time, and are initially defenseless, biting dipterans may feed more intensively on nestlings than feathered adult birds (Hassan et al. 2003, Cupp et al. 2004). Transmission experiments must be undertaken, however, to establish vector competence and eventually vectorial capacity of these two mosquito species.

A variety of ornithophilic black flies may vector *Leucocytozoon* and *Trypanosoma* spp. at our field sites. *S. arcticum* complex and *S. silvestre* / *S. craigi* may be good vector candidates, based on the large numbers of individuals recovered from light traps. In addition, their abundances are not affected by habitat type, and they reach peak abundances during session three (25 June – 14 July), coinciding when most birds are hatching and feeding susceptible year. Studies show that *S. silvestre* / *S. craigi* feeds on a variety of avian hosts (i.e. White-throated Sparrow, Common Grackle, American Robin, and Blue Grouse), and has been implicated as a vector for a multitude of *Leucocytozoon* spp (*L. fringillinarum*, *L. icteris*, *L. dubreuii*, and *L. lovati*). DNA from both *Leucocytozoon* spp. and passerine bird (Family: Turdidae) have been simultaneously sequenced from *S. silvestre* bloodfed individuals in other systems (Hellgren et al. 2008). However, parasite DNA amplified from the abdomens of bloodfed individuals does not provide proof of vector competence of *S. silvestre* (see Chapter 3). Not all *Leucocytozoon* species ingested with a bloodmeal can develop infectious stages in the salivary glands of a given black fly species and successfully transmit to the next avian host. Thus, these present only a tentative link among *S. silvestre* / *S. craigi*, passerine birds, and potential transmission of *Leucocytozoon* spp. in this system.

Another potential black fly vector species *S. canonicolum*, because it is moderately abundant across habitats and throughout most of the summer season. Finally,

because *S. irritatum* / *S. venustum*, *S. exulatum* / *S. pilosum*, and *S. vittatum* complex are moderately abundant, are present throughout the latter half of the summer season, and have their highest abundances in specific habitat types, they may be locally important *Leucocytozoon* vectors in forest, meadow, and willow habitat types, respectively. However, additional transmission experiments or studies utilizing molecular amplifications of parasite DNA from host-seeking individuals are required before we can establish true vector competence of these species for *Leucocytozoon* and *Trypanosoma* spp. in our study area.

Conclusions

Based on detailed prevalence and estimated abundance data for hemosporidian parasites, avian hosts, and potential arthropod vectors, we offer here a tentative framework of potential host-vector-parasite associations (Figure 3). We propose that each common habitat type (willow, meadow, and forest) may contain a transmission network consisting of the avian and dipteran species that have their maximum abundances associated with each habitat type. Transmission of different parasite genera is dependent upon the vectors associated with each habitat. Transmission of parasite genera among habitat types may be a function of bird and dipteran species that have abundances unaffected by habitat type. We propose that *Plasmodium* transmission may be maintained at low prevalence in forested habitats by the competent mosquito species, in particular *Oc. communis*, and is transmitted minimally to other habitat types by generalist bird species. *Leucocytozoon* and *Trypanosoma* spp. may be transmitted readily within each focus and among foci by the black fly species associated with each habitat

type, and possibly by the generalist, extremely abundant *S. arcticum* complex and *S. silvestre* / *S. craigi* (Figure 3).

However, to tease apart these proposed host-parasite-vector associations, additional studies that employ field, molecular and analytical techniques are needed. For example, molecular studies describing the parasite community from DNA amplified from both avian hosts and dipteran vectors are necessary. Parasite distribution can be influenced by many factors, including active vector preferences for particular avian hosts, the ability of each parasite to infect a specific vertebrate host, or climatic variables and microhabitat requirements that may place vertebrate hosts and vectors into frequent contact with each other (Hellgren et al. 2008). In addition, mathematical models that define the ecological, parasite, and host parameters that are most important for maintaining transmission on these field sites would be helpful. Finally, to begin to overlay the transmission ecology of these parasites with their effects on avian host ecology, we need comprehensive field experiments investigating the potential fitness effects of these parasites on their bird hosts.

Table 2.1 Sixteen bird species were infected with hemosporidians that also infect White-crowned Sparrows (given in bold). Numbers in parentheses following common bird and parasite species names indicate number of blood smears scored for that bird species and number of smears infected with that parasite species, respectively.

Family	Bird Species	Leucocytozoon	Haemoproteus	Plasmodium	Trypanosoma
Emberizidae	Fox Sparrow (8)	<i>L. fringillinarum</i> (2)			
		<i>L. majoris</i> (3)			
	Green-tailed Towhee (2)	<i>L. fringillinarum</i> (1)			<i>T. avium</i> (2)
		<i>L. majoris</i> (1)			
	Lincoln Sparrow (21)	<i>L. fringillinarum</i> (3)	<i>H. coatneyi</i> (1)	<i>P. relictum</i> (1)	<i>T. avium</i> (3)
Fringillidae		<i>L. majoris</i> (5)		<i>P. vaughani</i> (1)	
	White-crowned Sparrow (30)	<i>L. fringillinarum</i> (9)	<i>H. coatneyi</i> (1)	<i>P. vaughani</i> (3)	<i>T. avium</i> (6)
		<i>L. majoris</i> (12)			
	Vesper Sparrow	<i>L. fringillinarum</i>			<i>T. avium</i>
	Pine Siskin		<i>H. magnus</i>		
Hirundinidae	Tree Swallow (5)	<i>L. fringillinarum</i> (1)			<i>T. avium</i> (2)
		<i>L. majoris</i> (3)			
	Violet-green Swallow	<i>L. fringillinarum</i>	<i>H. hirundinis</i>		
Icteridae	Brown-headed Cowbird		<i>H. quiscalus</i>		
Parulidae	MacGillivray's Warbler (4)	<i>L. fringillinarum</i> (1)			<i>T. avium</i> (1)
	Orange-crowned Warbler (7)			<i>P. vaughani</i> (1)	<i>T. avium</i> (1)
	Wilson Warbler (3)				<i>T. avium</i> (2)
	Yellow Warbler (11)	<i>L. fringillinarum</i> (3)	<i>H. coatneyi</i> (1)	<i>P. relictum</i> (1)	<i>T. avium</i> (4)
Picidae		<i>L. majoris</i> (4)		<i>P. vaughani</i> (1)	
	Red-naped Sapsucker (10)		<i>H. magnus</i> (1)		<i>T. avium</i> (1)
Turdidae	American Robin (10)	<i>L. dubreutili</i> (4)	<i>H. attenuatus</i> (1)	<i>P. matutinum</i> (1)	<i>T. avium</i> (2)
		<i>L. majoris</i> (4)	<i>H. fallisi</i> (4)	<i>P. vaughani</i> (1)	
			<i>H. minutus</i> (4)		
Tyrannidae	Hermit Thrush	<i>L. dubreutili</i>	<i>H. neseri</i> (1)		<i>T. avium</i>
	Mountain Bluebird (2)	<i>L. majoris</i> (1)	<i>H. fallisi</i>		
	Swainson's Thrush			<i>P. hexamerium</i>	
Vireonidae	Willow Flycatcher (6)	unknown <i>L. spp.</i> (2)	<i>H. tyranni</i>		<i>T. avium</i> (3)
	Western Wood-Pewee (2)				<i>T. avium</i> (1)
	Warbling Vireo (2)	<i>L. majoris</i> (1)	<i>H. vireonis</i> (1)		<i>T. avium</i> (2)

Table 2.2 Estimates of bird abundance were most strongly affected by distance from the observer. The degrees of freedom of each fixed effect and samples sizes of each bird species are shown in parentheses below the variable and next to the species name, respectively. Significant results ($p < 0.05$) are in bold.

family	species	site (1, 1)		habitat type (1, 2)		distance (1, 3)		site x habitat type (1, 2)	
		χ^2	p	χ^2	p	χ^2	p	χ^2	p
Cardinalidae	Lazuli Bunting (304)	10.24	0.0014	2.54	0.2804	12.93	0.0048	6.48	0.0391
	Fox Sparrow (284)	12.25	0.0005	8.12	0.0173	21.01	0.0001	5.13	0.0770
Emberizidae	Gray-headed Junco (312)	2.56	0.1093	8.52	0.0141	12.87	0.0049	5.76	0.0562
	Green-tailed Towhee (312)	7.60	0.0058	8.21	0.0165	8.37	0.0390	9.51	0.0086
	Lincoln's Sparrow (317)	4.70	0.0301	1.05	0.5920	29.55	<0.0001	1.90	0.3861
	Mountain White-crowned Sparrow (331)	16.09	<0.0001	13.48	0.0012	26.73	<0.0001	5.17	0.0754
Fringillidae	Pine Siskin (311)	0.17	0.6804	2.13	0.3442	19.16	0.0003	2.53	0.2827
Hirundinidae	Tree Swallow (312)	0.89	0.3462	0.43	0.8047	6.83	0.0775	3.91	0.1418
	Violet-green Swallow (306)	0.40	0.5264	0.39	0.8211	11.33	0.0100	1.81	0.4050
Icteridae	Brown-headed Cowbird (327)	0.09	0.7656	10.42	0.0055	9.83	0.0200	0.21	0.9000
Paridae	Red-winged Blackbird (302)	1.39	0.2388	3.06	0.2166	4.86	0.1821	5.08	0.0790
	Mountain Chickadee (310)	1.17	0.2795	5.31	0.0704	9.85	0.0199	6.50	0.0389
Parulidae	Audubon's Warbler (328)	0.24	0.6236	12.27	0.0022	18.34	0.0004	3.88	0.1435
	MacGillivray's Warbler (312)	4.20	0.0404	3.15	0.2073	15.21	0.0016	0.60	0.7421
	Wilson's Warbler (309)	1.24	0.2653	10.28	0.0059	13.01	0.0046	1.43	0.4889
	Yellow Warbler (324)	4.46	0.0347	11.71	0.0029	27.27	<0.0001	7.72	0.0211
Picidae	Hairy Woodpecker (318)	4.48	0.0342	3.73	0.1545	6.98	0.0727	3.43	0.1803
	Red-naped Sapsucker (317)	1.60	0.2057	2.28	0.3200	13.64	0.0034	2.26	0.3229
Regulidae	Red-shafted Flicker (317)	0.00	0.9684	0.49	0.7834	19.13	0.0003	0.73	0.6959
	Ruby-crowned Kinglet (304)	1.25	0.2637	0.16	0.9213	12.42	0.0061	3.21	0.0709
Trochilidae	Broad-tailed Hummingbird (306)	0.17	0.6758	9.68	0.0072	23.72	<0.0001	1.06	0.5884
Troglodytidae	House Wren (315)	0.28	0.5935	9.85	0.0073	17.95	0.0005	0.87	0.6471
	American Robin (335)	10.11	0.0015	0.38	0.8286	26.24	<0.0001	2.55	0.2794
Turdidae	Dusky Flycatcher (308)	0.69	0.4075	0.82	0.6647	11.26	0.0104	0.19	0.9109
Tyrannidae	Western Wood-pewee (316)	0.10	0.7540	16.94	0.0002	51.89	<0.0001	3.32	0.1903
	Warbling Vireo (317)	0.07	0.7969	2.92	0.2327	29.01	<0.0001	6.61	0.0368

Table 2.3 Estimates of bird species abundance differed primarily between forest and non-forest habitat types, as reflected by Bonferroni post-hoc test results. The degrees of freedom and sample sizes of each bird species are shown in parentheses below the comparison and next to the species name, respectively. Significant results (adjusted $p < 0.0167$) are in bold.

family	species	forest: meadow (1, 1)		forest: willow (1, 1)		meadow: willow (1, 1)	
		χ^2	p	χ^2	p	χ^2	p
Emberizidae	Fox Sparrow (284)	3.20	0.0738	19.02	<0.0001	2.98	0.0842
	Gray-headed Junco (312)	9.49	0.0021	15.98	<0.0001	4.17	0.0411
	Green-tailed Towhee (312)	15.06	0.0001	2.12	0.1450	5.28	0.0216
	Lincoln's Sparrow (317)	0.11	0.7352	1.28	0.2582	0.79	0.37
Fringillidae	Mountain White-crowned Sparrow (331)	16.72	<0.0001	9.31	0.0023	1.17	0.2801
	Lazuli Bunting (304)	2.90	0.0887	1.18	0.2765	0.25	0.6193
	Pine Siskin (311)	1.77	0.1828	0.33	0.5647	0.96	0.3284
Hirundinidae	Tree Swallow (312)	0.22	0.6390	0.20	0.6511	0.53	0.4666
	Violet-green Swallow (306)	0.31	0.5780	0.00	0.9791	0.20	0.6525
Icteridae	Brown-headed Cowbird (327)	7.25	0.0071	14.59	0.0001	0.09	0.7671
Paridae	Red-winged Blackbird (302)	3.31	0.0688	0.58	0.4472	1.29	0.2567
	Mountain Chickadee (310)	7.51	0.0061	10.42	<0.0001	0.07	0.7907
Parulidae	Audubon's Warbler (328)	26.07	<0.0001	24.09	<0.0001	0.14	0.7088
	MacGillivray's Warbler 312)	2.59	0.1076	1.28	0.2582	0.79	0.3734
	Wilson's Warbler (309)	7.61	0.0058	32.56	<0.0001	7.28	0.0070
	Yellow Warbler (324)	7.19	0.0073	15.81	<0.0001	6.32	0.0119
Picidae	Hairy Woodpecker (318)	1.33	0.2482	4.18	0.0410	4.01	0.0452
	Red-naped Sapsucker (317)	2.43	0.1192	0.55	0.4569	0.27	0.6064
Regulidae	Red-shafted Flicker (317)	0.10	0.7499	0.45	0.5035	0.16	0.6860
	Ruby-crowned Kinglet (304)	0.07	0.7854	0.02	0.8939	0.21	0.6504
Trochilidae	Broad-tailed Hummingbird (306)	9.02	0.0027	8.13	0.0044	0.00	0.9648
Troglodytidae	House Wren (315)	14.44	0.0001	10.04	0.0015	2.92	0.0873
Turdidae	American Robin (335)	0.20	0.6530	0.08	0.7814	0.30	0.5835
Tyrannidae	Dusky Flycatcher (308)	0.42	0.5147	0.76	0.3846	0.18	0.6727
	Western Wood-pewee (316)	10.71	0.0011	7.38	0.0066	0.04	0.8415
Vireonidae	Warbling Vireo (317)	3.22	0.0685	1.44	0.2302	0.12	0.7312

Table 2.4 Estimated abundance of biting dipteran species were affected most by time of season, or sampling session, with little effect of site on abundance. The interaction that had the largest impact on dipteran abundance was between the effects of site and sampling session. The degrees of freedom of each fixed effect as well as sample sizes for each bird species are shown in the parentheses below the variable and next to the species name, respectively. Significant results ($p < 0.05$) are in bold.

family	species	site (1, 1)		session (1, 3)		habitat (1, 2)		site x habitat (1, 3)		site x session (1, 6)		session x habitat (1, 2)	
		X ²	p	X ²	p	X ²	p	X ²	p	X ²	p	X ²	p
Simuliidae	<i>Greiniera denaria</i> (55)	0.49	0.4852	26.61	<0.0001	0.96	0.6191	0.98	0.8072	5.11	0.5302	3.93	0.1405
	<i>Helodon onchodactylus</i> cpx (50)	0.13	0.7210	18.72	0.0003	0.37	0.8330	0.29	0.9623	3.78	0.1508	1.31	0.9709
	<i>Metacnephia jeanae</i> (63)	1.20	0.2726	28.74	<0.0001	4.35	0.1134	8.32	0.0399	7.42	0.2836	0.41	0.8149
	<i>Simulium arcticum</i> cpx (89)	1.82	0.1778	34.19	<0.0001	1.20	0.5486	2.54	0.4682	2.59	0.8580	2.12	0.3469
	<i>Simulium canonicolum</i> (79)	1.48	0.2233	9.03	0.0290	3.85	0.1462	7.76	0.0513	6.06	0.4163	5.57	0.0617
	<i>Simulium exulatum</i> / <i>pilosum</i> (84)	0.13	0.7178	26.16	<0.0001	6.04	0.0487	6.32	0.9690	6.95	0.3255	1.97	0.3728
	<i>Simulium irritatum</i> / <i>venustum</i> (61)	0.99	0.3191	34.54	<0.0001	6.58	0.0373	1.87	0.6006	12.01	0.0617	2.05	0.3587
	<i>Simulium silvestre</i> / <i>craigii</i> (97)	0.01	0.9112	22.68	<0.0001	0.11	0.9450	3.75	0.2894	7.71	0.2598	0.06	0.9691
	<i>Simulium vittatum</i> cpx (51)	0.17	0.6778	26.66	<0.0001	13.61	0.0011	2.31	0.5102	28.26	<0.0001	2.77	0.25
	<i>Culiseta alaskaensis</i> (49)	9.41	0.0022	29.55	<0.0001	20.09	<0.0001	24.70	<0.0001	32.28	<0.0001	9.33	0.0094
Culicidae	<i>Culiseta impatiens</i> (54)	0.02	0.8854	14.95	0.0019	8.12	0.0172	1.93	0.5869	16.60	0.0109	5.08	0.0789
	<i>Ochlerotatus cataphylla</i> (118)	1.04	0.3801	793.29	<0.0001	17.90	0.0001	118.53	<0.0001	34.81	<0.0001	1.94	0.3785
	<i>Ochlerotatus communis</i> (64)	1.08	0.2994	16.80	0.0008	19.87	<0.0001	7.52	0.0569	9.96	0.1262	2.92	0.2326
	<i>Ochlerotatus hexodontus</i> (60)	0.07	0.7913	43.69	<0.0001	7.00	0.0301	1.41	0.7028	21.60	0.0014	2.68	0.2624
	<i>Ochlerotatus impicatus</i> (121)	0.13	0.7178	23.43	<0.0001	3.24	0.1977	9.54	0.0229	2.94	0.8162	0.78	0.6788
	<i>Ochlerotatus incidens</i> (52)	1.03	0.3097	10.58	0.0142	13.83	0.0010	2.49	0.4770	22.46	0.0310	0.91	0.6341
	<i>Ochlerotatus pullatus</i> (61)	0.54	0.4642	12.72	0.0053	7.77	0.0206	3.64	0.3025	12.72	0.0476	0.85	0.654
	<i>Ochlerotatus punctor</i> (50)	0.02	0.9006	4.33	0.2280	3.53	0.1709	6.26	0.0995	7.60	0.2690	2.47	0.2901

Table 2.5 Estimates of dipteran species abundance differed predominantly between sampling sessions one and three (May 18th to June 4th vs. June 25th to July 14th), as reflected by the Bonferroni post-hoc test results. The degrees of freedom and sample sizes of each bird species are located in parentheses below the comparison and next to the species name, respectively. Significant results (adjusted $p < 0.0083$) are in bold.

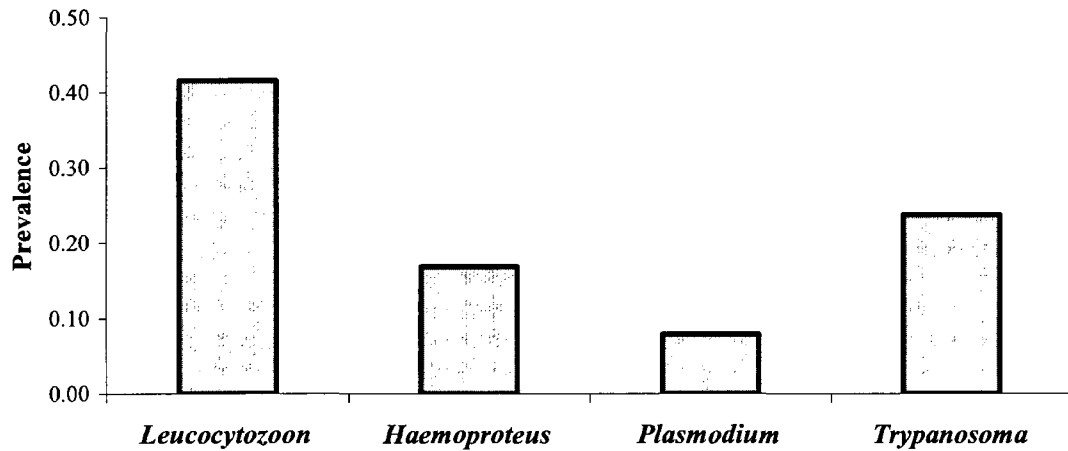
family	species	1 to 2		1 to 3		1 to 4		2 to 3		2 to 4		3 to 4	
		χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Simuliidae	<i>Greiniera denaria</i> (55)	9.52	0.0020	3.73	0.0533	3.73	0.0533	23.70	<0.0001	23.70	<0.0001	0.00	1.0000
	<i>Helodon onchodactylus</i> cpx (50)	0.00	0.9468	13.41	0.0003	0.01	0.9340	15.48	<0.0001	0.00	0.9858	15.10	0.0001
	<i>Metacnephia jeanae</i> (63)	3.00	0.0832	27.04	<0.0001	0.05	0.8277	12.53	0.0004	2.40	0.1214	25.95	<0.0001
	<i>Simulium arcticum</i> cpx (89)	0.02	0.8977	24.81	<0.0001	1.96	0.1616	24.44	<0.0001	1.62	0.2029	22.63	<0.0001
	<i>Simulium canonicolum</i> (79)	0.73	0.3936	3.98	0.0462	8.19	0.0042	1.17	0.2803	3.66	0.0557	0.79	0.3749
	<i>Simulium exultatum</i> / <i>pilosum</i> (84)	0.03	0.8555	4.89	0.0271	17.28	<0.0001	4.22	0.0399	16.12	<0.0001	3.27	0.0707
	<i>Simulium irritatum</i> / <i>venustum</i> (61)	0.00	0.9723	30.36	<0.0001	12.33	0.0004	31.16	<0.0001	12.47	0.0004	3.80	0.0511
	<i>Simulium silvestre</i> / <i>craigi</i> (97)	0.11	0.7376	16.91	<0.0001	7.69	0.0056	15.78	<0.0001	6.48	0.0109	3.44	0.0635
	<i>Simulium vittatum</i> cpx (51)	0.37	0.5456	26.36	<0.0001	0.39	0.5328	21.13	<0.0001	0.00	0.9768	20.19	<0.0001
Culicidae	<i>Culiseta alaskaensis</i> (49)	19.44	<0.0001	29.26	<0.0001	29.26	<0.0001	0.97	0.3247	0.97	0.3247	0.00	1.0000
	<i>Culiseta impatiens</i> (54)	6.93	0.0085	13.49	0.0002	10.81	0.0010	0.99	0.3208	0.39	0.5309	0.13	0.7144
	<i>Ochlerotatus cataphylla</i> (118)	54.46	<0.0001	42.70	<0.0001	5.54	0.0186	46.99	<0.0001	226.66	<0.0001	149.53	<0.0001
	<i>Ochlerotatus communis</i> (64)	5.31	0.0213	12.39	0.0004	0.82	0.3653	3.20	0.0738	3.50	0.0613	12.50	0.0004
	<i>Ochlerotatus hexodontus</i> (60)	44.93	<0.0001	3.29	0.0696	0.00	1.0000	24.81	<0.0001	44.93	<0.0001	3.29	0.0696
	<i>Ochlerotatus implicatus</i> (121)	14.39	0.0001	10.25	0.0014	0.38	0.3341	0.28	0.5956	15.34	<0.0001	9.73	0.0018
	<i>Ochlerotatus incidens</i> (52)	0.93	0.3341	9.60	0.0019	0.13	0.7150	4.81	0.0283	0.35	0.5540	7.42	0.0064
	<i>Ochlerotatus pullatus</i> (61)	5.50	0.0190	11.43	0.0007	0.53	0.4653	0.83	0.3634	2.58	0.1086	6.70	0.0096
	<i>Ochlerotatus punctor</i> (50)	1.80	0.1801	3.56	0.5910	0.13	0.7154	0.31	0.5783	0.94	0.3320	2.30	0.1293

Table 2.6 Estimates of mosquito species abundance differed primarily between forest and non-forest habitat types, as reflected by Bonferroni post-hoc test results. The degrees of freedom and sample sizes of each bird species are shown in parentheses below the comparison and next to the species name, respectively. Significant results (adjusted $p < 0.0167$) are in bold.

species	forest: meadow		forest: willow		meadow: willow	
	X^2	p	X^2	p	X^2	p
<i>Culiseta alaskaensis</i> (49)	14.47	0.0001	21.78	<0.0001	0.73	0.3937
<i>Culiseta impatiens</i> (54)	5.38	0.0204	7.10	0.0077	0.17	0.6786
<i>Ochlerotatus cataphylla</i> (118)	7.04	0.0080	7.73	0.0054	0.00	0.9806
<i>Ochlerotatus communis</i> (64)	19.91	<0.0001	11.61	0.0007	1.97	0.1600
<i>Ochlerotatus hexodontus</i> (60)	24.51	<0.0001	1.64	0.2006	25.61	<0.0001
<i>Ochlerotatus implicatus</i> (121)	2.73	0.0983	1.95	0.1627	0.12	0.7528
<i>Ochlerotatus incidens</i> (52)	12.66	0.0004	10.44	0.0012	0.10	0.7519
<i>Ochlerotatus pullatus</i> (61)	6.36	0.0017	5.52	0.0188	0.07	0.7894
<i>Ochlerotatus punctor</i> (50)	3.22	0.0726	2.14	0.1436	0.12	0.7261

Figure 2.1 A For all species except White-crowned sparrows (n = 102), prevalence was highest for *Leucocytozoon* spp. infections, lowest for infections with *Haemoproteus* spp. and *Plasmodium* spp., and intermediate for *Trypanosoma* spp. infections ($\chi^2_{1,3} = 27.37$, n = 102, p < 0.05). **B** Prevalence of hemosporidians for Mountain White-crowned Sparrows (n = 222) indicate that sparrows have higher prevalence of *Leucocytozoon* spp. and lower prevalence of *Haemoproteus* spp. and *Trypanosoma* spp. than other bird species ($\chi^2_{1,3} = 19.86$, n = 304, p < 0.05). If bars are represented by the same color, each bar represents a single parasite taxon (*H. coatneyi* and *T. avium*).

A



B

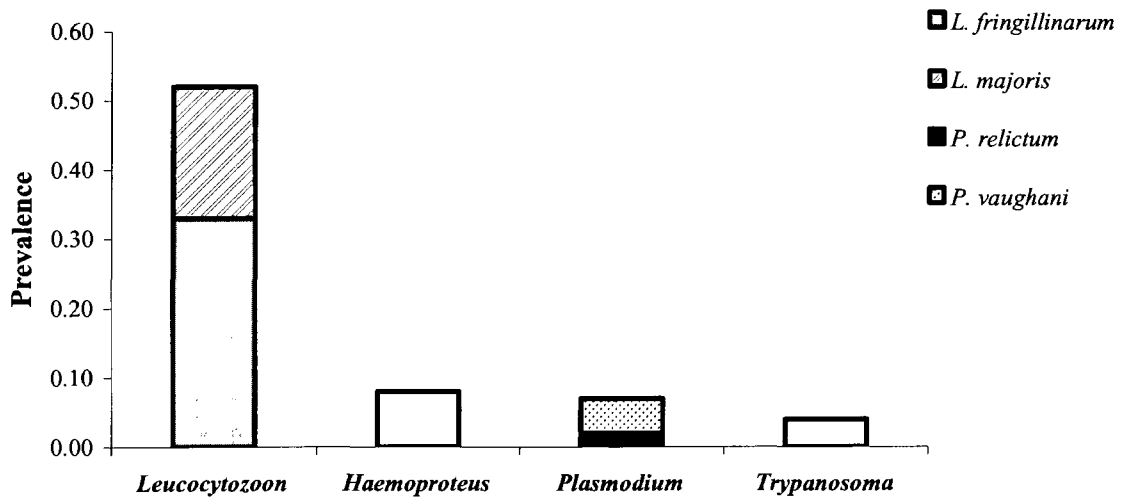


Figure 2.2 Estimated abundance of White-crowned Sparrows in each habitat type from the GLM analysis indicate that White-crowned Sparrows have higher abundances in meadow (Bonferroni Adjusted Post-Hoc Test: $\chi^2_{1,1} = 16.72$, $n = 331$, $p < 0.0001$) and willow habitats (Bonferroni Adjusted Post-Hoc Test: $\chi^2_{1,1} = 9.31$, $n = 331$, $p = 0.0023$) than forest habitats. Brackets indicate significant differences between groups at a Bonferroni adjusted significance level of $p < 0.0167$, and bars around the mean represent standard errors.

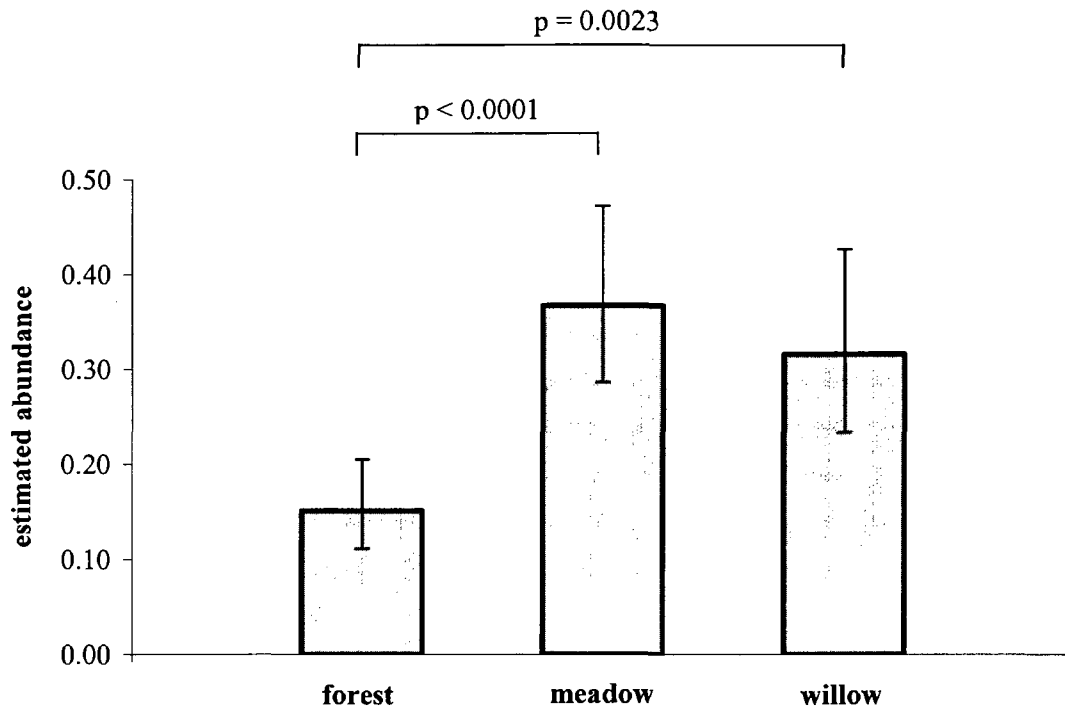
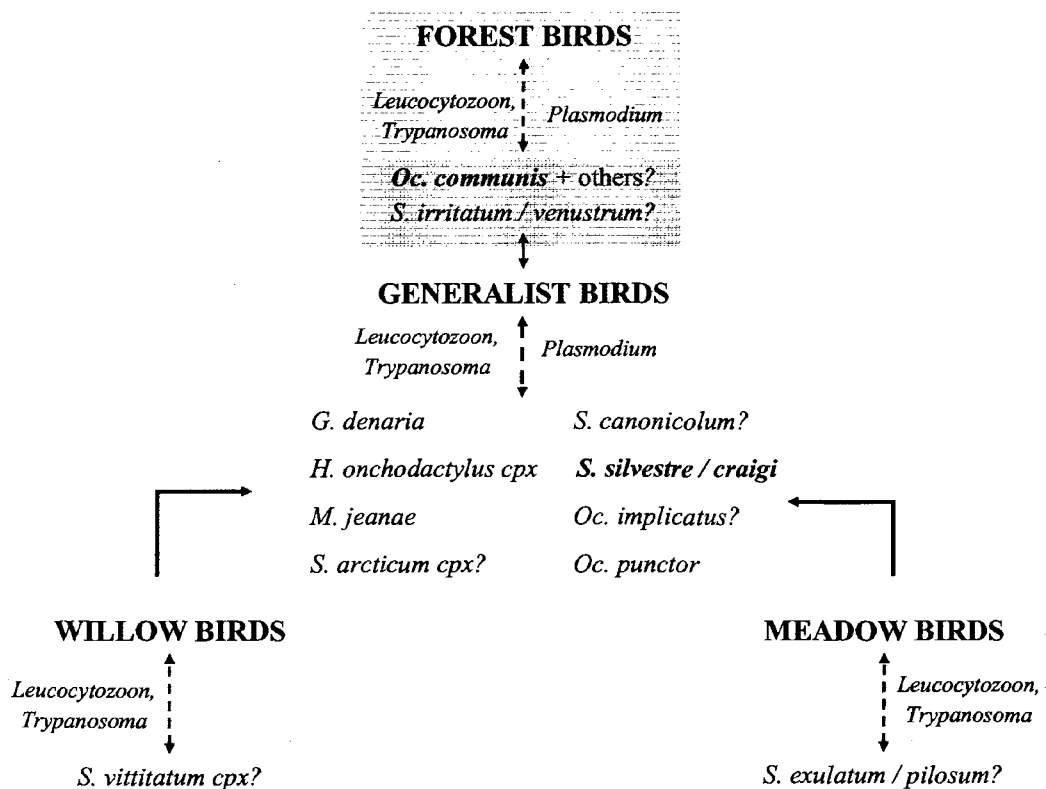


Figure 2.3 Transmission of hemosporidian parasites within each habitat type (focus) occurs through the interaction of the bird and dipteran species associated with each habitat type based on estimated abundances (dark gray). Transmission among foci is most likely through bird and dipteran species that are equally abundant across all habitat types (light gray). Bolded dipteran species represent species that are competent or have been linked to hemosporidian transmission in the literature. Question marks next to dipteran species indicate tentative suggestions on other possible vector species in this system based on their overall representation in the dipteran community, their abundances by habitat type, and their abundances across the summer season.



Chapter III

Molecular analyses on host-seeking black flies (Diptera: Simuliidae) reveal a diverse community of *Leucocytozoon* (Apicomplexa: Hemosporida) parasites

Introduction

Parasites belonging to the genus *Leucocytozoon* are avian hematozoan parasites that are blood-borne and vector-transmitted by biting dipterans; they are related to parasites in the genera *Plasmodium*, *Parahaemoproteus*, and *Haemoproteus*. All share a similar life cycle consisting of asexual reproduction and amplification in the vertebrate host, and sexual reproduction and asexual amplification in a dipteran vector.

Plasmodium is the only hematozoan genus that has blood-stage asexual reproduction.

The genus *Leucocytozoon* is a sister group to the other malaria parasite genera (Bensch et al. 2000, Perkins and Schall 2002, Waldenstrom et al. 2002, Martinsen et al. 2008b).

The genus *Leucocytozoon* is vectored by black flies (Diptera: Simuliidae) with a wide, global distribution, spanning from tropical to arctic regions (Valkiunas 2005a, Hellgren et al. 2007b). Although at least 35 morphologically defined species have been described to date (Valkiunas 2005a), molecular marker identification suggests the true diversity well exceeds the 35 species (Hellgren 2005, Valkiunas 2005a, Hellgren et al. 2007b). Further, several of the molecular lineages have demonstrated that the degree of host-specificity may vary; some lineages are found consistently only in one bird species while other lineages can develop successfully in a wide range of taxonomically-varied

hosts (Hellgren 2005, Hellgren et al. 2007b), and species of *Leucocytozoon* apparently can complete development across a large range of simuliid vectors (Desser and Yang 1973, Valkiunas 2005a). Because both vertebrate host and dipteran vector are essential for the complete development of *Leucocytozoon* spp. (Valkiunas 2005a), the distribution of parasites can be influenced by all of the following: active vector preferences for certain avian hosts, the ability of the parasite to infect a vertebrate host, or other factors such as climatic variables and microhabitat requirements that may place vertebrate hosts and vectors into frequent contact with each other (Hellgren et al. 2008).

We sampled black flies and potential bird hosts from a variety of sites and habitats in an alpine ecosystem located in the Colorado Rocky Mountains. Here we first outline the diversity of the potential vector community for *Leucocytozoon* at these sites. We then describe the parasite distribution of *Leucocytozoon* lineages from the abundant, ornithophilic black fly species. Finally, we explore whether the distribution of *Leucocytozoon* lineages is most influenced by avian host species, microhabitat, or time of season.

Materials and Methods

Field site description and study design

Black flies were collected from two field sites within the vicinity of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Gunnison County, Colorado, U.S.A between May 15th – August 12th 2007. One field site was located approximately one mile south valley from RMBL (UTM: N 4312713 E 327700) and the other field site located in the adjacent, Washington Gulch valley (UTM: N 4311531 E 325807). Elevation ranges between 2902 m - 2987 m asl. Both field sites are a mosaic of alpine meadows, forest

stands, and riparian willow thickets. The latter are dominated by bog birch (*Betula glandulosa*), mountain alder (*Alnus tenuifolia*), and several species of willow (*Salix* spp.). Forest habitats are composed of conifer (*Picea engelmannii*) and aspen (*Populus tremuloides*) stands at higher elevations; meadow habitats consist of a diversity of herbaceous vegetation across a range of elevations.

To ensure a representative selection of the bird and black fly communities, we sampled across a variety of common habitats in each of our two field sites from May 15th – August 12th, 2005 and 2007. Each field site was first stratified by the following broad habitat types: willow, alpine meadow, and forest habitat types. We then took a random sample ($n = 2$) of the habitat types from each field site. Thus, each field site had a total of six sampled patches with two patches per habitat type.

Black fly collection methods

We collected black flies from CO₂ baited Center for Disease Control (CDC) miniature light traps (John W. Hock Company, No. 512 fine mesh collection cups, Gainesville, FL, USA) (Service 1976). CDC traps were set and checked every 24 hours. Due to the possibility of trap failure, traps were paired approximately 50 m from each other within each sampled patch. We trapped each habitat patch for two consecutive nights over an interval of eight days. After each two-day trapping session, traps were pulled and rotated to the other field site to minimize any potential effects of season or weather on trap success. Sampling occurred from May 19th – May 26th, June 8th – June 17th, July 1st – July 12th, and July 22nd – July 31st in 2007.

Within two hours of capture in the field, all biting dipterans were placed into a garbage bag and exposed to cotton soaked in triethylamine (Fisher Scientific, Amber

Glass, No. BP616-500, Pittsburgh, PA, USA) for five minutes in a well-ventilated area. Once flies were immobilized, we separated the black flies from other biting dipterans and stored them immediately in 95% ethanol for future identification and parasite DNA analyses. Female black flies were identified to species or species complex based on structural characters (Adler et al. 2004). Identifications were facilitated by genital preparations of selected specimens. Representative specimens have been deposited in the Clemson University Arthropod Collection, Clemson, South Carolina.

Bird collection methods

During the summer of 2005 (June 18th – July 16th, July 29th – August 12th), we captured birds between 06:00 and 12:00 each day in millet-baited, single- and double-cell (18 cm × 18cm × 18 cm per cell), galvanized wire Potter traps as well as nylon mist nets (Avinet, 38mm mesh, 6 m wide, No. BH06, Dryden, NY, USA). All birds were marked with a uniquely numbered metal U.S. Fish and Wildlife Service band (Bird Banding Laboratory, Patuxent, MD, USA). Upon capture, we sexed each bird, collected standard morphometric measures, and took blood samples from the brachial vein with a sterile 26-gauge hypodermic needle (Fisher Scientific Precision Glide, No. 14-826-15, Pittsburgh, PA, USA) to determine infection status. Blood samples were collected in 70 µL heparinized microcapillary tubes (Fisher Scientific, No. 22-362-566, Pittsburgh, PA, USA) and temporarily stored in a cooler with ice. A small portion of the blood sampled from each bird was used to prepare a microscope slide (Fisher Scientific, No. 12-542-5, Pittsburgh, PA, USA), which was air-dried and stained with a Fisher Hema 3 Stat packTM (Fisher Scientific, No.22-122911, Pittsburgh, PA, USA). We determined infection status by identifying hemosporidian blood parasites to the morphological species level

(Valkiunas 2005a). Slides were scanned for blood parasites for a total of 15 min. Further, a small portion of blood was used to make blood dots on sterile filter paper (Fisher Scientific, Fisherbrand plain circles, PS grade, No. 09-801C, Pittsburgh, PA, USA) for future molecular analysis.

DNA extraction and amplification methods

In the lab, we screened and sequenced hemosporidian parasites from our most abundant ornithophilic black fly species and those birds that were identified as having single infections with *Leucocytozoon* species. We minced the selected black flies and combined them into pools of five individuals. We took a small sample of dried blood dot from each bird that scored positive for single infections with *Leucocytozoon* spp. We then extracted DNA from each using the DNeasy Animal Tissue extraction kit from QIAGEN (Valencia, CA, USA) per the manufacturer's protocol. We conducted a polymerase chain reaction (PCR) on the extracted DNA using primers "Co1midF" and "Co1inR" (Perkins et al., 2007) that amplify a 498 basepair portion of the parasites' mitochondrial gene cytochrome oxidase I (*coxI*). The PCR was set up using PureTaq Ready-to-Go PCR beads (GE Biosciences) in 25 µl reactions using 1 mM of each primer and 2 µl of DNA extracted from the pooled black fly samples. PCR conditions were an initial denaturation of 4 min at 94°C, followed by 35 cycles of 90°C for 30 sec, 48°C for 15 sec, and 68°C for 45 sec, with a final extension at 68°C for 10 min.

Negative and positive controls were always included in PCR reactions to detect possible contamination. Amplified DNA was visualized on a 1% agarose gel with CyberSafe (10%; Invitrogen, Carlsbad, CA; 10 µl per 100 µl of gel), and positive amplifications were cleaned with the AMPure reagent (Agencourt, Beverly,

Massachusetts) and sequenced in both directions using BigDye v. 3.1 (Applied Biosystems, Foster City, California) with the same primers that were used in amplification. We re-amplified and re-sequenced any samples that revealed ambiguous base calls. If base calls continued to be ambiguous, they were discarded from the study, because this suggests a mixed-species infection. Sequences were cleaned with CleanSeq (Agencourt, Beverly, Massachusetts) and run on an ABI 3730xl automated sequencer. Sequences were edited in Sequencher (GeneCodes, Madison, Wisconsin).

Phylogenetic analyses

All clean sequences generated for the *col* gene were incorporated in phylogenetic analyses. We included *Plasmodium vivax* (GenBank Accession number AY598140) as our outgroup taxon to root the tree, because it is closely related to, but not contained within, the *Leucocytozoon* parasite clade (Perkins and Schall 2002). We did not use *Theileria* or *Toxoplasma* to root our phylogeny (although parasites of these genera have previously been used for this purpose, Escalante et al. 1998, Perkins and Schall 2002), because they are very distantly related and resulting alignments are problematic (Martinsen et al. 2008b). Additional taxa of hemosporidian parasites for which *col* sequences were available on GenBank were also included in the analysis. These consisted of the following species: *Plasmodium floridense* (GenBank accession number NC_00961), *Plasmodium mexicanum* (GenBank accession number NC_009960), *Plasmodium juxtannucleare* (GenBank accession number NC_008279), *Plasmodium gallinaceum* (GenBank accession number NC_008288), *Parahaemoproteus* sp. “jb2.SEW514” (GenBank accession number AY733087), and *Parahaemoproteus* sp. “jb1.JA27” (GenBank accession number AY733086).

We conducted a maximum parsimony analysis using PAUP* v.4.0 (Sinauer, Sunderland, MA, USA) using equal weighting for all characters, 30 random addition sequences, and the “MulTrees” option, also performing a bootstrap analysis of 100 replicates of the full heuristic search to assess nodal support. We performed maximum likelihood (ML) analyses with RaxML software (Stamatakis 2006) as implemented on the Cyberinfrastructure for Phylogenetic Research portal (CIPRES; <http://www.phylo.org/news/RAxML>). Models and proportion of invariant sites were estimated by the program. Bootstrap support was assessed using an automatic cut-off by the program (Stamatakis et al. 2008). Once the tree was generated, we visually inspected the resulting topology to see if clades of parasite haplotypes corresponded to avian host or vector species, or environmentally shaped by time of season or habitat types avian hosts and black flies were sampled from on our field sites.

Results

Black fly community and avian prevalence

We collected a total of 2,951 black flies belonging to 18 different species of which 10 feed predominantly on birds (Table 1). We chose to sample a total of 800 black flies belonging to two ornithophilic species, *Simulium silvestre* / *S. craigi* and *Greeneira denaria* for the presence of *Leucocytozoon* DNA. *S. silvestre* / *S. craigi* was the most abundant black fly species on our field sites, based on the sheer number of individuals captured in the CO₂ baited light traps, and was abundant throughout most of the summer season. *G. denaria* was not as abundant as some of the other black fly species captured, however this species reached peak abundance earlier in the summer season and allowed us to determine if there were any effects of time of season on *Leucocytozoon* distribution

(CM unpublished data, see Chapter 2). Out of 160 pools of black flies, we obtained *Leucocytozoon* sequence data from 94 out of 145 pools (725 individuals) of *S. silvestre* / *S. craigi*, and 7 out of 15 pools (75 individuals) of *G. denaria*.

We captured, took blood samples, and determined the infection status of a total 130 birds from 23 species, including 28 White-crowned Sparrows (Table 2). Data generated from scoring microscope slides visually for parasites indicate that *Leucocytozoon* spp. (46%) infections had the highest prevalence among all bird species sampled, while *Haemoproteus* (14%) and *Plasmodium* (8%) had significantly lower prevalences (CM unpublished data, see Chapter 2). Because of the large number of birds hosting mixed infections, we only were able to obtain *coI* sequences from 10, singly infected bird hosts. For a small sample ($n = 2$) we were not able to amplify the *coI* gene at all.

Parasite distribution

Figure 1 presents the full phylogeny of parasite *coI* genes amplified from both black fly and avian hosts. Only nodes with bootstrap support values greater than or equal to 70% are included. We also obtained *Parahaemoproteus* sequences from two birds, a Lincoln Sparrow (*Melospiza lincolnii*) and a Warbling Vireo (*Vireo gilvus*), which clustered with existing *Parahaemoproteus* sequences found on GenBank. The *Leucocytozoon* spp. sequenced fell into three divergent clades. The first *Leucocytozoon* clade (Figure 1A), containing parasites sampled from both black fly species, is the most divergent of the clades. The other two *Leucocytozoon* clades are more closely related, demonstrating a large diversity of *Leucocytozoon* haplotypes. One clade (Figure 1B) contained related parasite sequences that amplified from *S. silvestre* / *S. craigi* and a

variety of avian hosts, suggesting that these sequences may be morphologically consistent with either “*Leucocytozoon majoris*” or “*Leucocytozoon fringillinarum*” (Valkiunas 2005a). Furthermore, three of the parasite sequences amplified from *S. silvestre* / *S. craigi* were identical to parasite sequences amplified from a Green-tailed Towhee (*Pipilo chlorurus*) and a Mountain White-crowned Sparrow (*Zonotrichia leucophrys oriantha*) visually identified as infected with *L. majoris*, and a Vesper Sparrow (*Pooecetes gramineus*) visually identified as infected with *L. fringillinarum*. These results suggest that this clade may include parasite strains that predominantly infect passerine birds. The third *Leucocytozoon* clade (Figure 1C) contained parasite sequences amplified from *S. silvestre* / *S. craigi* only.

Effects of habitat and time of season

We did not see strong evidence for any effects habitat on the topology of the resulting tree. The second *Leucocytozoon* clade (Figure 1B) has a total of 21 parasite haplotypes. Of these, 11 and eight strains were amplified from hosts captured in meadow and willow habitats, respectively. There were only two parasite strains obtained from hosts sampled from forest habitats. The other two *Leucocytozoon* clades (Figure 1A and 1C) appear to have a more even representation of habitats. The first clade (Figure 1A) has a total of 9 haplotypes of which three, four, and two strains were amplified from hosts captured in meadow, willow, and forested habitats, respectively. The third clade (Figure 1C) with 21 haplotypes has a higher representation of parasite strains from hosts found in forested habitat types (9 haplotypes), and five and seven strains from hosts captured in meadow and willow habitats, respectively. Finally, we saw no definitive trends for the effects of time of season on distribution of *Leucocytozoon* haplotypes. Only one clade

(Figure 1C) was potentially constrained by time of season, with all haplotypes sampled from hosts captured during the month of July. The other two clades contained parasite haplotypes sampled from black flies and birds captured across the entire summer season (June, July and August).

Discussion

To our knowledge this is the first study to amplify parasite DNA from non-bloodfed, host-seeking dipterans. Assuming one black fly in each positive pool was infected with *Leucocytozoon*, the minimum prevalence of *Leucocytozoon* in *S. silvestre* / *S. craigi* and *G. denaria* was 12.6% and 9.3%, respectively. In Algonquin, Ontario, 90% to 100% of ornithophilic black fly species had *Leucocytozoon* sporozoites present in their salivary glands (Bennett and Squires-Parsons 1992). Additionally, Hellgren et al. (2008) found 62% of blood-fed black flies (n = 38) were positive for *Leucocytozoon* spp. infections. However, the prevalence of infectious black flies infected with a particular species of *Leucocytozoon* may be much lower, because both of these studies examined multiple black fly and *Leucocytozoon* species (also see Chapter three). Thus, the prevalence of *Leucocytozoon* in both, *S. silvestre* / *S. craigi* and *G. denaria*, is probably higher than previously recognized in light of this research, and due to the possibility of having more than one individual in a pool positive for *Leucocytozoon*.

We amplified a large diversity of *Leucocytozoon* haplotypes from *S. silvestre* / *S. craigi*, which suggests *S. silvestre* / *S. craigi* is probably an important vector species for a multitude of *Leucocytozoon* spp. on our field sites. Studies have shown that *S. silvestre* / *S. craigi* feeds on a variety of avian hosts (i.e. White-throated Sparrow, Common Grackle, American Robin, and Blue Grouse), and has been implicated as a vector for a

number of *Leucocytozoon* spp: *L. fringillinarum*, *L. icteris*, *L. dubreuilii*, and *L. lovati* (Adler et al. 2004). *S. silvestre* / *S. craigi* is the most abundant ornithophilic black fly species on our field sites, based on the sheer number of individuals recovered from light traps, and its abundance was not restricted by habitat type. Further, *S. silvestre* / *S. craigi* reaches peak abundance during the time of season (June 25th – July 14th) when most bird species are hatching and feeding susceptible young of the year on our field sites (CM unpublished data, see Chapter 2).

Previous studies on field captured black flies amplified a diversity of *Leucocytozoon* DNA from bloodfed individuals (Hellgren et al. 2008). However, parasite DNA amplified from the abdomens of bloodfed individuals does not provide proof of vector competence of *S. silvestre* (see Chapter 3). Not all *Leucocytozoon* species ingested with a bloodmeal can develop infectious stages in the salivary glands of a given black fly species and successfully transmit to the next avian host. These results present only tentative links among *S. silvestre*, passerine birds, and potential transmission of *Leucocytozoon* spp. In fact, because black flies in our study were collected in CO₂ baited CDC light traps and were not blood-fed, most can be identified as host-seeking individuals. Thus, parasite sequences amplified from these individuals may potentially be generated from parasite transmission stages, and indicate that *S. silvestre* / *S. craigi* is a competent vector for these *Leucocytozoon* strains. This may also explain why *Leucocytozoon* is the most prevalent of hemosporidian parasites in the avian community on our field sites.

Additionally, identical matches between parasite sequences amplified during our study from *S. silvestre* / *S. craigi* and avian hosts indicate potential host-vector

associations in this system. We had identical matches between haplotypes amplified from *S. silvestre* / *S. craigi* and some of the avian hosts sampled in this study that were morphologically consistent with *L. fringillinarum* and *L. majoris* (Valkiunas 2005a). Identical black fly and avian parasite haplotypes were sampled from individuals captured in the same habitat (forest, meadow, or willow) types (Figure 1), suggesting there may be some avian host or habitat restrictions on parasite distributions. However, due to the large number of mixed infections, our sample size of parasite sequences from avian hosts is small, resulting in only a few identical matches between black fly and avian parasite haplotypes. More sampling is needed of singly infected avian hosts or the use of cloning techniques to separate out sequences from mixed infections to truly confirm this assertion.

The clade containing parasite haplotypes amplified from both black fly and avian host individuals (Figure 1B) may represent a clade of parasite haplotypes hosted by passerine birds that *S. silvestre* / *S. craigi* had once fed upon in meadow and willow habitats (Table 1). The other two clades (Figure 1A and 1C) may have parasite haplotypes from bird species that we did not sample, which may explain why parasite haplotypes amplified from our avian hosts did not match with parasite haplotypes in these two clades. The third, most distant clade (Figure 1A) may actually be an undescribed genus of malarial parasite (Martinsen et al. 2008a). The presence of parasite strains amplified from *G. denaria*, a black fly species that reaches peak abundance early in the summer season (CM unpublished data, see Chapter 2), further indicates that these parasite haplotypes may come from non-passerine bird hosts (such as resident species or bird species in different orders) we were unable to sample in the field. In conclusion, a

wider sampling of the avian and ornithophilic black fly communities is needed to achieve a concrete understanding of how avian hosts, black fly vectors, or environmental factors may shape the distribution of *Leucocytozoon* spp. on our field sites.

Table 3.1 gives a complete list of black fly species and number of individuals recovered in CO₂ baited light traps throughout the summer on our two field sites. Ornithophilic black fly species are in bold.

Black fly species	RMBL Count	WA Count
<i>Greniera denaria</i>	29	30
<i>Helodon onchodactylus</i> cpx	5	3
<i>Metacnephia jeanae</i>	70	47
<i>Prosimulium exigins</i>	194	65
<i>Prosimulium fulvum</i>	1	0
<i>Prosimulium hirtipes</i> group	22	43
<i>Prosimulium uinta</i>	0	1
<i>Simulium arcticum</i> cpx	339	175
<i>Simulium canonicolum</i>	91	31
<i>Simulium decorum</i>	0	2
<i>Simulium exulatum</i> / <i>pilosum</i>	112	97
<i>Simulium hunteri</i>	0	1
<i>Simulium irritatum</i> / <i>venustum</i>	25	34
<i>Simulium piperi</i>	19	20
<i>Simulium silvestre</i> / <i>craigi</i>	861	553
<i>Simulium vandalicum</i>	19	19
<i>Simulium vittitatum</i> cpx	6	7

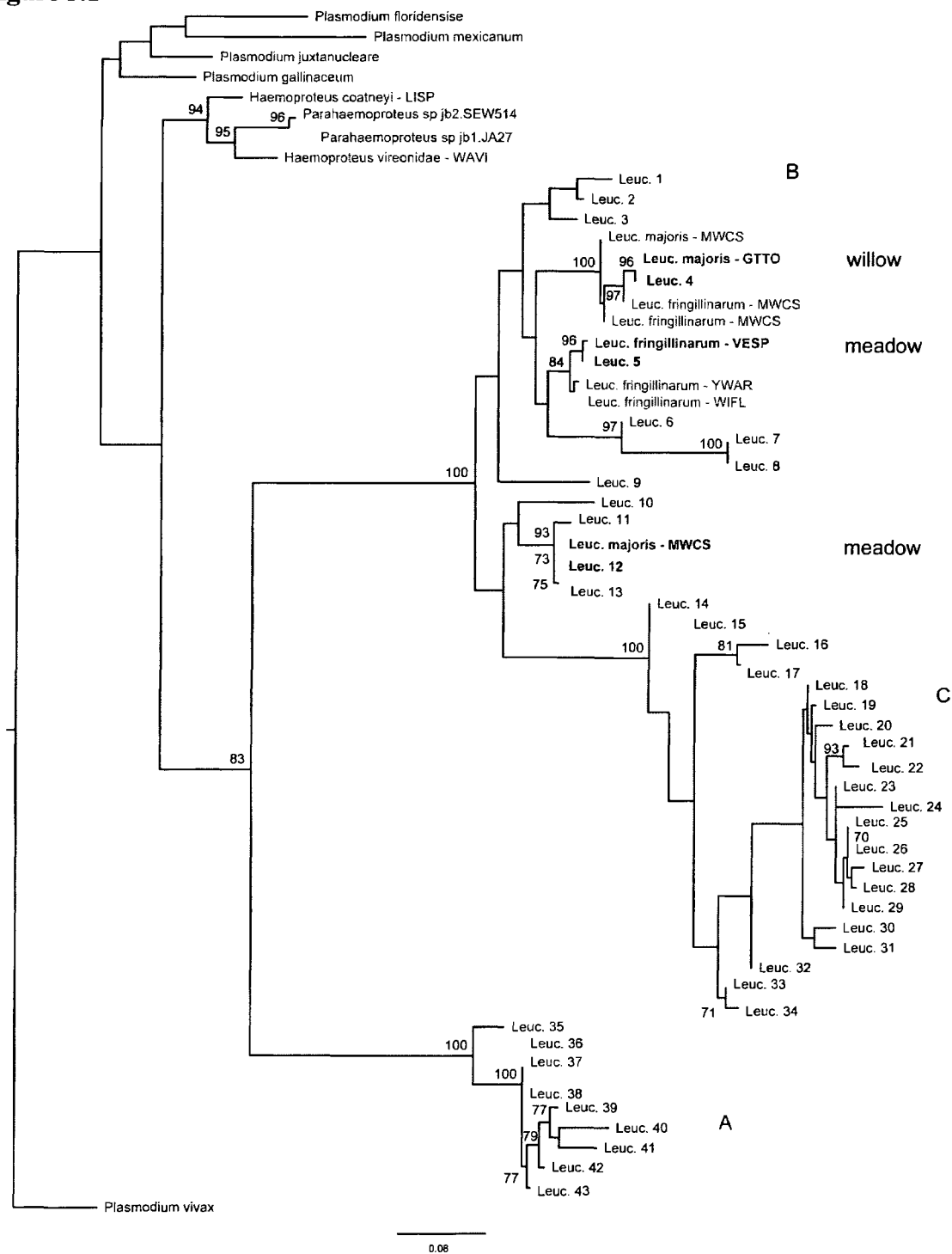
Table 3.2 List of all morphologically identified species of *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* spp. found in birds captured during summer 2005. Numbers in parentheses following common bird species name and parasite species name indicate number of microscope smears scored for that bird species and number of smears infected with that parasite species, respectively.

Bird Family	Bird Species	<i>Leucocytozoon</i>	<i>Haemoproteus</i>	<i>Plasmodium</i>
Emberizidae	Fox Sparrow (8)	<i>L. fringillinarum</i> (2)		
		<i>L. majoris</i> (3)		
	Green-tailed Towhee (2)	<i>L. fringillinarum</i> (1)		
		<i>L. majoris</i> (1)		
	Lincoln Sparrow (21)	<i>L. fringillinarum</i> (3)	<i>H. coatneyi</i> (1)	<i>P. relictum</i> (1)
		<i>L. majoris</i> (5)		<i>P. vaughani</i> (1)
	Mountain White-crowned Sparrow (30)	<i>L. fringillinarum</i> (9)	<i>H. coatneyi</i> (1)	<i>P. vaughani</i> (3)
		<i>L. majoris</i> (12)		
	Vesper Sparrow (1)	<i>L. fringillinarum</i> (1)		
Fringillidae	Pine Siskin (1)		<i>H. magnus</i> (1)	
Hirundinidae	Tree Swallow (5)	<i>L. fringillinarum</i> (1)		
		<i>L. majoris</i> (3)		
	Violet-green Swallow (1)	<i>L. fringillinarum</i> (1)	<i>H. hirundinis</i> (1)	
Icteridae	Brown-headed Cowbird (1)		<i>H. quisculus</i> (1)	

Bird Family	Bird Species	Leucocytozoon	Haemoproteus	Plasmodium
Parulidae	MacGillivray's Warbler (4)	<i>L. fringillinarum</i> (1)		
	Orange-crowned Warbler (7)			<i>P. vaughani</i> (1)
	Yellow Warbler (11)	<i>L. fringillinarum</i> (3) <i>L. majoris</i> (4)	<i>H. coatneyi</i> (1)	<i>P. relicum</i> (1) <i>P. vaughani</i> (1)
Picidae	Red-naped Sapsucker (10)		<i>H. magnus</i> (1)	
Turdidae	American Robin (10)	<i>L. dubreuilii</i> (4) <i>L. majoris</i> (4)	<i>H. attenuatus</i> (1) <i>H. fallisi</i> (4) <i>H. minutus</i> (4) <i>H. neseri</i> (1)	<i>P. matutinum</i> (1) <i>P. vaughani</i> (1)
	Hermit Thrush (1)	<i>L. dubreuilii</i> (1)	<i>H. fallisi</i> (1)	
	Mountain Bluebird (2)	<i>L. majoris</i> (1)		
	Swainson's Thrush (1)			<i>P. hexamerium</i> (1)
Tyrannidae	Willow Flycatcher (6)	unknown <i>L. spp.</i> (2)	<i>H. tyranni</i> (1)	
Vireonidae	Warbling Vireo (2)	<i>L. majoris</i> (1)	<i>H. vireonis</i> (1)	

Figure 3.1 *col* phylogeny of hemosporidian parasites from both black fly vector and avian host species suggests *Simulium silvestre* / *S. craigi* may vector a diversity of *Leucocytozoon* strains in our field sites. This phylogeny was determined by maximum likelihood analysis. Boot strap values above 70% are included in the tree topology and located next to the associated nodes. Parasite haplotypes were amplified from two black fly species, *Simulium silvestre* / *craigi* (**black**) and *Greeneira denaria* (**green**), and from 10 avian hosts (**red**). The species of avian host parasite haplotypes are denoted by the American Ornithologists' Union species shortcode (**GTTO** = Green-tailed Towhee, **LISP** = Lincoln's Sparrow, **MWCS** = Mountain White-crowned Sparrow, **WAVI** = Warbling Vireo, **WIFL** = Willow Flycatcher, and **YWAR** = Yellow Warbler). Full species names of *Leucocytozoon* haplotypes amplified from avian hosts were determined through morphological identification. Bolded haplotypes indicate an identical match between parasite DNA amplified from a black fly and avian host species, and the habitats we captured their hosts from are listed to the right of each pair. There are three divergent *Leucocytozoon* clades. The first clade **A** contains parasites sampled from both black fly species and is the most divergent of the clades, suggesting parasites in this clade may be from an undescribed genus of malarial parasite. The second clade **B** includes parasite DNA amplified from avian hosts and *S. silvestre* / *S. craigi*, suggesting this clade may comprise *Leucocytozoon* spp. that predominantly infect passerine birds living in willow and meadow habitats. The third clade **C** contains parasite DNA amplified only from *S. silvestre* / *S. craigi*, implying these *Leucocytozoon* parasites may infect bird hosts that we did not sample at our field sites.

Figure 3.1



Chapter IV

A transmission model for the ecology of an avian hemosporidian parasite in a temperate ecosystem

Introduction

Currently there are over 200 morphologically defined species of avian hemosporidian parasites of the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* (Apicomplexa: Haemosporida), which can have broad global distributions (Valkiunas 2005a). However, molecular analyses of parasite mitochondrial DNA suggest that there likely exist many more malarial species than are currently recognized (Ricklefs and Fallon 2002, Bensch et al. 2004, Hellgren et al. 2007a). While much empirical work has been done on the ecology of avian haemosporidian parasites and the effects these parasites have on host fitness (e.g. van Riper et al. 1986, Atkinson and van Riper III 1991, Valkiunas 2005a), our general knowledge across different groups of hemosporidians remains unevenly distributed, with most attention devoted toward human malaria and the genus *Plasmodium* (Valkiunas 2005a).

Avian hemosporidian infections have been characterized by severe pathology in the acute phase (Desser and Ryckman 1976, Atkinson and van Riper III 1991, Atkinson et al. 2001) and high population prevalence (Valkiunas 2005a). Although acutely infected young birds can succumb to hemosporidians, surviving adults typically carry chronic, sublethal infections. However, even birds infected with chronic hemosporidian

infections may experience reduced breeding success (Stjernman et al. 2004, Marzal et al. 2005, Tomas et al. 2007), body condition (Bonier et al. 2007), immunity (Millington et al. 2007), and survival (Sol et al. 2003, Marzal et al. 2008).

The density of parasite stages in the blood (parasitemia) changes dynamically throughout the course of an infection (Figure 1). Upon infection, a bird enters the acute phase of the infection, which is characterized by an initial spike in blood stage parasitemia; the acute phase ranges from one week to several months depending on the parasite species, vertebrate host, and environmental factors. After the acute phase of the infection ends, parasitemia decreases and birds enter the chronic phase of infection which also varies in duration. The chronic phase is characterized by low parasitemia, roughly 1-3 gametocytes per 10,000 red blood cells (Valkiunas 2005a). Upon exiting the chronic phase of infection, the bird then enters a latent stage of infection, when parasites disappear from the peripheral blood and persist in non-circulating tissues, such as the internal organs (Valkiunas 2005a).

Much of what we know about avian hemosporidian parasites comes from the Hawaiian Islands where the recently introduced *P. relictum*, in conjunction with habitat degradation, invasive mosquito vectors, and avian pox, has lead to the endangerment or extinction of many endemic bird species (van Riper et al. 1986, Atkinson et al. 1995, van Riper and Scott 2001, Kilpatrick et al. 2006, Aruch et al. 2007). However, while we have a reasonable understanding of transmission ecology of invasive avian malaria in Hawaii, we know much less about the ecology of endemic hemosporidian infections (especially for the parasite genera *Leucocytozoon* and *Haemoproteus*) in continental bird species. Such mainland systems, characterized by greater parasite diversity and longer

evolutionary history of host-parasite interactions, are the rule rather than the exception. Additionally, in light of the abundance of empirical work done on certain hemosporidian systems, there is a surprising lack of theoretical work (either conceptual or analytical) describing transmission dynamics for any of these parasites (exceptions are Beaudoin et al. 1971, Allan and Mahrt 1989).

Here we develop a model for hemosporidian transmission in a songbird population breeding in a temperate ecosystem, in which different conditions prevail. In temperate regions, the majority of avian hemosporidians undergo seasonal relapses (Huff 1942, Chernin 1952, Desser et al. 1968, Khan and Fallis 1970, Applegate 1971, Alverson and Noblet 1977, Valkiunas 2005a). Latently infected birds (characterized by no parasite blood stages) that were infected for the first time during the prior breeding season, return to the breeding grounds and experiencing a relapse. Relapse is an increase in parasite blood stages prior to the emergence of biting dipteran vectors (Khan and Fallis 1970). As compared to the period of chronic parasitemia, transmission of parasites from relapsing hosts to dipteran vectors increases during the spring relapse, because infectivity to the vector is positively related to hemosporidian parasitemia in the peripheral bloodstream of the host (Applegate et al. 1971, Mackinnon and Read 1999). Additionally, the parasitemia associated with the relapse is lower (Figure 1), but of longer duration than acute infections (Khan and Fallis 1970). This seasonal elevation of parasitemia is initiated by seasonal increases in sexual hormones and corticosterone in early spring; these hormones stimulate asexual reproduction of the parasite in non-circulating and circulating (for *Plasmodium* spp. only) tissues in latently infected birds (Applegate 1970, Applegate and Beaudoin 1970). In addition, some studies have suggested that dipteran

vectors may feed more easily on nestlings than adult birds (Blackmore and Dow 1958). Thus, seasonal relapses are considered to be an adaptive strategy to time transmission effectively on temperate breeding grounds with the introduction of the susceptible, young of the year (YOY) birds to the system (Beaudoin et al. 1971).

Our model considers the transmission of *Leucocytozoon* in a passerine population breeding in an alpine ecosystem. Transmission in our model depends upon the interaction between an age-structured (adult and YOY birds), bird reservoir population and a sympatric black fly vector population. We had the following objectives: 1) build a basic model for this system, 2) run a sensitivity analysis to determine the parameters that most influence transmission, and 3) validate the model with field data and estimate any unknown parameters. We address the following questions with this model: 1) Do nude nestlings, that are easily accessible to biting vectors, affect overall transmission dynamics? 2) How important is the relapse of chronically infected individuals for maintaining transmission dynamics in this system?

Materials and methods

We constructed a variation of the standard Susceptible (*S*), Exposed (*E*), Infectious (*I*) model for the transmission of *Leucocytozoon fringillinarum*. We parameterized the model with demographic and haemosporidian data collected from a Mountain White-crowned Sparrow (*Zonotrichia leucophrys oriantha*) host population and a local black fly (*Simulium silvestre* / *S. craigi*) vector population breeding on three field sites. All field sites were within the vicinity of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Gunnison County, Colorado, U.S.A. Two field sites were located approximately one mile south (UTM: N 4312713 E 327700) and one mile north

from RMBL (UTM: N 4314126.9 E 327648.3), and the third field site was located in the adjacent, Washington Gulch valley (UTM: N 4311531 E 325807). Elevation of the three study sites ranges between 2902 m to 2987 m above sea level.

Focus and development of the simple single season model

We chose to model this system because there exists a substantial foundation of baseline knowledge on White-crowned Sparrow habitat preferences, food habits, behavior, breeding, physiology, and predators (for reviews see Chilton et al. 1995a, Morton 2002). In addition, we chose *S. silvestre* / *S. craigi* as a potential vector because it is the most abundant ornithophilic black fly species at these field sites, is present throughout the majority of the breeding season (June – August), host-seeking individuals have been found to harbor a large diversity of *Leucocytozoon* spp. (CM unpublished; see Chapter three), and has been linked to transmission of *L. fringillinarum* in other systems (Adler et al. 2004). Finally, the most prevalent morphologically-defined haemosporidian parasite species (Valkiunas 2005a) within the sparrow population is *L. fringillinarum* (CM unpublished).

We ran the model in Berkeley-Madonna (Macey and Oster 2001), a software package designed to model dynamical systems. The model contains three interacting modules: adult bird, young of the year (YOY) bird, and the black fly vector populations (Figure 2). We constructed the model to run across a transmission season from May to September (200 days) in each year. We incorporated age structure by including two modules for the bird populations, adult and YOY birds, and had a third module for the simuliid vector population.

The adult sparrow module includes sparrows entering the breeding season as either susceptible adults or chronically infectious adults; this module comprises infection, natural death, and parasite-induced death. The YOY sparrow module incorporates infection, natural and parasite-induced death, and birth (nestling hatching), which is a function of the size of the adult bird population and time (Figure 3). The third module (for the simuliid vector population) includes infection, natural and parasite-induced death, and black fly emergence, which is a function of time (Figure 3). Both the sparrow birth function and the black fly emergence function were parameterized from empirical nest monitoring and insect data (Table 1; also Appendices C and F).

Susceptible birds (adults and YOY), either remain susceptible throughout the entire season or become infected. Once infected, sparrows become acutely infectious after a set prepatent period (Figure 1). Birds with acute infections move into a chronically infectious compartment. YOY birds enter as nude nestlings and transition into either susceptible feathered juveniles or exposed feathered juveniles (Figure 2). For details on differential equations used in the simple, one season model, refer to Appendix D.

Extensions of the simple model

From the basic single season transmission model, we extended the model to run for multiple years (360 days each), with a transmission season from May through September (150 days); as a result we included some additional compartments in the adult sparrow and YOY modules (Figure 4). We included a relapsing infectious adult compartment because *L. fringillinarum* causes spring relapses in its hosts after migration to the breeding grounds; thus, latently infected YOY and adults return the following

season, relapse, and reintroduce infection on the breeding grounds (Figure 4). Because the majority of the rate parameters in the model caused linear or percent changes and exponential distributions in their associated periods, we added chains in the relapsing and chronically infectious adult bird compartments to ensure the associated periods followed a gamma distribution and all chronically infectious birds become latently infected before migrating to their wintering grounds (Jacquez 1996, Lloyd 2001).

To simulate overwintering bird populations we used four *ovens*, or compartments for latent birds of different age (*adult* or *YOY*) and infection (*susceptible* or *infected*) statuses (see Appendix E). *Ovens*, in Berkeley-Madonna, simulate biological lag times; they are compartments that fill with individuals for a specified amount of time (*fill time*), hold the individuals that enter for a time interval (*cook time*), and then release all individuals at once into a specified compartment. Susceptible YOY and adults, and latently infectious juveniles and adults, each enter into the corresponding overwinter *oven* at the end of the breeding season (day 200). To account for overwinter mortality, we had 70% of the YOY birds die overwinter (Morton 2002). To balance the sparrow population's hatch and death rates, 5% of the overwintering adults died as well. The overwintering susceptible YOY birds that survive leave their *ovens* to return to the transmission locality the following season as susceptible adults, while surviving, overwintering latently infected YOY and adults return as relapsing infectious adults the following season (Figure 4). For details on the differential equations and ovens used in the extended model, see Appendix E.

Parameter selection

In both models, transmission of *L. fringillinarum* in this system is represented through the interactions of the modules and the number of infectious individuals in each population. The final model contains 20 fixed parameters, 6 time-dependent parameters, and four overwinter periods, susceptible and latently infected YOY, and susceptible and latently infected adult birds.

We shaped our model with parameters and initial conditions taken from the literature and field data collected on both sparrow (summers 1999-2008) and vector populations (summer 2005 and 2007) from field sites in Colorado when possible. Some parameters that were taken from the literature, such as the duration of the chronically and relapsing infectious stages, were adjusted within their possible ranges to ensure all birds were entered into their associated, overwintering compartment by day 200 of the season. The remaining parameter values were estimated based on model performance and whether they produced reasonable model outputs (see Appendix F for details). The model uses a fourth-order Runge-Kutta algorithm (Press 1992) to simultaneously iterate all 26 parameters (Table 1) each time step (1 day).

Model assumptions

We make the following assumptions in this model: 1) We have no recovery in this model for either the bird host and black fly vector. Substantial evidence demonstrates that most bird species do not develop complete immunity to avian hemosporidian parasites and remain chronically infected throughout the duration of their lifespans (Atkinson and van Riper III 1991, Valkiunas 2005a). Additionally, we assume that relatively short-lived black flies (longevity is 10-35 days depending on the species) die before clearing the parasite (Adler et al. 2004). 2) We also assume no variation in

immunity among birds. 3) Due to the relative helplessness of nestlings and lack of feathers in the first 6-7 days of life (Morton 2002), we assume that the accessibility of nestlings to biting vectors and the probability of transmission from an infectious vector are higher than adults. 4) Black fly per capita death rates are based on black fly life history stage (nonengorged, host-seeking or engorged, resting individuals) and infection status (susceptible or infectious, host-seeking individuals): host-seeking infectious individuals suffer the highest death rate while engorged, resting individuals have the lowest death rates whether they are susceptible or exposed. 5) Individuals in each module interact through random mixing.

Sensitivity and elasticity analyses

We conducted sensitivity analyses on all 26 parameters in the model by changing the value of each parameter several times over a 10-fold range, or over the entire range of the parameter, if changes were not feasible within a 10-fold range. We then determined how each parameter affected a number of output estimates representative of transmission in this system: final number of latently infected adult and YOY birds, mean number of infectious black flies, final prevalence of infected birds, and mean prevalence of infectious black flies. We estimated parameter sensitivity by determining the mean percentage change in these five outputs from a 1% change in the parameter of interest; an elasticity analysis prevents any issues that may arise during the sensitivity analysis due to differences among parameter units.

Results

Fifteen of the 26 model parameters had some effect on the output statistics, the final number of latently infected YOY and adult birds, the mean number of infectious

black flies, the final prevalence of infected birds, and the mean prevalence of infectious black flies (Table 2). Thus, 11 out of 26 parameters had no strong effects on any of the output statistics, or resulted in less than a half percent change in the output statistics with a one percent change in the parameter value; these included eight avian specific parameters and three black fly specific parameters (Table 2). The mean number and prevalence of infectious black flies were the most sensitive output statistics to changes in the model parameters, while the final number of latently infected adults was the least sensitive of all output statistics to changes in the model parameters, with the number of black fly bites bird⁻¹ day⁻¹ having the largest positive effect (half percent increase) in this output statistic (Table 2). Appendix G includes a summary of the sensitivity analysis and the resulting parameter plots.

Only one parameter, the number of black fly bites bird⁻¹ day⁻¹ (r), significantly affected all output statistics (Table 2). With one percent increases in the parameter r , there are corresponding increases in the avian and black fly output statistics by approximately half a percent and over one percent, respectively. However, beyond an r of three (three bites bird⁻¹ day⁻¹) the output statistics decrease in sensitivity to further changes in this parameter (Figure 5). This parameter is thus an important factor to measure if other systems have vectors taking fewer than three bites per host on a daily basis.

Avian specific parameters

Of the avian specific parameters, three parameters governing compartmental transitions within the bird modules were important (Table 2). An increase of one percent in the rate susceptible birds transition into the overwintering compartment decreases the

final number of latently infected YOY birds by half a percent. A one percent increase in the rates relapsing birds move into the overwintering, latent compartment (δ_R) and acutely infectious birds transition into the chronically infectious stage (δ_A), both decrease the mean prevalence of infectious black flies by approximately half a percent. Surprisingly, a one percent increase in the natural death rate of YOY nestlings, the natural death rate of YOY and adult birds, and the parasite-induced death rate does not strongly affect any of the output statistics.

The parameters of the nestling hatch function do influence some of the output statistics. A one percent change in the height of the nestling hatch curve (A_B), or the number of nestlings that hatch female⁻¹ day⁻¹, results approximately in a one percent increase in the final number of latently infected YOY birds (Figure 6). A one percent increase in the spread of the nestling hatch function (c_B) also results roughly in a half percent increase in this output statistic. Finally, a one percent increase in the day the nestling hatch function is centered on increases the mean number of infectious black flies by approximately a half percent (Table 2). Surprisingly, the final prevalence of infected birds and the mean prevalence of infectious black flies appear to be relatively insensitive to changes in these parameters (Figure 6).

Black fly specific parameters

Of the black fly specific parameters, two parameters governing rates of compartmental transition within the simuliid module have significant impacts on the sensitivity of the output statistics (Table 2). A one percent increase in the probability of transmission from an acutely infectious bird to a susceptible black fly (b_A) results in a half percent increase in the mean prevalence of infectious black flies. Additionally, a one

percent increase in the probability of transmission from a chronically infectious bird to a susceptible black fly (b_C) corresponds to a half percent increase in both the mean number and prevalence of infectious black flies. The natural and parasite-induced death rates of black flies appear to only affect the sensitivity of the black fly output statistics (Table 2).

Unlike the avian death rates, a one percent increase in the natural death rate of host-seeking black flies results in a decrease by roughly a half percent in the mean number of infectious black flies and an increase of over a half percent in the mean prevalence of infectious black flies (Table 2). This result is not surprising; if the death rate of susceptible, host-seeking black flies increases, the proportion of infectious black flies in the simuliid population will increase. As the death rate of exposed, resting black flies increases by one percent, there is a decrease in the mean prevalence of infectious black flies by half a percent. The black fly output statistics were the most sensitive to increases in the parasite-induced death rate of infectious, host-seeking black flies. As this parameter increased by one percent, there were corresponding decreases of over one percent in the mean number and prevalence of infectious black flies (Table 2).

The parameters of the black fly emergence curve also had strong impacts on both the black fly output statistics but some of the avian outputs statistics (Table 2). A one percent increase in the height of the black fly emergence curve (A_S), or the number of black flies that emerge day^{-1} , results in a one percent increase in the mean number of infectious black flies (Figure 7). Further, a one percent increase in the spread of the black fly emergence curve (c_S) corresponds to a half percent increase in the mean number of infectious black flies. Again, there are no effects of these emergence parameters on the mean prevalence of infectious black flies even though increases of these parameters

increase the mean number of infectious black flies (Figure 7). However, the day the emergence function centers (q_S) on has large effects on four out of five output statistics. A one percent shift later in the season of the black fly emergence function results in a half percent decrease in the final number of latently infected YOY, the mean number of infectious black flies, and the final prevalence of infected birds. It also results in almost a one percent decrease in the mean prevalence of infectious black flies (Table 2).

Discussion

The parameter values we chose for our model yielded final prevalence of infected birds of 70% and a mean prevalence of infectious black flies of 23%. Empirical data collected during the summers of 2003, 2004, and 2005 indicate that the prevalence of sparrows infected with *L. fringillinarum* on these sites is on average 50% (Murdock unpublished). Our model may overestimate true prevalence. However, if our field sample contains some chronically infectious birds across each summer, the empirical data may underestimate prevalence; our visual scoring method can miss infections with low parasitemia (1-3 gametocytes per 10,000 red blood cells), resulting in false negatives.

We feel the model estimate for mean prevalence of infectious black flies is reasonable, even though there is a considerable lack of empirical data on the prevalence of *Leucocytozoon* spp. in black fly populations. In Algonquin, Ontario, 90% to 100% of ornithophilic black fly species had *Leucocytozoon* sporozoites present in their salivary glands (Bennett and Squires-Parsons 1992). Hellgren et al. (2008) found 62% of blood-fed black flies ($n = 38$) were positive for *Leucocytozoon* spp. infections. However, the prevalence of infectious black flies infected with a particular species of *Leucocytozoon*

may be much lower, because both of these studies examined multiple black fly and *Leucocytozoon* species (also see Chapter three).

Discussion of sensitivity and elasticity analyses

The parameter r

The final number of latently infected YOY and adult birds, the final prevalence of infected birds, the mean number of infectious black flies, and the mean prevalence of infectious black flies were most sensitive to the parameter (r), the number of black fly bites bird⁻¹ day⁻¹. As the number of black fly bites bird⁻¹ day⁻¹ increases from zero bites to three bites, all of the output statistics increase from a half to one percent, suggesting that an increase in contact rate between birds and vectors significantly increases overall infection in both the bird and black fly population. However, above three black fly bites bird⁻¹ day⁻¹, the output statistics are relatively insensitive to further increases in the daily bite rate (Figure 5). This highlights the importance of estimating the parameter r , if the natural range of the daily bite rate for a particular vector falls within one to three bites per host.

To address this uncertainty in our model system, we attempted to rerun our model with a daily bite rate of one bite bird⁻¹. This model resulted in a significant drop in prevalence of infected birds and infectious black flies, a delay in transmission to susceptible bird hosts, and a lag in the development of acute infections. As a result, at the end of the season (day 200) there were acutely and chronically infectious birds that did not enter the overwinter stage, and transitioned in those states into the following breeding season. When we adjusted the parameters to ensure all birds entered the overwinter

period as latently infected birds, the parameters were no longer within their natural ranges. This suggests that in this system, black flies must be taking at least two to three bites bird⁻¹ day⁻¹. The next step will be to rerun the sensitivity and elasticity analyses with a daily bite rate of three bites bird⁻¹ day⁻¹; if there are significant changes in the elasticity of the output statistics with an r of three, future models need to take care in estimating the value for this parameter. Fitting this model to field prevalence data may aid in estimating the value of this parameter, and may inform sparse empirical data on black fly biting rates.

The probabilities of transmission associated with host-vector contact

It is not surprising that as the probability of transmission from acutely (b_A) and relapsing (b_R) infectious birds to susceptible black flies increases, there is a corresponding increase in the mean number and prevalence of infectious black flies. However, the output statistics are not sensitive to increases in the probability of transmission from chronically infectious birds to susceptible black flies (b_C), which is unexpected. This may be due to the fact that the peak of the black fly emergence curve (the peak of susceptible black flies) does not tightly overlap with the peak of chronically infectious YOY birds, which occurs later in the season. In each season, the majority of chronic infections are due to the susceptible YOY birds becoming infected for the first time. Thus, chronically infectious birds may play only a minor role in transmission dynamics at this site, not only due to the low parasitemia and transmissibility associated with chronic infections (Khan and Fallis 1970), but also in relation to when susceptible vectors emerge and experience their highest abundance (see Chapter 2).

Unexpectedly, the output statistics are relatively insensitive to increases in the probabilities of transmission from an infectious black fly to a nestling YOY (b_N) or feathered (b_F) susceptible bird. There may be a variety of reasons. The output statistics may be insensitive to changes in b_N because the distinction between unfeathered and feathered YOY birds may not be meaningful for transmission in this system. Nestlings are without feathers for a relatively brief window of time during the breeding season, and an increase in the probability of transmission during this interval does not significantly affect overall infection in both the bird and black fly population. This is further supported by the fact that the output statistics are insensitive to increases in the rate nestlings acquire feathers (F). The output statistics may also be insensitive to changes in b_F because its current setting is at the upper end of the range where further increases in the parameter do not result in changes in the output statistics. However, the output statistics significantly increase when b_F increases from zero to 0.05. This is another parameter for which careful estimation may be required if the natural range falls below a five percent probability of transmission.

Compartmental transition and death parameters

Increases in the rate acutely (δ_A) and relapsing infectious (δ_R) birds transitioned into the chronically infectious state (CIJ and CIA) and latently infected state (LIJ and LIA), respectively, resulted in a decrease in mean prevalence of infectious black flies. These compartments (chronically infectious and latently infected) are associated with lower or no probability of transmission to biting, susceptible black flies. Additionally, increases in the rate at which susceptible birds transition into the overwintering stage (γ)

also lead to decreases in the final number of latently infected YOY, but did not strongly affect overall final prevalence of infected birds (< half percent) or infectious black flies.

Increases in the death rates of exposed (d_5) and infectious black flies (d_6) caused a decrease in the mean number of infectious black flies overall, which resulted in a significant decrease in mean prevalence of infectious black flies in a season. Increases in the natural death rate of host-seeking black flies (d_4), also led to decreases in the mean number of infectious black flies. However, this decrease resulted in an increase in mean prevalence of infectious black flies, most likely due to the decrease in the proportion of susceptible individuals in the black fly population due to natural death. Instead of having the parameter for parasite-induced death include the natural background death rate of the population, infectious individuals simply experienced a higher death rate than uninfected individuals. Thus, this result may not be realistic and instead may be a consequence of how we assigned death rates in our model. Surprisingly, output statistics were insensitive to changes in the avian natural and parasite-induced death rates.

Parameters of the nestling hatch and black fly emergence function

The height, spread, and time of season the nestling hatch and black fly emergence functions reach their peaks affect the output statistics in values. Increases in the height and spread of the nestling hatch (A_B , c_B) and black fly (A_S , c_S) emergence functions resulted in an increase in the number of susceptibles that enter the breeding season at a given rate and an elevated final number of latently infected YOY and mean number of infectious black flies. Unexpectedly, this increase in infection in the bird and black fly population did not translate into a higher final prevalence of infected birds or mean prevalence of infectious black flies (Figures 6 and 7). These results suggest that the ratio

of infected to susceptible individuals in the bird and black fly population remain balanced across changes in these parameters.

As the time of season black flies reach their peak emergence (q_s) shifts later in the transmission season, there is a decrease in the final number of latently infected YOY birds and the mean number of infectious black flies. Transmission declines as the degree of overlap decreases between the nestling hatch function and the black fly emergence function. As a result, the final prevalence of infected birds and mean prevalence of infectious black flies is reduced. However, this occurs only when the degree of overlap is minimized with shifts in the emergence function toward later in the breeding season. If the emergence function is shifted earlier in the breeding season, to the time when black flies are peaking on day 0 (the beginning of May), we see an increase in parasite prevalence in both the bird and black fly populations. When the peak of the black fly emergence curve is shifted earlier in the season, susceptible black flies interact with relapsing infectious birds and become infected earlier in the season, which results in the number of infectious black flies reaching their daily peak at approximately the same time as nestlings begin to hatch during the breeding season (Figure 9). This result also indicates that the bird-feeding black flies of overall lower abundances, but emerge and reach peak abundances earlier in the breeding season (*G. denaria* and *Metacnephia jeanae*), may play significant roles in reintroducing *L. fringillinarum* to young of the year birds from relapsing infectious adult birds.

This has implications for potential warming at high elevation systems. Avian timing of reproduction is minimally influenced by changes in temperature; instead reproduction is regulated by seasonal changes in corticosterone and sex hormones, which

are driven by changes in day length (Lack 1954, Wingfield et al. 2003, Ramenofsky and Wingfield 2007). In contrast, dipteran development is more sensitive to changes in temperature, with increases in temperature within some range resulting in an increase in growth and development (Becker 1973, Merritt et al. 1982, Ross and Merritt 1988, Adler et al. 2004). Parasite development within the vector also increases with temperature within some range (Ball and Chao 1964, Valkiunas 2005a). As warming occurs at higher elevations, black flies emerge earlier in the season, which results in an increase in parasite prevalence in the bird and black fly population, and an earlier peak in acutely infectious adults. Surprisingly, this results in fewer nestlings hatching on a daily basis, because more adults are dying of parasite-induced death (Figure 8). Thus, warming at higher elevations may increase parasite prevalence and decrease sparrow recruitment.

Significance of relapse and YOY birds

Based on the sensitivity and elasticity analyses, both the relapse phenomenon and YOY birds are crucial in maintaining seasonal transmission on the breeding grounds. Of the parameters for the probability of transmission upon vector-host contact and the rates governing compartmental transitions, the mean prevalence of infectious black flies was the most sensitive to changes in the probability of transmission from a relapsing infectious bird to a susceptible black fly (b_R), and the rate at which relapsing infectious individuals transition into the latently infected overwinter stage (δ_R). In addition, when we remove the relapsing infectious adult compartment (and instead have birds infected in past summer seasons return to subsequent seasons as chronically infectious adults) we do not get persistence across summer seasons. Because chronic infections are characterized by low parasitemia and decreased infectivity to biting vectors, there is a delay in

transmission to susceptible YOY birds, and a lag in the development of acute infections. Again, we do not have birds entering the overwinter compartment at the appropriate time of season (day 200). Parameter adjustments to ensure all birds entered the overwinter period as latently infected birds did not fall within their natural ranges, thus we conclude a scenario without relapse is unlikely.

We can also conclude that YOY birds play an important role in persistence of seasonal transmission of *L. fringillinarum*. Our sensitivity and elasticity analysis demonstrated that the degree of seasonal overlap between the peaks of daily nestling hatching and black fly emergence was important for both the final prevalence of infected birds and the mean prevalence of infectious black flies. YOY birds, especially in temperate systems, represent a pulse of susceptible hosts each season, and other analytical studies have demonstrated their significance in maintaining seasonal transmission (Lord and Day 2001b, Unnasch et al. 2006). Adults, once infected, remain infected throughout the remainder of their life span and are immune to further infection with the same parasite strain (Atkinson and van Riper III 1991, Valkiunas 2005a). In addition, adults are seasonally faithful to their breeding territories and do not disperse to new areas (Morton 2002). Thus, new susceptible birds are introduced solely through the nestlings produced each season, or through susceptible YOY birds from the preceeding season that are dispersing from their natal breeding grounds. Additionally, when we remove the possibility of transmission between infectious black flies and YOY birds from the model, *L. fringillinarum* quickly fades after a couple of seasons due to the exhaustion of susceptible birds.

However, distinguishing between nude nestlings and feathered YOY is unnecessary, as shown by the insensitivity of our output statistics to the daily rate nestlings acquire feathers (F) and the probability of transmission from an infectious black fly to a nude nestling (b_N). This contrasts with results from models for St. Louis encephalitis virus (Lord and Day 2001b, a), West Nile virus (Lord and Day 2001a), and Equine encephalitis models (Unnasch et al. 2006), which found nestlings to be important for transmission dynamics. However, these models incorporated mosquito feeding preferences for nude nestlings and the development of nestling immunity; in the encephalitic disease systems, nestling birds develop infections faster, maintain higher and longer viremias than adult birds, and consequently are good amplifying hosts in these systems. The development of immunity in YOY birds and any potential vector feeding preferences for YOY birds may be important factors to incorporate in future models on hemosporidian transmission.

Conclusions and future directions

This model reflects transmission of *L. fringillinarum* from a potential vector species, *S. silvestre* / *S. craigi*, and a population of breeding White-crowned Sparrows in a temperate system. *L. fringillinarum* can infect multiple bird species in the studied community (see Chapter two), and potentially multiple common vector species. This model can be easily modified to investigate the roles of other vector and bird species in the overall transmission of hemosporidian parasites. Using a modified version of this model, it may be possible to predict the important host-vector pairs that are most important in maintaining hemosporidian transmission in a system. In addition, YOY birds disperse from their natal territories and return to nearby sites as breeding adults the

following season (Morton 2002); a metapopulation structure could be incorporated with the rates of exposure and infection varying by transmission locality (Figure 9). The different breeding sites can then be connected by varying rates of immigration and emigration of susceptible and latently infected YOY birds.

To our knowledge, this is the first model developed for simulating avian hemosporidian transmission, and it provides an in depth look at the ecological, parasite, and host factors that are important for hemosporidian transmission outside of the Hawaiian Island system. With this multi-year model, we determined that both the relapse phenomenon and YOY birds are essential for parasite persistence from year to year in temperate, seasonal systems. In addition, as temperatures warm at high elevation sites, we predict that parasite prevalence may increase in both the host and vector populations on these sites, resulting in an overall decline in avian recruitment.

Table 4.1 Parameter values for the model are presented below. Initial conditions for the model are the following: 300 susceptible adults and 100 relapsing infectious adults. All other populations initially had zero individuals at the beginning of the season.

Initial values of parameters			
Bird Parameters	Value	Definition	Reference
b_N	0.10	transmission probability from infectious black fly to susceptible nestling	model driven
b_F	0.05	transmission probability from infectious black fly to susceptible feathered bird	model driven
λ_B	0.18 day ⁻¹	rate an exposed bird becomes acutely infectious	1
δ_A	0.09 day ⁻¹	rate an acutely infectious bird becomes chronically infectious	2
δ_R	0.14 day ⁻¹	rate relapsing infectious birds become latently infected	2
σ_I	0.5 week ⁻¹	rate chronically infectious YOY birds become latently infected	model driven
σ_A	0.23 week ⁻¹	rate chronically infectious adult birds become latently infected	model driven
γ	0.007 day ⁻¹	rate a susceptible bird becomes an overwintering, susceptible bird	model driven
F	0.17 day ⁻¹	rate nude nestlings acquire feathers	3
d_1	0.004 day ⁻¹	natural death rate of YOY nestlings	3
d_2	0.002 day ⁻¹	natural death rate of feathered YOY and adults	3
d_3	0.01 day ⁻¹	death rate of acutely infectious birds	model driven
A_B	0.17 YOY female ⁻¹ day ⁻¹	the height of our nestling hatch function	4
q_B	57 day	the peak day during of nestling hatching	4
c_B	150	controls the width of the nestling hatch function	4
susceptible birds	180, 180 day	# days oven fills & cooks for the susceptible juvenile & adult oven	3
latently infectious birds	200, 160 day	# days oven fills & cooks for the latently infectious juvenile & adult oven	3

¹Valkiunas 2005, ²Allan & Mahrt 1989, ³Morton 2002, ⁴Foufopoulos unpublished, ⁵Adler et al. 2004, ⁶Mackinnon & Read 2004,

⁷Murdock unpublished

Initial values of parameters			
Black Fly Parameters	Value	Definition	Reference
r	$2 \text{ fly}^{-1} \text{ bird}^{-1} \text{ day}^{-1}$	# bites a black fly takes per bird per day	model driven
b_A	0.10	transmission probability from an acutely infectious bird to susceptible fly	2, 6
b_R	0.05	transmission probability from a relapsing infectious bird to susceptible fly	2, 6
b_C	0.01	transmission probability from a chronically infectious bird to susceptible fly	2, 6
λ_S	0.20 day ⁻¹	rate of an exposed simuliid becoming infectious	1, 5
d_4	0.05 day ⁻¹	natural death rate of non-engorged, host-seeking flies	5
d_5	0.01 day ⁻¹	death rate of engorged, resting black flies	model driven
d_6	0.10 day ⁻¹	death rate of infectious, non-engorged, host-seeking black flies	model driven
A_S	60 flies ⁻¹ day ⁻¹	the height of our black fly emergence function	7
q_S	60 day	the peak day of black fly emergence	7
c_S	400	controls the width of the black fly emergence function	7

¹Valkiunas 2005, ²Allan & Mahrt 1989, ³Morton 2002, ⁴Foufopoulos unpublished, ⁵Adler et al. 2004, ⁶Mackinnon & Read 2004,

⁷Murdock unpublished

Table 4.2 Bolded output statistics experienced a half to one percent change in response a one percent change in parameter value.

Elasticity (% change with 1% change in parameter value)							
Driver	Number			Prevalence			
	final latent infected YOY	final latent infected adults	mean infectious simuliids	final infected birds	mean infectious simuliids		
Avian Parameters							
b_N = probability of transmission to a susceptible nestling	0.05	0.01	0.06	0.03	0.00		
b_F = probability of transmission to a susceptible feathered bird	0.19	0.19	0.23	0.29	0.00		
λ_B = rate a bird becomes infectious	0.01	0.01	0.06	-0.10	0.00		
δ_A = rate an acutely infectious bird becomes chronically infectious	0.01	0.02	-0.22	-0.03	-0.45		
δ_R = rate a relapsing infectious bird becomes a latently infected overwintering bird	-0.04	0.18	-0.07	0.03	-0.53		
σ_J = rate a chronically infectious YOY becomes latently infected overwintering bird	0.01	0.00	-0.01	0.00	-0.04		
σ_A = rate a chronically infectious adult becomes latently infected overwintering bird	-0.04	0.02	-0.01	0.01	-0.04		
γ = rate a susceptible bird becomes an overwintering bird	-0.47	-0.33	-0.05	-0.32	-0.18		
F = the rate nude nestlings become feathered YOY birds	-0.04	0.00	-0.03	-0.03	-0.04		
d_1 = the natural death rate of YOY nestlings	-0.02	0.00	0.00	-0.01	0.00		
d_2 = the natural death rate of feathered YOY and adult birds	0.00	0.00	0.00	0.00	0.00		
d_3 = the death rate of acutely infectious YOY and adult birds	-0.11	-0.06	-0.02	-0.03	-0.04		
A_B = the height of the nestling hatch function	0.94	-0.02	-0.16	0.00	-0.13		
c_B = the spread of the nestling hatch function	0.46	-0.01	-0.09	0.01	-0.09		
q_B = the day the nestling hatch function centers on	-0.09	0.07	0.43	0.20	0.36		

Elasticity (% change with 1% change in parameter value)		Number			Prevalence		
Driver		final latent infected YOY	final latent infected adults	mean infectious simuliids	final infected birds	mean infectious simuliids	
Simuliid Parameters							
r = number of black fly bites bird ⁻¹ day ⁻¹		0.44	0.40	1.21	0.43	1.11	
b_A = probability of transmission from an acutely infectious bird to a susceptible simuliid		0.09	0.05	0.36	0.07	0.52	
b_R = probability of transmission from a relapsing infectious bird to a susceptible simuliid		0.10	0.12	0.53	0.14	0.77	
b_C = probability of transmission from a chronically infectious bird to a susceptible simuliid		0.01	0.00	0.03	0.00	0.00	
λ_S = rate a simuliid becomes infectious		0.03	0.06	0.13	0.04	-0.15	
d_4 = the natural death rate of uninfected, host-seeking simuliids		0.09	0.04	-0.62	0.06	0.89	
d_5 = the death rate of infected, resting simuliids		-0.01	-0.01	-0.06	-0.01	-0.51	
d_6 = the death rate of infectious, host-seeking simuliids		-0.26	-0.15	-1.05	-0.29	-1.41	
A_S = the height of the simuliid emergence function		0.00	-0.03	1.00	0.00	0.00	
c_S = the spread of the simuliid emergence function		0.15	-0.14	0.58	-0.04	-0.09	
q_S = the day the simuliid emergence function centers on		-0.46	-0.35	-0.51	-0.40	-0.81	

Figure 4.1 is a typical representation of how parasitemia of hemosporidian parasites change throughout the course of infection and is adapted from Valkiunas' figure 16 (2005). The phases of infection are the following: prepatent period (**I**); primary parasitemia (**II**) consisting of the acute (**a**) and chronic (**b**) phases; latent phase of infection (**III**); and secondary parasitemia resulting from seasonal relapse (**IV**). The x-axis represents the calendar year and the y-axis is density of blood stages, or parasitemia. Infected birds exhibit the highest parasitemia during the acute phase of the infection, and lowest and intermediate parasitemia during the chronic and relapse phase, respectively. Thus, birds are most infectious during the acute phase of infection and are least infectious during the chronic phase of infection.

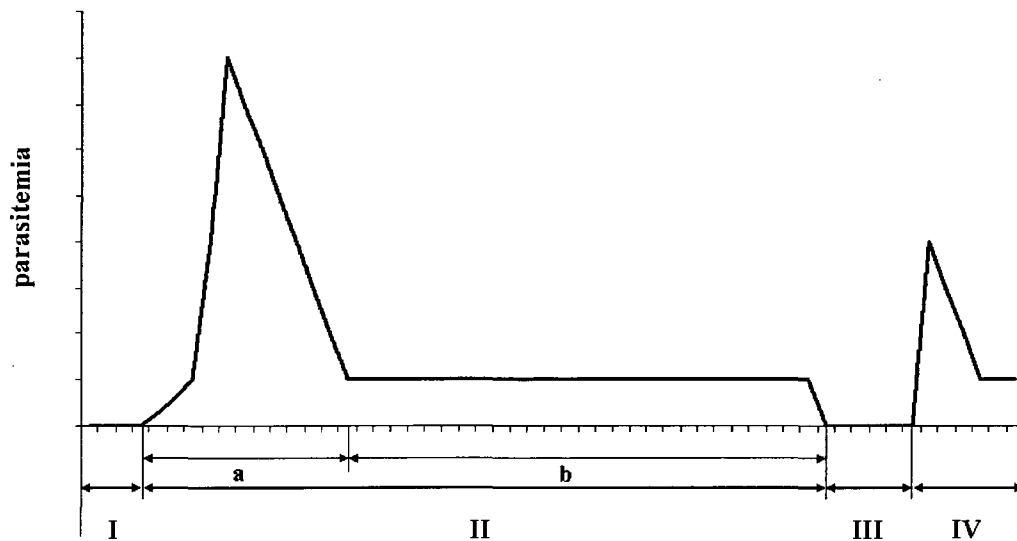


Figure 4.2 The three modules are represented by different colored boxes (white = YOY birds, light gray = adult birds, and dark gray = simuliid vectors). Arrows denote flows of individuals entering or leaving compartments within each module over time. As shown in the module headings, **B** and **S** represent the total number of individuals in the bird and simuliid vector populations, respectively. The first one or two letters of each compartment label corresponds to the infection status of individuals entering or leaving that compartment (**S** = susceptible, **E** = exposed, **I** = acutely infectious, and **CI** = chronically infectious). The last letter in each compartment label corresponds to the population the module represents (**N** = nude nestling and **J** = feathered juvenile populations in the YOY module, **A** = feathered adult bird population, and **S** = simuliid vector population). In the sparrow modules, **BI** represents the total number of infectious birds (**IJ** + **CIJ** + **IA** + **CIA**). Initial conditions for the compartments in the modules are the following: 300 sparrows begin as susceptible adults (**SA**) and 100 sparrows enter as chronically infectious adults (**CIA**). All other compartments initially begin with zero individuals.

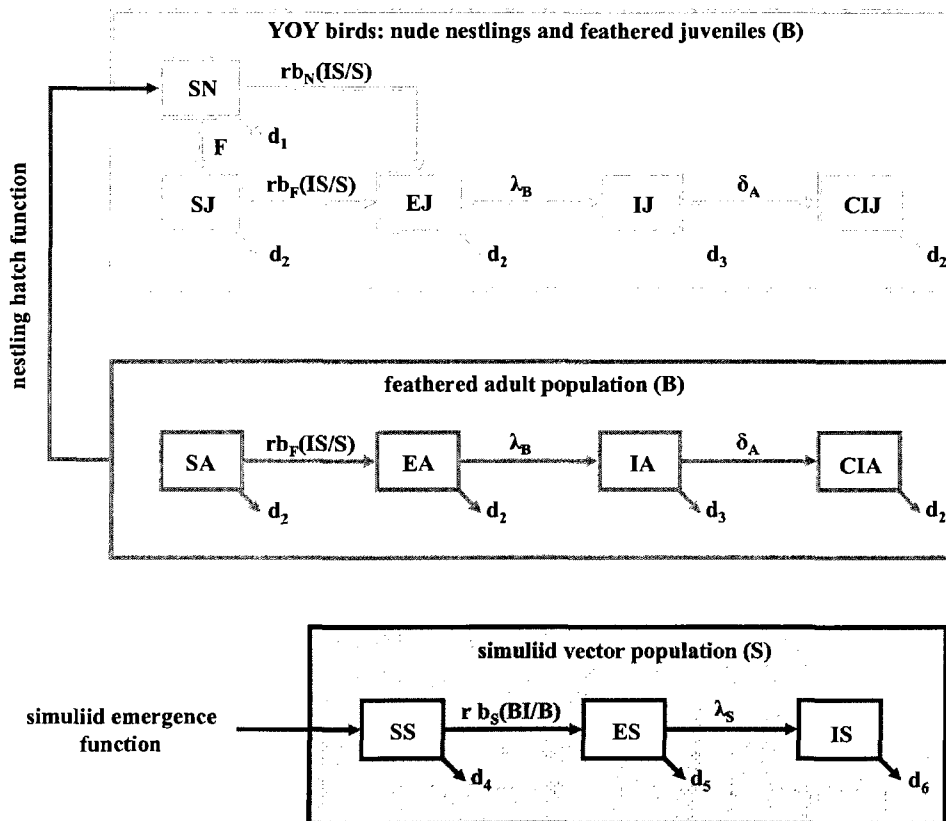


Figure 4.3 shows the daily nestling (**A**) and black fly field data (**B**) overlapping with the corresponding nestling hatch and black fly emergence functions. Empirical field data used to parameterize both functions are represented by gray bars, while the nestling hatch and black fly emergence functions are represented by black bars. Day zero corresponds to May 1st in both figures.

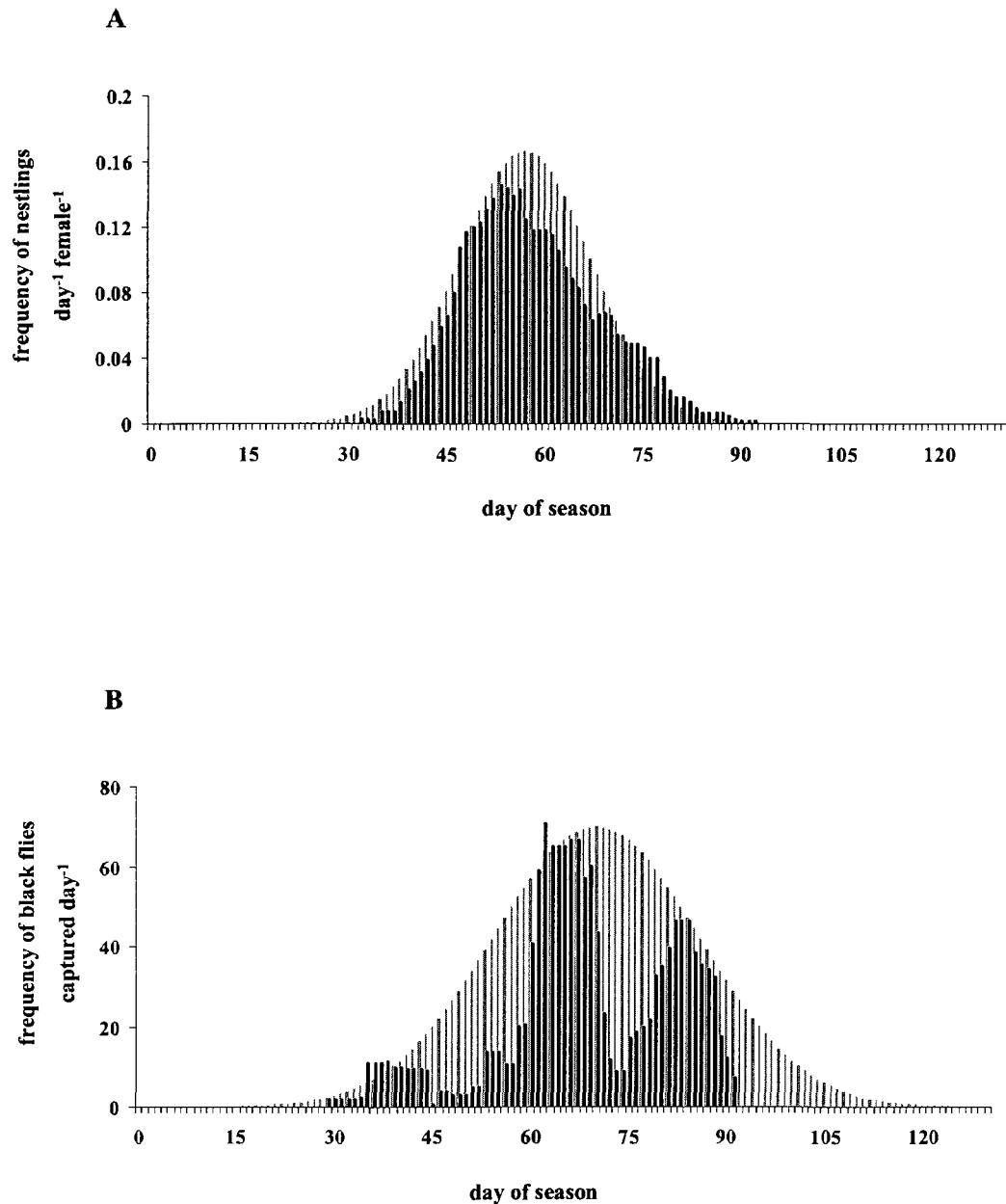


Figure 4.4 Extending the model from Figure 2 to a multi-year model adds a relapsing infectious adult bird compartment and chains to the chronically infectious YOY and adult bird compartments. The three modules are represented by different colored boxes (white = YOY birds, light gray = adult birds, and dark gray = simuliid vectors). Dashed compartments, or *ovens*, represent overwintering birds in various states (adult or YOY birds that are susceptible or latently infected). Arrows denote flows of individuals entering or leaving compartments within each module over time. As shown, in Figure 2, **B** and **S** in the module headings represent the total number of individuals in the bird and simuliid vector populations, respectively. The first one or two letters of each compartment label corresponds to the infection status of individuals entering or leaving that compartment (**S** = susceptible, **E** = exposed, **I** = acutely infectious, **CI** = chronically infectious, and **RI** = relapsing infectious). The last letter in each compartment label corresponds to the population the module represents (**N** = nude nestling and **J** = feathered juvenile populations in the YOY module, **A** = feathered adult bird population, and **S** = simuliid vector population). In the multi-year model sparrow modules, **BI** represents the total number of infectious birds ($IJ + CIJ_{1-4} + IA + CIA_{1-4} + RIA_{1-20}$). To ensure approximately all relapsing and chronically infectious individuals transition into the corresponding overwinter oven, we added chains or compartments to these stages (four and 20 for chronically and relapsing infectious state, respectively). Initial conditions for the compartments in the modules are the following: 300 sparrows begin as susceptible adults (**SA**) and 100 sparrows enter as relapsing infectious adults (**RIA**). All other compartments initially begin with zero individuals.

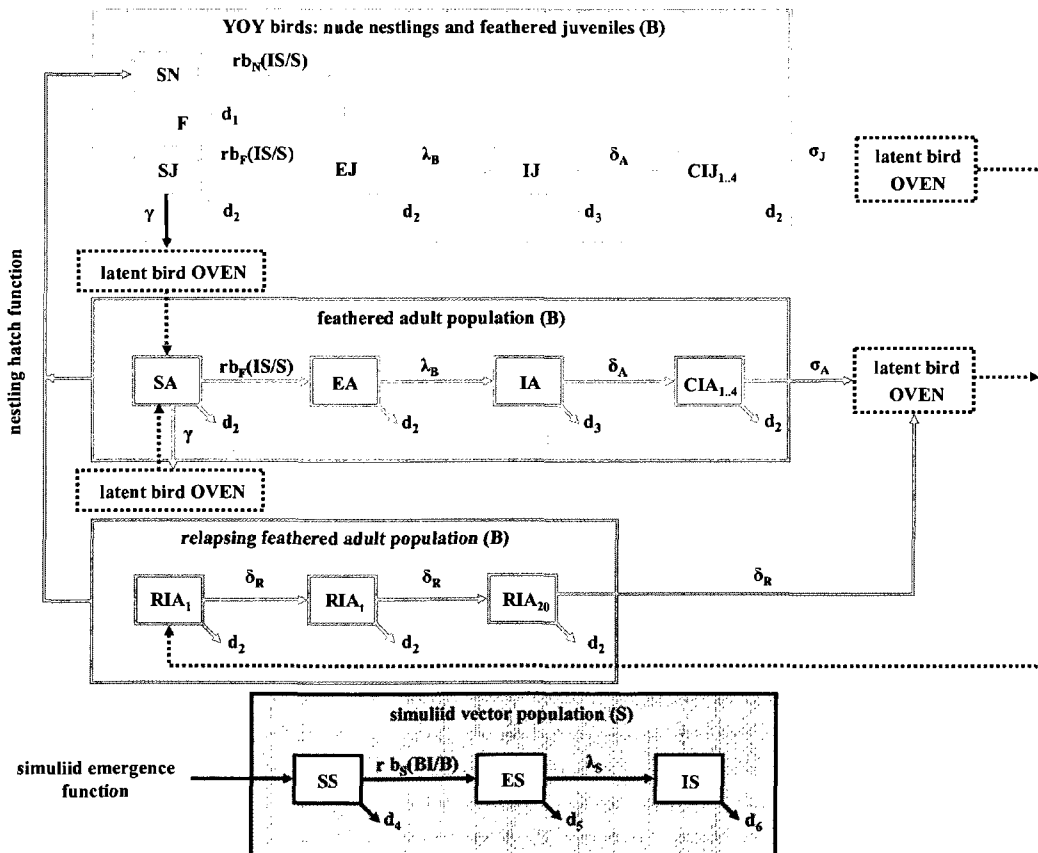


Figure 4.5 As the number of black fly bites bird⁻¹ day⁻¹ increases from zero bites to three bites, **A** the final number of YOY, the final number of adult birds, the mean number of infectious black flies, **B** and the final prevalence of infected birds and mean prevalence of infectious black flies, all increase from a half to one percent. The sensitivity of the output statistics within this range suggests that an increase in contact rate between birds and vectors significantly increases overall infection in both the bird and black fly population.

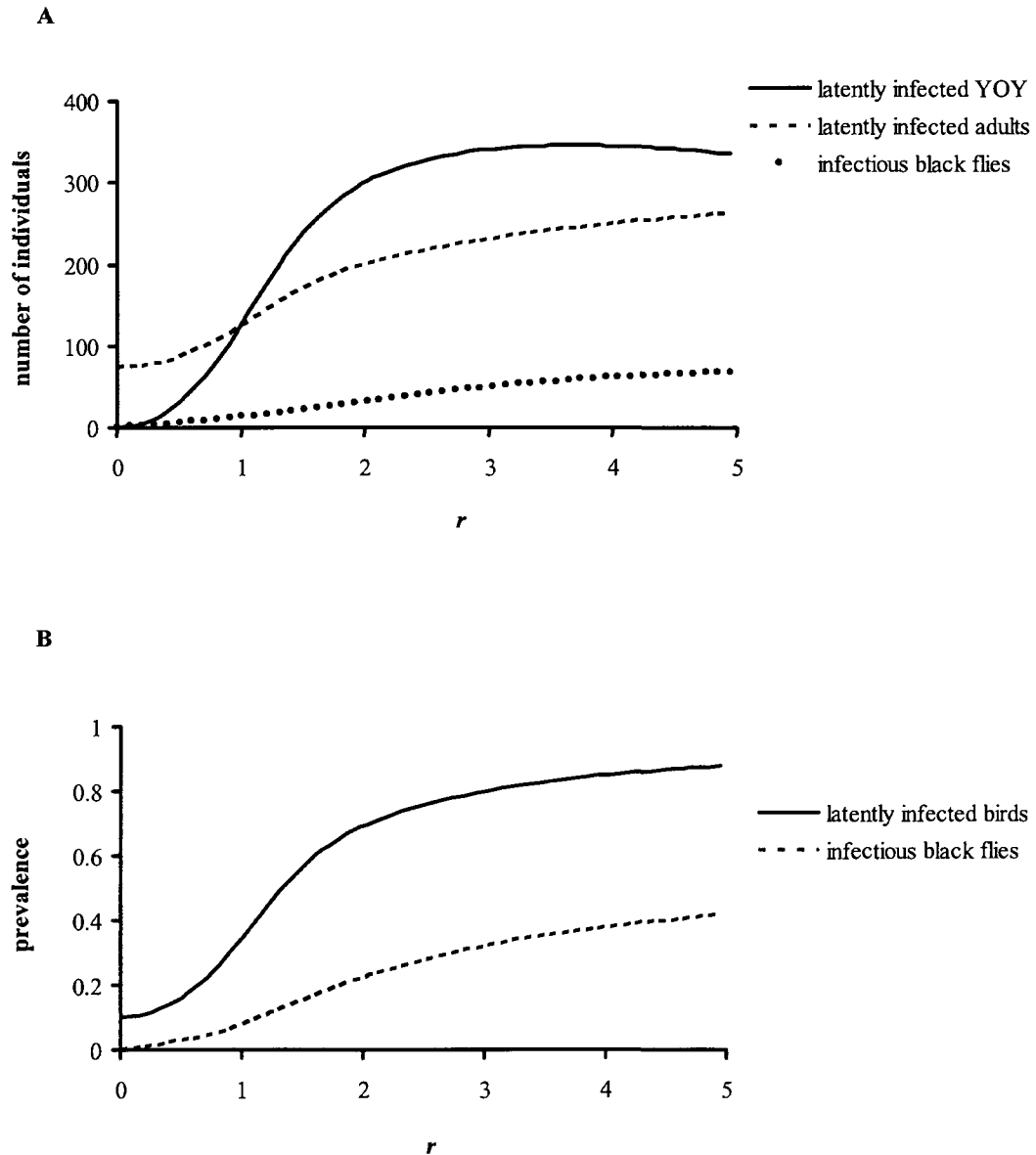


Figure 4.6 Increases in the peak number of nestlings hatched female⁻¹ day⁻¹ (A_B) increases the final number of latently infected YOY birds. However, this increase does not translate into a significant increase in final prevalence of infected birds or mean prevalence of infectious black flies, which suggests that the ratio of infected to susceptible individuals in the bird and black fly population remain balanced across changes in this parameter.

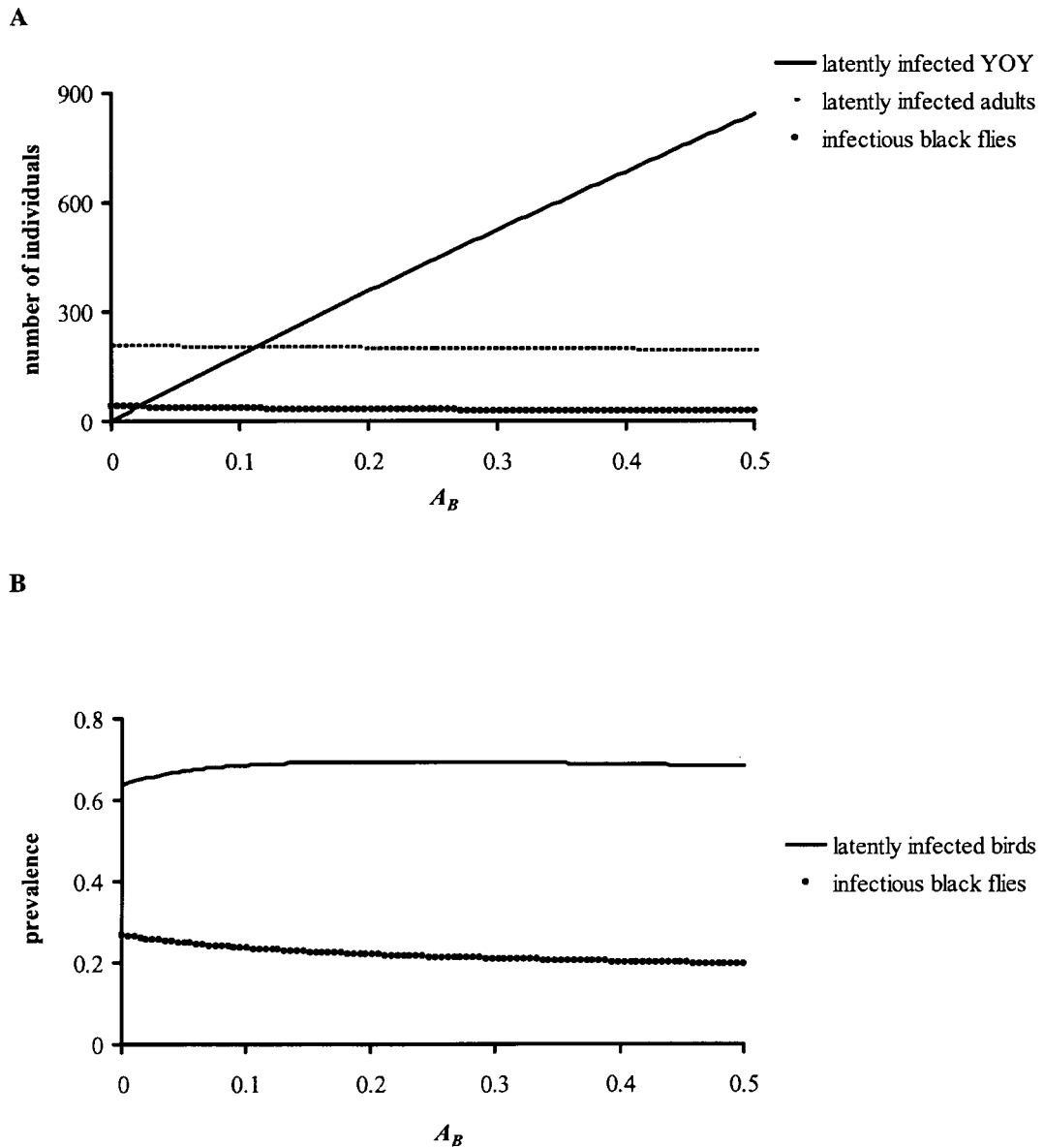


Figure 4.7 Increases in the peak number of black flies that emerge day⁻¹ (A_S) result in increases in the mean number of infectious black flies. However, this increase does not translate into a significant increase in final prevalence of infected birds or mean prevalence of infectious black flies, which suggests that the ratio of infected to susceptible individuals in the bird and black fly population remain balanced across changes in this parameter.

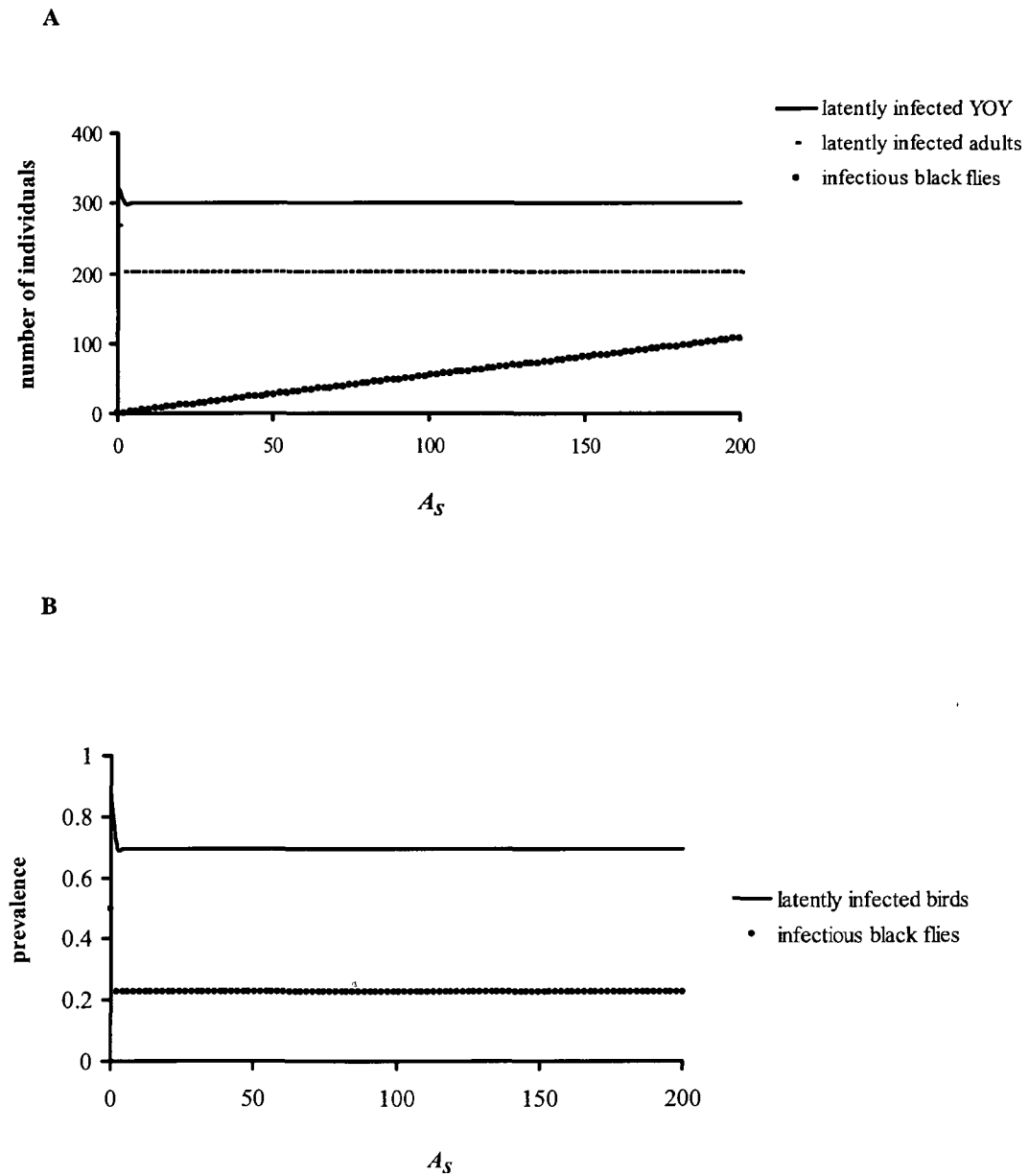


Figure 4.8 **A** is the model output when the peak of black fly emergence (q_S) occurs on day 0, while **B** is the model output when the peak of black fly emergence (q_S) occurs at the initial value of day 60. As the peak of black fly emergence shifts earlier in the season, the number of susceptible black flies interacting with relapsing infectious adults increases, which results in an earlier peak in the number of infectious black flies. This peak shift in infectious black flies coincides closely with the arrival of susceptible nestlings, which results in an overall increase in final prevalence of infected birds and mean prevalence of infectious black flies **C**. In addition, because more adults are becoming acutely infectious and dying of parasite-induced death, there is a subsequent decrease in the number of nestlings hatching female⁻¹ day⁻¹.

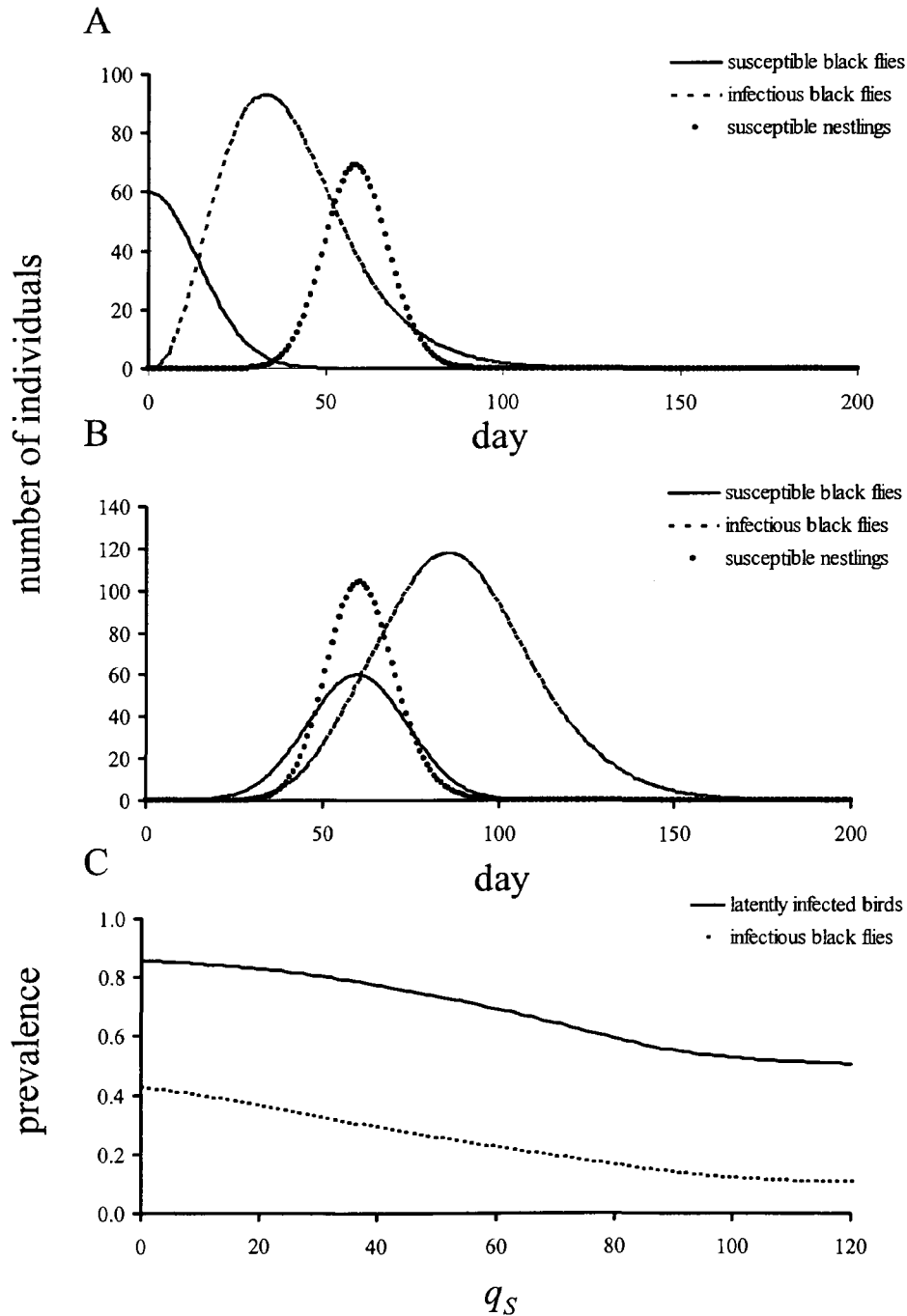
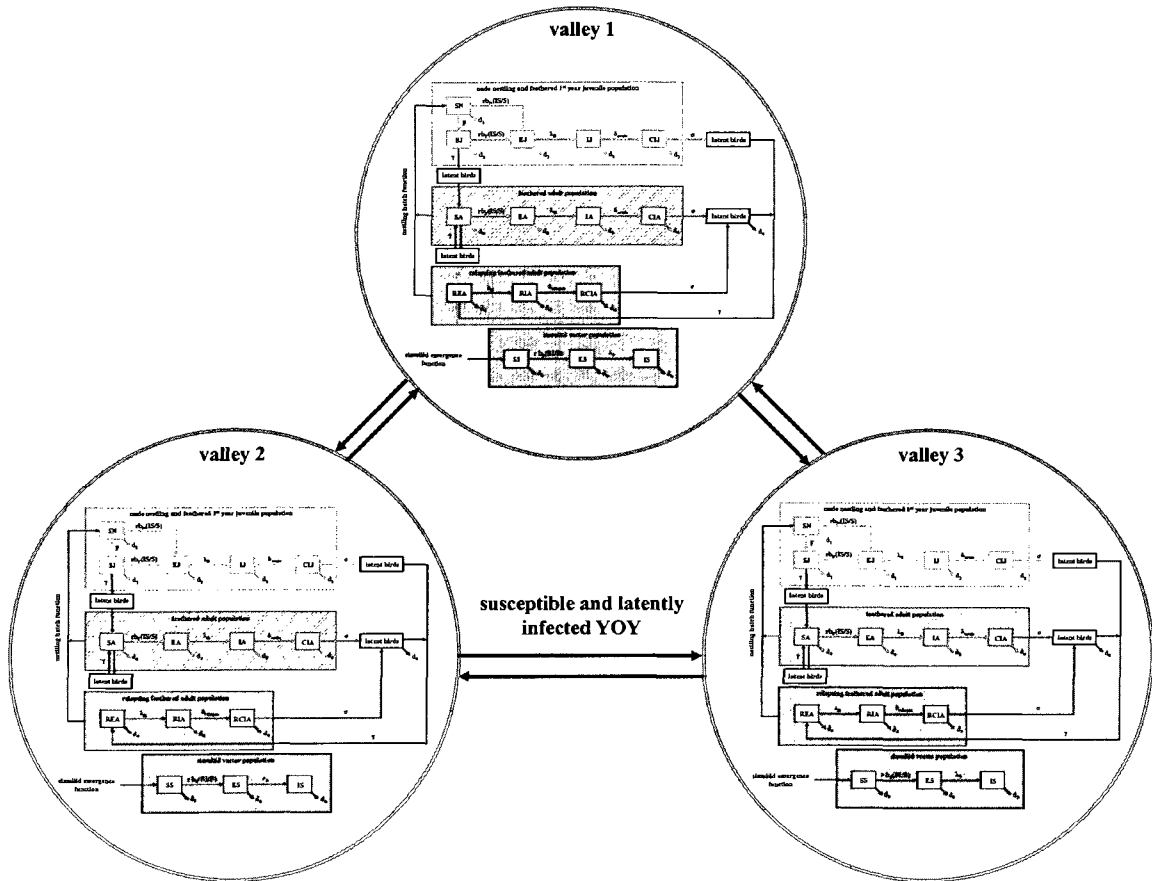


Figure 4.9 A model schematic showing the extension of the original model by incorporating a metapopulation consisting of three transmission localities, or neighboring valleys, which are connected through varying rates of immigrating and emigrating susceptible and latently infected YOY birds.



Chapter V

Food availability and parasitism affect stress and immune response in song birds

Introduction

Free-ranging vertebrates balance life-history demands with ever-present environmental constraints. Arguably the most important constraints are nutritional limitations, dictated by food shortages and competition for food (Newton 1998), and chronic infection with parasites (Moller 1997). Food limitation is extremely important, and many bird species breeding in temperate zones time their reproduction around seasonal peaks in food availability (Lack 1954). Birds with access to high-quality food resources often breed earlier (Boutin 1990, Schoech et al. 2004) and rear larger clutches (Clinchy et al. 2004). Food resources also positively affect body condition (Schoech 1996) and survival (Scott and Dobson 1989, Sinclair and Arcese 1995).

Parasitism is ubiquitous – there are more parasitic than nonparasitic organisms in the world – and the selective pressures imposed by parasites shape host defenses and life-history strategies (Moller 1997, Schmidt and Roberts 2000). Avian hemosporidian parasites of the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* have global distributions (Valkiunas 2005a). Although young birds acutely infected can die from hemosporidians, adults often carry chronic, sublethal infections which may cause reduced breeding success (Stjernman et al. 2004, Marzal et al. 2005), poorer body condition (Bonier et al. 2007), or reduced survival (Sol et al. 2003, Marzal et al. 2008).

Food availability and infection with micro- or macroparasites may play an important role in the stress response and immune system function, two physiological systems that mediate survival and reproduction. The vertebrate stress response is a highly-conserved set of physiological processes that results in an increase in circulating glucocorticoids that release endogenous energy resources, allowing organisms to tolerate or escape sudden environmental challenges such as inclement weather, predation, and intra-specific competition (Wingfield et al. 1998, Wingfield and Kitaysky 2002). Moderate food restriction affects avian stress response by elevating baseline corticosterone (the primary avian glucocorticoid, i.e. Mench 1991, Kitaysky et al. 2001) and stress-induced CORT concentrations (CORT, Romero and Wikelski 2001, Clinchy et al. 2004). Unpredictability of food resources also affects stress response through elevation of baseline CORT concentrations (Pravosudov et al. 2001). Chronic infection with parasites may also be a significant source of physiological stress (Oppliger et al. 1998, Morales et al. 2004).

Recent advances in the use of standardized immunological field assays have allowed ecologists to elucidate the importance of immunocompetence in controlling parasitic infections (Moller et al. 2003, Tschirren and Richner 2006, Tomas et al. 2007), as well as the energetic costs of maintaining an effective immune system (Cook 1991, Klasing 1998, Lochmiller and Deerenberg 2000). Laboratory evidence demonstrates that if animals do not meet minimum energetic requirements, they may experience suppressed immunity and become more susceptible to new parasitic infections (Chandra and Newberne 1977, Vestey et al. 1993). Chronic infection with parasites –alone or in conjunction with low food availability – can, in turn, suppress immune function and may

increase susceptibility to secondary parasitic infections (Wakelin 1997, Millington et al. 2006, Millington et al. 2007).

Many of the effects of food availability or chronic parasitic infection on stress and immunity have been investigated singly under laboratory conditions; very little empirical work has been done to elucidate these relationships in wild populations. The aim of this study is to determine the effects of two environmental challenges (food scarcity and chronic hemosporidian infection) on stress response and immune function in a wild population of White-crowned Sparrows (*Zonotrichia leucophrys oriantha*) breeding in the Colorado Rocky Mountains. In the early breeding season when sparrows first arrive, food resources are limited and unpredictably available. The site is snow-covered with little emergent vegetation, arthropod abundance is low, and inclement weather (i.e. rapidly advancing snow storms and freezing rain) occurs throughout early summer (CM, pers. observ.). Six morphologically-distinct species of hemosporidian parasites (*Leucocytozoon fringillinarum*, *L. majoris*, *Haemoproteus coatneyi*, *Plasmodium relictum*, *P. vaughani*, and *Trypanosoma avium*) are prevalent in this study population.

We investigated how sparrow stress responses and immune function (measured by the standardized stress series protocol and the phytohemagglutinin immune challenge, respectively) responded to two experimental treatments (food supplementation and antimalarial drug treatment), designed to buffer sparrows from naturally occurring food shortages and chronic infection with blood parasites. We propose two hypotheses: 1) food scarcity and unpredictability in the early breeding season results in chronic stress and decreased immune function, and 2) infection with blood parasites produces chronic stress and decreased immune function. From these hypotheses we generated the

following predictions. Control birds exposed to the background food and parasite stressors should experience the highest baseline CORT and stress-induced CORT concentrations compared to sparrows in other treatment groups. Because food restriction and blood parasite infection may have a negative effect on immune function, control birds should have the smallest wing web swellings. In contrast, birds that received both the food supplementation and drug treatments should have the lowest baseline CORT and stress-induced CORT concentrations, as well as the highest wing-web swellings. Birds that received either food supplementation or drug treatment, but not both, should have intermediate baseline CORT concentrations, stress-induced CORT concentrations, and wing-web swellings.

Materials and Methods

Experimental design and treatments

We conducted a field experiment in the vicinity of the Rocky Mountain Biological Laboratory in Gothic (38°57'33"N, 106°59'21"W), Gunnison County, Colorado, U.S.A. on four plots that have been used for long-term studies of individually-marked White-crowned Sparrows. The plots are located in the East River valley and are bisected by a dirt road; they vary in size from 483 m to 966 m in road length. Each plot is separated from the others by a buffer zone of at least 400 m to ensure that bird territories do not overlap neighboring plots (Ralph and Pearson 1971, Patterson and Petrinovich 1978). Elevation of the study plots ranges from 2902 m to 2987 m asl.

The experiment was a split-plot design consisting of a food supplementation treatment and an antiprotozoal drug treatment, with the plot and the individual bird as the large and small experimental units, respectively. We applied a standardized stress series

protocol to measure baseline and stress-induced CORT concentrations and administered phytohemagglutinin (PHA) challenges to measure immune function. Each sparrow received no more than one standardized stressor or PHA immune challenge throughout the duration of the study. To avoid any measurement interference, we ran the standardized stress series in the early (May 29th – June 11th) and late (July 6th – July 31st) breeding season and applied immune challenges in the interval (June 12th – July 5th).

In each year, two non-adjacent experimental plots received food supplementation treatment while the other two plots served as controls. Food supplementation consisted of walking the treated plots daily and throwing one handful of white millet seed (*Panicum miliaceum*) on supplementation sites (approximately 30-40 sites dispersed over each plot) throughout the breeding season. We chose to supplement with white millet because it is a well-balanced supplement (carbohydrate 74%, protein 14.4%, fat 5.4%, and fiber 4.2%) rich in minerals (Ravindran 1991), wild birds gain weight on diets made up exclusively of white millet (Madison and Robel 2001), and this supplement is comparable to natural seed sources for wild sparrows. We initiated food supplementation after sparrows had established breeding territories to avoid any influence on territory selection. Within each plot, we administered an oral, antiprotozoal drug treatment (an aqueous solution of sulfadimethoxine [50 mg / kg] and pyrimethamine [1 mg / kg]) to birds with odd-numbered metal bands, while birds with even-numbered bands received a water placebo of equal volume.

Bird capture and measurement methods

We captured birds between 06:00 and 12:00 each day in millet-baited, single- and double-cell (18 cm × 18cm × 18 cm per cell), galvanized wire Potter traps. Sparrows were marked with a unique combination of three colored leg-bands and one metal U.S. Fish and Wildlife Service band (Bird Banding Laboratory, Patuxent, MD, USA). Upon capture, we sexed each bird, collected standard morphometric measures, and took blood samples from the brachial vein with a sterile 26-gauge hypodermic needle (Fisher Scientific Precision Glide, No. 14-826-15, Pittsburgh, PA, USA) to quantify the stress response and to determine infection status. Blood samples were collected in 70 µL heparinized microcapillary tubes (Fisher Scientific, No. 22-362-566, Pittsburgh, PA, USA) and temporarily stored in a cooler with ice.

Estimating intensity of blood parasite infection

Because the administered drug treatment is not 100% effective, we did not assume drug-treated birds were uninfected and assessed parasite burden visually through blood smears. A small portion of the blood sampled from each bird was placed on a microscope slide (Fisher Scientific, No. 12-542-5, Pittsburgh, PA, USA), air-dried, and stained with a Fisher Hema 3 Stat packTM (Fisher Scientific, No.22-122911, Pittsburgh, PA, USA). We determined infection severity by identifying hemosporidian blood parasites to the species level (Valkiunas 2005a), and by counting the total number of gametocytes and meronts of all blood parasite taxa encountered in a sample of 10,000 red blood cells. We calculated total parasitemia (an aggregate measure of infection intensity) instead of species-specific parasitemia to quantify total burden of infection because

prevalences (proportion of sparrows infected) of individual species of blood parasite were too small for meaningful analysis.

Measuring the stress response - standard stress series protocol

We checked our trap lines frequently enough to ensure that no bird was left in a trap for more than 15 min because previous research has shown that the CORT concentrations of White-crowned Sparrows left in Potter traps for 15 min do not differ from those removed immediately after capture (Romero and Romero 2002). Breeding White-crowned Sparrows have no significant increase in CORT titers within three min of experiencing a stressor (Romero and Reed 2005) – the approaching researcher in our case. Thus, we collected all baseline blood samples (60 – 120 μ L) within three min of first sight of a trapped bird. After taking the baseline circulating CORT sample, we applied a standardized stressor (placing the sparrow in a shaded, dark cloth bag for 30 min) to quantify the individual's stress response to a novel external challenge (Romero et al. 1997). This standardized stress series protocol yields two measures of the stress response. The baseline or pre-stressor CORT concentration reflects the cumulative effect of daily stressors experienced by an individual. The second measurement, stress-induced CORT concentration, reflects the sensitivity of an individual to a novel environmental stressor (Wingfield and Romero 2001).

Within seven hours of collection in the field, we spun the chilled blood in a centrifuge (Damon IEC MB) for 5 min at 5000 rpm to separate the red blood cells from the plasma. We extracted plasma from the capillary tubes using a 100 μ L Hamilton syringe (Hamilton Company, Reno, NV, USA), placed the plasma in a 1 mL NUNC cryo tube (Fisher Scientific, No. 12-565-168B, Pittsburgh, PA, USA), and stored it in a freezer

at -20 °C. We measured plasma CORT concentrations by radioimmunoassay (Wingfield et al. 1992). A small amount of titrated steroid was added to each sample to monitor percent recovery. We then extracted CORT with redistilled dichloromethane, dried samples in a stream of nitrogen, and reconstituted samples in an aqueous buffer. Endogenous CORT then displaced labeled steroid from binding to a CORT antibody (B3-163 from Endocrine Sciences) and sample concentrations were determined by comparison to a standard curve. We analyzed samples in 5 assays with intra- and inter-assay variabilities less than 8 and 15%, respectively.

Measuring immune responsiveness – PHA immune challenge

In the summer of 2004, we assessed the strength of T-cell-mediated immunity and inflammation by measuring the local skin swelling response to 100 µL of phytohemagglutinin (PHA, Sigma H 9017, 5 mg mL⁻¹) in a phosphate-buffered saline (PBS) solution injected intradermally into the right wing-web with a with a 28 G 1cc U-100 insulin syringe (Fisher Scientific, No. 14-829-1B, Pittsburgh, PA, USA). After injection the bird was released and recaptured 24 hours later. Pre- and post-injection wing web thicknesses were measured with a digital spessimeter (Model PK – 0505, Mitutoyo Corporation, Kawasaki, Japan). We calculated the strength of an individual's immune response to the injection as the difference in average wing-web thickness measures before and after injection with PHA (Smits et al. 1999). Because the response to PHA injection stimulates infiltration of the injection site with many immune cell types, constituting innate as well as cell-mediated immune components (Martin et al. 2006), we interpreted the amount of swelling to reflect the general immune responsiveness of an individual to a novel, mitogenic challenge.

Statistical analyses

To compare baseline CORT concentrations, stress-induced CORT concentrations, and wing-web swellings among our four treatment groups, we used linear mixed model (LMM) analysis in SAS (Version 9.1.2). All response variables were approximately normally distributed (verified with Q-Q plots). Dates were assigned a numerical value (May 1st – July 31st = days 1 – 92). Fixed factors in all models included food treatment (*No Food* vs. *Food*), drug treatment (*No Drug* vs. *Drug*), year (2003 vs. 2004) and sex of bird (*Male* vs. *Female*). Plot was included as a random factor to account for the non-independence among birds within a plot. Time of season (*Early* vs. *Late* and *Day of Season*), log-transformed total blood parasitemia, and total amount of drug treatment received across the season were included as covariates (see Appendix H for more detail). After noting a sex effect ($F_{1, 105} = 5.76$, $n = 118$, $p = 0.0182$) in our initial LMM analyses with stress-induced CORT as the dependent variable, we separated by sex and re-ran the LMMs with baseline and stress-induced CORT concentrations as the response variables for each sex separately (cf. Romero et al. 1997, Bonier et al. 2007).

Results

Baseline plasma corticosterone concentrations

We collected baseline CORT concentrations from 109 sparrows (Table 1). For males, we found that the only significant predictor of an effect on baseline CORT concentration was total drug dose (the total amount of drug a sparrow received throughout the breeding season). As the total amount of drug a male received increased there was a corresponding decrease in baseline CORT concentration (Table 1). This relationship was strongest in drug-treated males (drug control males excluded) (Figure 1,

Adjusted Regression: $F_{1,15} = 13.29$, $n = 20$, slope = -11.5930, $p = 0.002$). In contrast, none of the main factors or covariates was significantly related to baseline CORT concentrations in females (Table 1).

Stress-induced plasma corticosterone concentrations

We sampled stress-induced CORT concentrations from 118 sparrows (Table 1). In males only, time of season significantly affected mean stress-induced CORT concentrations (Table 1). In early breeding season, males had significantly higher mean stress-induced CORT concentrations than in late breeding season. Furthermore, males during early breeding season had higher stress-induced CORT concentrations than both early- (Tukey Adjusted Post-hoc Test: $t_{1,105} = 2.89$, $n = 118$, $p = 0.0238$) and late-season females (Tukey Adjusted Post-hoc Test: $t_{1,105} = 4.72$, $n = 118$, $p < 0.0001$). In contrast, female sparrows not only had lower overall mean stress-induced CORT concentrations, but time of season also had no significant effect (Figure 2). Additionally, in males only, there was a significant effect of the interaction between year and drug treatment on stress-induced CORT concentrations (Table 1). Males who received drug treatment in 2003 had significantly lower average stress-induced CORT concentrations than no-drug males (Tukey Adjusted Post-hoc Test: $t_{1,61} = 2.86$, $n = 72$, $p = 0.0287$); no such effect was detected in 2004. There was no significant effect of drug treatment on female stress-induced CORT concentrations. Overall, these results suggest that males are more responsive to novel environmental stressors than females, and that this relationship is seasonally driven.

PHA immune challenge - wing-web swelling

We administered immune challenges to 33 sparrows in 2004. Food supplementation ($F_{1,8} = 7.64$, $n = 33$, $p = 0.025$) and antimalarial drug treatment ($F_{1,21} = 4.55$, $n = 33$, $p = 0.045$) enhanced immune responsiveness in sparrows of both sexes. Thus, sparrows that received both experimental treatments had higher wing web swellings than control sparrows (Tukey Adjusted Post-Hoc Test: $t_{1,22} = -2.87$, $n = 33$, $p = 0.0418$) (Figure 3a). A significant sex-by-drug treatment interaction in our model revealed that the overall effect of drug treatment was primarily driven by a strong female response ($F_{1,21} = 7.52$, $n = 33$, $p = 0.033$). Thus, drug-treated females had significantly higher wing-web swellings than non-drug treated females (Tukey Adjusted Post-Hoc Test: $t_{1,21} = -2.87$, $n = 33$, $p = 0.0418$), while drug treatment had no significant impact on wing-web swellings in male sparrows (Figure 3b).

Discussion

Baseline plasma corticosterone concentrations

Male and female *Z. leucophrys oriantha* did not differ significantly in their baseline CORT concentrations. Food supplementation did not affect baseline CORT concentrations in either sex. This result was unexpected, considering that the existing literature suggests that ample food availability (i.e. Mench 1991, Kitaysky et al. 2001, Lynn et al. 2003), predictability of food resources, (Pravosudov et al. 2001), and food supplements in a variety of wild populations are associated with decreases in baseline CORT concentrations (Clinchy et al. 2004, Schoech et al. 2004, Pedersen and Greives 2008).

Two reasons may explain why food supplementation did not affect baseline CORT concentrations in this system. Most previous supplementation studies used custom-made high fat, high protein supplements (Clinchy et al. 2004, Schoech et al. 2007). White millet is not as high in fat and protein and therefore may not sufficiently buffer our study population from food scarcity and unpredictability during the early breeding season. Alternatively, food scarcity and unpredictability in the early breeding season may not constitute a significant source of stress for our study population. Because food availability is consistently unreliable in the early breeding season, alpine sparrows may be well adapted to the widely fluctuating food resources. Our results differ from the Clinchy et al. (2004) study that demonstrated for Song Sparrows (*Melospiza melodia*) breeding in climatically stable, maritime locations, food scarcity was associated with elevated baseline CORT concentrations.

Treatment with sulphadimethoxine / pyrimethamine has been shown to suppress protozoan infections in birds (Huchzemeyer 1996, Fukui et al. 2002, Gilman et al. 2007). In our study, drug-treated male sparrows have significantly lower mean total parasitemia than untreated males (Independent samples T test: $t_{1,96} = 2.838$, $n = 98$, $p = 0.006$). The effect of drug treatment on baseline CORT concentrations is consistent with our prediction: if infection with blood parasites represents an important source of environmental stress, elimination or reduction of parasite burden in drug-treated sparrows should result in a decrease in baseline CORT concentrations. We failed, however, to detect a corresponding positive relationship between total parasitemia and baseline CORT concentrations in control birds. This issue is most likely explained by the fact that the antimalarial treatment reduces not only protozoan stages circulating in the peripheral

blood system, but also fixed tissue stages - found in the lining of the brain capillaries, spleen, and lungs (Valkiunas 2005a) – that are invisible with conventional microscopy. Thus, birds with drug-reduced parasitemia may experience an increased benefit due to the reduction of deep-tissue parasite populations, in comparison to control birds with comparable parasitemia (Huchzemeyer 1996). These hidden benefits of antimalarial treatment may be reflected in the reduced baseline CORT concentrations in drug-treated birds.

Stress-induced plasma corticosterone concentrations

Effects of sex and season on stress-induced corticosterone concentrations

We observed a seasonal difference in how males and females responded to the standardized stress series protocol: males showed high early season responses that then diminished over the course of the summer, while females maintained fairly stable, low responses throughout the summer. A proximate mechanism underlying this sex difference may be due to seasonal changes in sex steroids, such as testosterone, and their interactions with corticosterone, both of which fluctuate across the breeding season in sex-specific ways (Wingfield et al. 1984). For example, increases in testosterone stimulates the HPA axis toward enhanced secretory capacity (Schoech et al. 1999). Consequently, the high early summer stress-induced CORT concentrations observed in male sparrows may simply be a byproduct of elevated testosterone concentrations occurring during this period (Wingfield and Farner 1978, Morton 2002).

The physiological mechanism underlying the seasonal sex difference in stress-induced CORT concentrations may ultimately be linked to the distinct energetic demands males and females experience during breeding. Life-history theory predicts that the sex

investing most in parental effort should experience the greatest fitness cost if reproduction is sacrificed (Roff 1992). Due to the compressed breeding season and short lifespan of these sparrows, high levels of circulating CORT can lead to nest abandonment and a considerable loss in lifetime reproductive success. Thus, one would expect to observe a down-regulation of the stress response during the breeding season (Wingfield and Sapolsky 2003). Studies on White-crowned Sparrows (Holberton and Wingfield 2003) and other bird species (Wingfield et al. 1992, Wilson and Holberton 2004) breeding in harsh environments (e.g. high latitude or elevation) suggest that females, having invested more parental effort in reproduction, will benefit most from down-regulation of the stress response.

Treatment effects on stress-induced corticosterone concentrations

We found no significant impact of food supplementation treatment on stress-induced CORT concentrations in either males or females, counter to our initial predictions. Other studies have found mixed effects of food supplementation on stress-induced CORT concentrations. For example, there was no difference in stress-induced CORT concentrations among wild Florida Scrub-jays fed high fat - high protein, high fat - low protein, and unsupplemented, control diets (Schoech et al. 2007). In contrast, Song Sparrows (*Melospiza melodia*) without access to food supplements had significantly higher stress-induced CORT concentrations than non-supplemented individuals (Clinchy et al. 2004), suggesting sparrows with access to predictably abundant food resources are buffered from chronic environmental stress caused by food shortage and predator pressure. Our results reinforce the notion that for this study population, subject to highly

variable environmental conditions, unpredictable and scarce food resources do not constitute a significant source of stress.

Drug treatment significantly affected male stress-induced CORT concentrations in 2003 only, with drug-treated males experiencing lower stress-induced CORT concentrations than males that did not receive drug treatment. Although it is not clear why this pattern was not significant in 2004, these results imply that males infected with blood parasites mount stronger responses to novel environmental stressors. In line with our predictions, this result suggests that blood parasites may represent a significant source of stress for this study population. Similarly, Dunlap & Schall (1995) found that male fence lizards (*Sceloporus occidentalis*) infected with *Plasmodium mexicanum* had higher stress-induced CORT concentrations than uninfected lizards.

Immune responsiveness

Food supplementation and antimalarial drug treatment significantly enhanced the strength of sparrow non-specific immune response; the factorial design of this study suggests that treatments had an additive effect on wing web swelling. Past research indicates that an individual requires sufficient energy resources to mount a strong immune response to a new, antigenic challenge (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Norris and Evans 2000). Our results demonstrate that birds with access to predictably abundant food supply were allocating more resources in an immune response toward a novel antigen, while continuing to perform other energetically costly activities like reproduction. Drug treatment also increased immune responsiveness, but only in females. This suggests that breeding females chronically infected with hemsporidian parasites may only be able to mount an attenuated response

to novel antigens. This argument assumes that mounting an immune response to PHA injection is energetically costly and there may be a trade-off between resources allocated toward parental effort and immune responsiveness to novel antigens (see Appendix I).

Relatively few studies have focused on the effects of chronic parasitism on immune function in free-ranging organisms (see i.e. Owen and Clayton 2007). Both observational and experimental studies have shown that hosts infected with parasites often have lower immune responsiveness to PHA challenges than uninfected hosts (i.e. Johnsen and Zuk 1999, Navarro et al. 2003, Lindstrom et al. 2004). Yet none of these studies actually differentiate between two alternative explanations: active immunosuppression by existing parasite infections or reallocation of resources to different defenses of a functioning immune system (Owen and Clayton 2007). If blood parasites were immunosuppressive, we would expect drug treatment to positively affect both male and female immune responsiveness to PHA challenge. Food supplementation significantly increased wing web swelling in both sexes, suggesting that energy resources are important for mounting a strong response to PHA challenge. Thus, our results suggest that female sparrows are not immunosuppressed, but instead allocate resources first toward reproduction and current immunogenic challenges, such as infection with blood parasites. Future research should investigate how breeding females with chronic blood parasite infections respond to novel parasitic challenges, and whether females cleared of chronic infections are indeed more responsive to those parasitic challenges than females that maintain chronic blood parasite infections.

Conclusions

Food scarcity and unpredictability in the early breeding season may be so commonplace that they do not comprise a significant source of stress for our study population. The insensitivity of baseline and stress-induced CORT concentrations to supplemented food may simply reflect the adaptation of these sparrows to the poor and widely fluctuating food resources of alpine ecosystems during the early breeding season. However, breeding birds exposed to unpredictable food shortages throughout the season may not have as many resources to devote to mounting strong immune responses to novel antigens, potentially increasing susceptibility to acquiring new parasitic infections. Once infected, blood parasites represent a significant source of environmental stress for pre-parental male sparrows; they can also decrease the resources females have available to allocate outside of breeding to other energetically demanding activities, such as mounting an immune response to novel antigenic challenges. Finally, the theme of sex differences resonates throughout our results; we urge future studies of physiological and behavioral responses to environmental challenges should consider the distinct demands males and females face throughout their life cycles.

Table 5.1 Results, separated by sex, from the linear mixed models with baseline and stress-induced CORT concentrations as the response variables with significant results shown in bold.

baseline CORT concentrations						
male n = 71, female n = 38						
effects	males			females		
	F	d.f.	p	F	d.f.	p
year	0.14	1, 8	0.718	0.22	1, 8	0.315
food	0.63	1, 8	0.509	0.15	1, 8	0.057
drug	0.00	1, 56	0.961	0.87	1, 26	0.727
season	1.22	1, 56	0.275	0.72	1, 26	0.954
total drug dosage	5.27	1, 56	0.026	0.83	1, 26	0.674
log parasitaemia	1.15	1, 56	0.288	0.49	1, 26	0.450

stress-induced CORT concentrations						
male n = 72, female n = 46						
	F	d.f.	p	F	d.f.	p
year	0.08	1, 8	0.787	1.15	1, 8	0.654
food	0.94	1, 8	0.939	4.92	1, 8	0.057
drug	3.69	1, 61	0.059	0.12	1, 36	0.359
season	25.09	1, 61	<0.0001	0.00	1, 36	0.472
total drug dosage	3.36	1, 61	0.072	0.18	1, 36	0.372
log parasitaemia	3.94	1, 61	0.052	0.58	1, 36	0.489
drug x year	6.88	1, 61	0.011	-	-	-

Figure 5.1 Baseline CORT concentrations of drug-treated males decreased as total drug dose increased (Adjusted Regression: $F_{1,15} = 13.29$, $n = 20$, slope = -11.593, $p = 0.002$). The model-predicted adjusted relationship between total drug dose and baseline CORT concentration for drug-treated males only, is shown by plotting the residuals for total drug dose against the residuals for baseline CORT concentration from our linear mixed model.

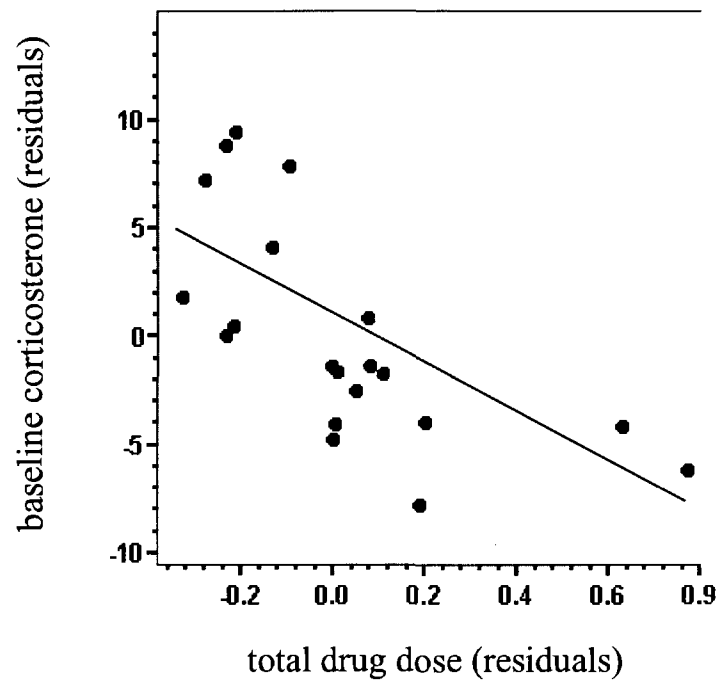


Figure 5.2 Early season males were on average more responsive to environmental stressors than late season males (Tukey Adjusted Post-hoc Test: $t_{1,105} = 5.12$, $n = 118$, $p < 0.0001$), early season females (Tukey Adjusted Post-hoc Test: $t_{1,105} = 2.89$, $n = 118$, $p = 0.0238$), and late season females (Tukey Adjusted Post-hoc Test: $t_{1,105} = 4.72$, $n = 118$, $p < 0.0001$). Time of season had no significant effect on female responsiveness to environmental stressors. Brackets indicate significant differences between groups at a significance level of $p < 0.05$, and bars around the mean represent standard errors.

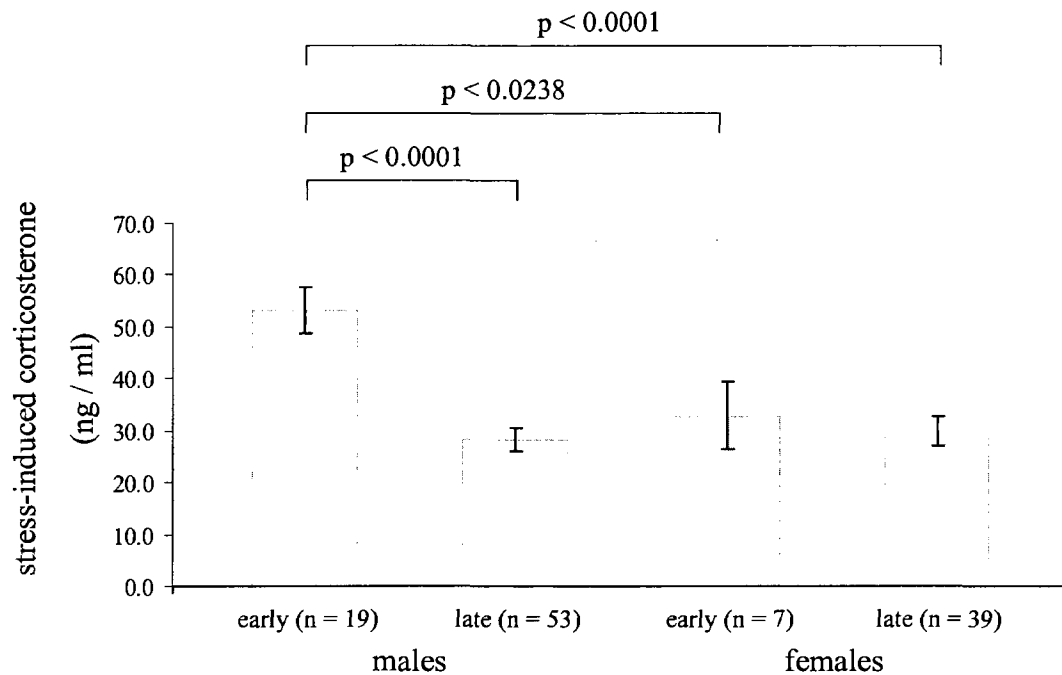
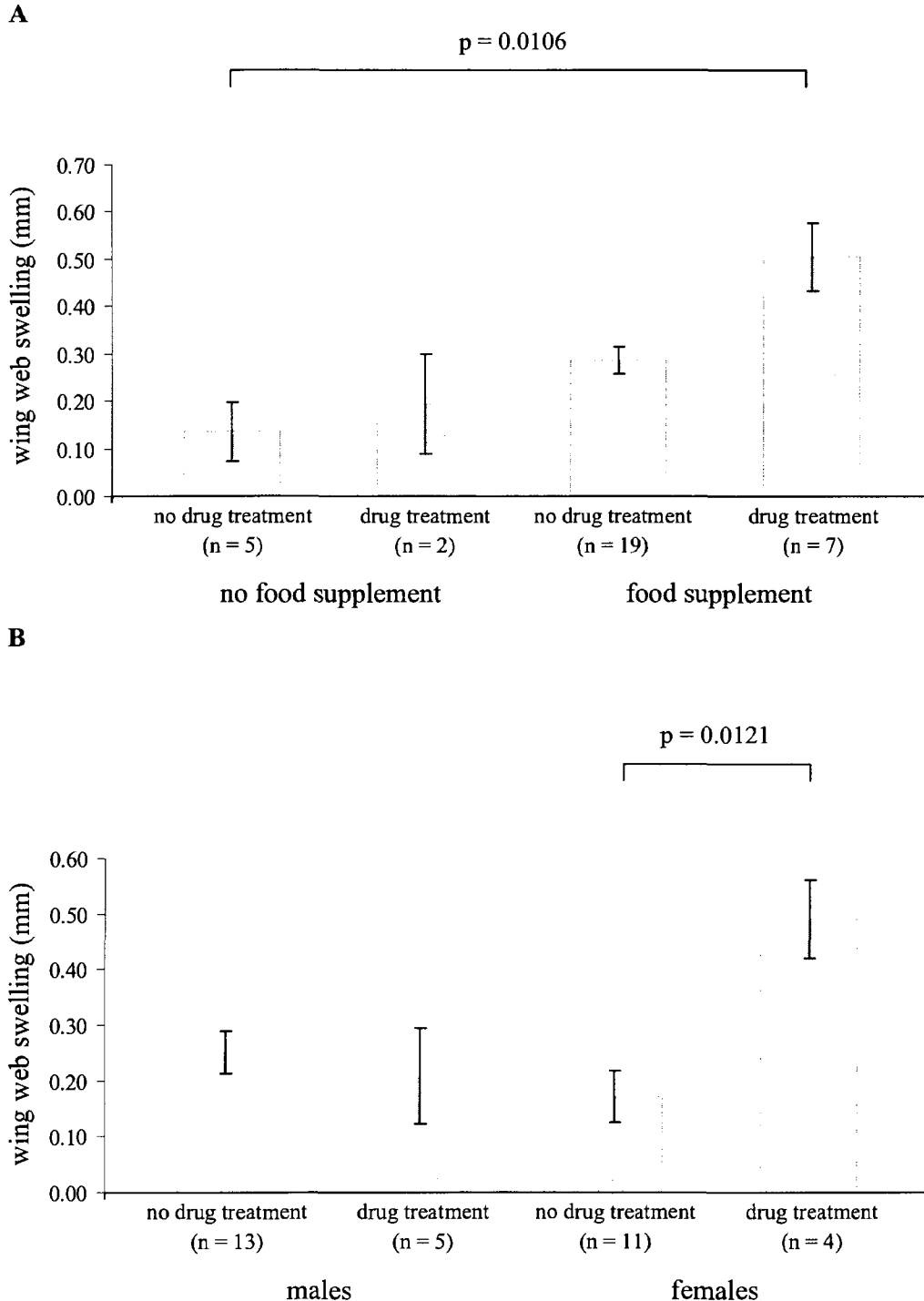


Figure 5.3 A Both food supplementation and drug treatment increased average immune responsiveness in all birds. Sparrows that received both experimental treatments had significantly higher wing web swellings than control birds (Tukey Adjusted Post-hoc Test: $t_{1,21} = -3.50$, $n = 33$, $p = 0.0106$). **B** Females that received drug treatment were on average more immune responsive than non-drug treated females (Tukey Adjusted Post-hoc Test: $t_{1,21} = -3.44$, $n = 33$, $p = 0.0121$). Brackets denote significance level of $p < 0.05$ and bars around the mean represent standard errors.



Chapter VI

Conclusion

We conducted a series of studies (throughout the summers of 2003 – 2007) on a population of Mountain White-crowned Sparrows (*Zonotrichia leucophrys oriantha*) breeding in the Colorado Rockies to better understand the ecology of avian hemosporidian parasites. The first study (Chapter two) described the avian host, potential dipteran vector, and parasite community present on our field sites. Overall, *Leucocytozoon* spp. were the most prevalent of hemosporidian parasites in the avian community, *Plasmodium* spp. and *Haemoproteus* spp. were the least prevalent, and *Trypanosoma avium* had an intermediate prevalence. White-crowned Sparrows were infected with six morphologically defined hemosporidian species: *Leucocytozoon fringillinarum*, *L. majoris*, *Haemoproteus coatneyi*, *Plasmodium relictum*, *P. vauhani*, and *Trypanosoma avium*. The most prevalent of hemosporidian parasites in the sparrow population was *Leucocytozoon* spp. (*L. fringillinarum* had the highest prevalence overall), while sparrows had lower prevalence of *Trypanosoma avium* and *Haemoproteus* spp. than the rest of the avian community. The higher prevalence of *Leucocytozoon* and *Trypanosoma* spp. is most likely due to the abundance of ornithophilic black fly species breeding on these sites, and the fact that *Leucocytozoon* spp. can complete development across a wide-range of black fly species, including those with mammal-feeding preferences (Desser and Yang 1973, Adler et al. 2004). Additionally, the low prevalence

of *Plasmodium* spp. in the avian community is expected due to the mammalian feeding preferences of the mosquitoes captured on our field sites. This suggests *Leucocytozoon* spp. may have more important effects on the avian community as a whole than the other hemosporidian parasites.

Based on detailed prevalence and estimated abundance data for hemosporidian parasites, avian hosts, and potential arthropod vectors, we outlined a tentative framework of potential host-vector-parasite associations. We proposed the existence of three possible transmission foci falling within the three common habitat types (forest, meadow, and willow) present on our field sites. Each transmission focus is maintained by the avian and dipteran species that have their maximum abundances associated with that habitat type. Transmission of different parasite genera is dependent upon the vectors associated with each habitat. Transmission of parasite genera among foci may be a function of bird and dipteran species that are equally abundant across all habitat types. We propose that *Plasmodium* transmission may be maintained at low prevalence in forested habitats by the competent mosquito species, in particular *Ochlerotatus communis*, and is transmitted minimally to other habitat types by generalist bird species and potentially *O. implicatus* because its abundance is minimally affected by habitat type. *Leucocytozoon* and *Trypanosoma* spp. may be transmitted readily within each focus and among foci by the black fly species associated with each habitat type, and possibly by the generalist, extremely abundant *Simulium arcticum* complex and *S. silvestre* / *S. craigi*.

However, parasite distribution across a landscape can be influenced by active vector preferences for certain avian hosts, the ability of the parasite to infect a vertebrate

host, or by other factors such as climatic variables and microhabitat requirements that may place vertebrate hosts and vectors into frequent contact with each other (Hellgren et al. 2008). Thus, to further tease apart the proposed host-parasite-vector associations for *Leucocytozoon*, we conducted a molecular study (Chapter three) to describe the *Leucocytozoon* community from DNA amplified from the avian host, as well as the dipteran vectors (*S. silvestre* / *S. craigi* and *Greeneira denaria*). We described three divergent clades of *Leucocytozoon* spp. from *col* sequences amplified from avian host species and primarily *S. silvestre* / *S. craigi*. One clade contained parasites sampled from both black fly species and was the most divergent of the clades, suggesting parasites in this clade may be from an undescribed genus of malarial parasite. Another clade included parasite DNA amplified from avian hosts and *S. silvestre* / *S. craigi*, suggesting this clade may comprise *Leucocytozoon* spp. that predominantly infect passerine birds living in willow and meadow habitats. This clade also included haplotypes of avian host species that were morphologically consistent with *L. fringillinarum* and *L. majoris* and were identical to sequences amplified from *S. silvestre* / *S. craigi*. Thus, *S. silvestre* / *S. craigi* may be an important vector of these two parasite species that infect the White-crowned Sparrow population on our field sites. The final clade was comprised of parasite DNA amplified only from *S. silvestre* / *S. craigi*, implying these *Leucocytozoon* parasites may infect bird hosts that we did not sample at our field sites. Because DNA was amplified from non-bloodfed, host-seeking black flies, this study established that both black fly species are potential vectors for a large diversity of *Leucocytozoon* haplotypes on our field sites.

Once we established a link between *L. fringillinarum*, the White-crowned Sparrow population, and a potential vector species, *S. silvestre* / *S. craigi*, we developed a mathematical model (Chapter three) to define the ecological, parasite, and host parameters that are important for maintaining transmission of *L. fringillinarum* in this system. With this multi-year model, we determined that both the relapse phenomenon and YOY birds are essential for parasite persistence from year to year in temperate, seasonal systems. Of the parameters for the probability of transmission upon vector-host contact and the rates governing compartmental transitions, the mean prevalence of infectious black flies was the most sensitive to changes in the probability of transmission from a relapsing infectious bird to a susceptible black fly (b_R), and the rate at which relapsing infectious individuals transition into the latently infected overwinter stage (δ_R). Further, when we removed the relapsing infectious adult compartment we did not get persistence across summer seasons.

We also concluded that YOY birds play an important role in persistence of seasonal transmission of *L. fringillinarum*. The degree of seasonal overlap between the peaks of daily nestling hatching and black fly emergence was important for both the final prevalence of infected birds and the mean prevalence of infectious black flies. YOY birds, especially in temperate systems, represent a pulse of susceptible hosts each season (Lord and Day 2001b, Unnasch et al. 2006). When the possibility of transmission between infectious black flies and YOY birds was eliminated from the model, *L. fringillinarum* quickly fades after a couple of seasons due to the exhaustion of susceptible birds. However, distinguishing between nude nestlings and feathered YOY was unnecessary, as shown by the insensitivity of our output statistics to the daily rate

nestlings acquire feathers (F) and the probability of transmission from an infectious black fly to a nude nestling (b_N).

The model also indicated that the number of black fly bites bird⁻¹ day⁻¹ (r) had the greatest influence of the parameters on all of the output statistics, especially within the range one to three black fly bites bird⁻¹ day⁻¹. However, above three black fly bites bird⁻¹ day⁻¹, the output statistics were relatively insensitive to further increases in the daily bite rate (Figure 5). Thus, *S. silvestre* / *S. craigi* likely is taking on average two to three bites bird⁻¹ day⁻¹, because when the model was run with an r of one bite bird⁻¹ day⁻¹, we were unable to adjust the remaining parameters within their natural ranges to ensure all birds entered the overwinter period as latently infected birds.

Unexpectedly, we discovered as the time of season black flies reach their peak emergence (q_s) shifts earlier in the transmission season, we see an increase in parasite prevalence in both the bird and black fly populations. When the peak of the black fly emergence curve was shifted earlier in the season, susceptible black flies interact with relapsing infectious birds and become infected earlier in the season, which results in the number of infectious black flies reaching their daily peak at approximately the same time as nestlings begin to hatch during the breeding season. Further, because more acutely infectious adults were dying from parasite-induced death the number of nestlings hatching on a daily basis decreases. Thus, knowing that the cues birds and black flies use to time reproduction are distinct, as warming occurs at higher elevations we predicted parasite prevalence to increase in both the host and vector populations on these sites, resulting in an overall decline in avian recruitment. This also indicates that the bird-feeding black flies that are of lower abundance but emerge and reach peak abundances

earlier in the breeding season (*G. denaria* and *Metacnephia jeanae*), may play a significant role in reintroducing *L. fringillinarum* to young of the year birds from relapsing infectious adult birds.

Finally, to begin to overlay the transmission ecology of these parasites with their effects on avian host ecology, we conducted a comprehensive field experiment (Chapter five) to investigate the potential fitness effects of these parasites on their bird hosts. We investigated how food scarcity and chronic infection with *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* parasites (manipulated by food supplementation and antimalarial drug treatments) affected sparrow stress (corticosterone) and immune responses (wing web swelling), respectively. Both of which are physiological mediators of survival and reproduction.

The insensitivity of baseline and stress-induced CORT concentrations to supplemented food treatment simply reflected the adaptation of these sparrows to the poor and widely fluctuating food resources of alpine ecosystems during the early breeding season. Thus, food scarcity and unpredictability in the early breeding season may be so commonplace that they do not comprise a significant source of stress for our study population. However, breeding birds, especially female sparrows, exposed to unpredictable food shortages throughout the season may not have as many resources to devote to mounting strong immune responses to novel antigens, potentially increasing their susceptibility to new parasitic infections. Once infected, blood parasites did represent a significant source of environmental stress for pre-parental male sparrows; they also decreased the resources females have available to allocate outside of breeding to other energetically demanding activities, such as mounting an immune response to

novel antigenic challenges. Finally, the theme of sex differences resonates throughout our results from this study; future studies of physiological and behavioral responses to environmental challenges must consider the distinct demands males and females face throughout their life cycles.

In conclusion, this series of studies revealed that the hemosporidians transmitted by black fly vectors, *Leucocytozoon* and *Trypanosoma* spp., are the most prevalent of hemosporidians in this high-elevation, avian community. This research also indicates that the bird-feeding black fly species *S. silvestre* / *S. craigi* is an important vector for a diversity of *Leucocytozoon* spp. in the community, due to its overall high abundance, lack of habitat preferences, and time of season when peak abundance is attained. However, black fly species that emerge and reach peak abundance early in the season, such as *G. denaria* and *M. jeanae*, may also play a significant role in reintroducing *Leucocytozoon* spp. from relapsing infectious adults to young of the year birds. Once infection is reseeded, the highly abundant *S. silvestre* / *S. craigi* may continue the transmission cycle throughout the remainder of the summer season. In White-crowned Sparrows, roughly half of the population was infected with *Leucocytozoon* spp. throughout the summer season, while the proportions of the population infected with other hemosporidian parasites were significantly smaller. With warming at higher elevations a certainty, black fly vectors may emerge and reach peak abundances earlier in the summer season resulting in an increase in prevalence of *Leucocytozoon* spp. If prevalence does increase, sparrows may face added costs of parasitism through increased stress levels and decreased energy available for other energetically expensive activities, such as reproduction, which may result in decreased population size over time.

Appendix A

Family	Species	RMBL Counts	WA Counts
	Red-shafted Flicker	18	15
Regulidae	Golden-crowned Kinglet	2	2
	Ruby-crowned Kinglet	9	24
Scolopacidae	Common Snipe	0	3
Strigidae	Flammulated Owl	0	1
Tetraonidae	Blue Grouse	4	7
Thraupidae	Western Tanager	0	5
Trochilidae	Broad-tailed Hummingbird	25	19
Troglotyidae	House Wren	34	19
Turdidae	American Robin	82	45
	Hermit Thrush	1	0
	Mountain Bluebird	1	1
	Swainson's Thrush	24	0
	Townsend's Solitaire	1	0
Tyrannidae	Cordilleran Flycatcher	3	3
	Dusky Flycatcher	13	7
	Hammond's Flycatcher	1	0
	Olive-sided Flycatcher	2	5
	Western Wood-pewee	25	27
	Willow Flycatcher	10	0
Vireonidae	Warbling Vireo	44	52

Appendix B

Family	Species	RMBL Count	WA Count
Culicidae	<i>Culex tarsalis</i>	3	0
	<i>Culiseta alaskaensis</i>	11	3
	<i>Culiseta impatiens</i>	40	42
	<i>Culiseta incidins</i>	2	0
	<i>Culiseta inornata</i>	4	1
	<i>Ochlerotatus cataphylla</i>	1041	828
	<i>Ochlerotatus communis</i>	72	37
	<i>Ochlerotatus dorsalis</i>	0	2
	<i>Ochlerotatus fitchii</i>	5	0
	<i>Ochlerotatus hexodontus</i>	27	40
	<i>Ochlerotatus impiger</i>	0	1
	<i>Ochlerotatus implicatus</i>	952	1053
	<i>Ochlerotatus intrudens</i>	14	4
	<i>Ochlerotatus melanimon</i>	1	1
	<i>Ochlerotatus pionips</i>	1	0
	<i>Ochlerotatus pullatus</i>	31	17
	<i>Ochlerotatus punctor</i>	6	6
	<i>Ochlerotatus schizopinax</i>	0	2
Simuliidae	<i>Greniera denaria</i>	29	30
	<i>Helodon onchodactylus</i> cpx	5	3
	<i>Metacnephia jeanae</i>	70	47
	<i>Prosimulium exigins</i>	194	65
	<i>Prosimulium fulvum</i>	1	0
	<i>Prosimulium hirtipes</i> group	22	43
	<i>Prosimulium uinta</i>	0	1
	<i>Simulium arcticum</i> cpx	339	175
	<i>Simulium canonicolum</i>	91	31
	<i>Simulium decorum</i>	0	2
	<i>Simulium exulatum</i> / <i>pilosum</i>	112	97
	<i>Simulium hunteri</i>	0	1
	<i>Simulium irritatum</i> / <i>venustum</i>	25	34
	<i>Simulium piperi</i>	19	20
	<i>Simulium silvestre</i> / <i>craigi</i>	861	553
	<i>Simulium vandalicum</i>	19	19
	<i>Simulium vittatum</i> cpx	6	7

Appendix C

During the summers of 1999 – 2008, sparrows were captured between 06:00 and 12:00 each day in millet-baited, single- and double-cell (18 cm x 18 cm x 18 cm), galvanized wire Potter traps. Sparrows were marked with a unique combination of three colored leg-bands and one metal U.S. Fish and Wildlife Service band (Bird Banding Laboratory, Patuxent, MD, USA). Upon capture, we sexed each bird, collected standard morphometric measures, and took blood samples from the brachial vein with a sterile 26-gauge hypodermic needle (Fisher Scientific Precision Glide, No. 14-826-15, Pittsburgh, PA, USA) to determine infection status. Blood samples were collected in 70 μ L heparinized microcapillary tubes (Fisher Scientific, No. 22-362-566, Pittsburgh, PA, USA) and temporarily stored in a cooler on ice. A small portion of the blood sampled from each bird was placed on a microscope slide (Fisher Scientific, No. 12-542-5, Pittsburgh, PA, USA), air-dried, and stained with a Fisher Hema 3 Stat packTM (Fisher Scientific, No. 22-122911, Pittsburgh, PA, USA). We identified *Leucocytozoon* parasites to species (Valkiunas 2005b) and counted the total number of gametocytes out of 10,000 red blood cells to quantify intensity of infection.

Each year we searched for nests from June 5 through July 30. We located nests during building, egg-laying, incubation, and feeding of nestlings via territory searches and bird behavioral cues (Martin and Geupel 1993). We alternated which plots were searched every three days to eliminate temporal bias. For each nest, we recorded the color-band combinations, if available, of the associated male and female, and marked its

location. We checked each nest once every two to three days and recorded date, time, stage of nest, number of eggs or young, and estimated age of the young. We measured nestling mass and tarsus seven days after hatching (Morton 2002) because nestlings usually fledge on day nine. Nests were “successful” if we observed one or more of the following pieces of evidence: a flattened nest rim and fecal droppings in the nest, nearby adult alarm calls, begging calls of the young, or observed fledgling feedings (Martin and Geupel 1993). For failed nests, “failure” was recorded as “predation” if there were signs of predator disturbance and missing eggs or nestlings or “desertion” if cold eggs or dead nestlings remained in the nest.

To ensure a representative sample of the black fly community in our field sites, we sampled black flies at regular intervals from May 15th – July 31st in 2005 and 2007, across a variety of common habitats. Each field site was first stratified by the following broad habitat types: willow, alpine meadow, and forest habitat patches. We then took a random sample from each field site of the habitat patches. At varying intervals throughout the summer, we sampled blood-fed mosquitoes from the selected habitat patches in both field sites. We collected black flies from carbon-dioxide baited Center for Disease Control (CDC) miniature light traps (John W. Hock Company, No. 512 fine mesh collection cups, Gainesville, FL, USA) (Service 1976). CDC traps were set and checked every 24 hours. Due to the chance of trap failure, traps were paired approximately 50 m from each other within each sampled patch. We trapped each habitat patch for two consecutive nights over an interval of eight days. After each two-day trapping session, traps were pulled and rotated to the other field site to minimize any potential effects of season or weather on trap success. Sampling occurred at regular

intervals from May 19th – July 31st each year. Within two hours of capture in the field, all biting dipterans were placed into a garbage bag and exposed to cotton soaked in triethylamine (Fisher Scientific, Amber Glass, No. BP616-500, Pittsburgh, PA, USA) for five minutes in a well-ventilated area. Once flies were immobilized, we separated out the black flies from other biting dipterans and stored them immediately in 95% ethanol for future identification and parasite DNA analyses. Female black flies were identified to species or species complex based on structural characters presented in the keys and illustrations of Adler et al. (2004). Identifications were facilitated by genitalic preparations of selected specimens. Representative specimens have been deposited in the Clemson University Arthropod Collection, Clemson, South Carolina.

Appendix D

Bird Modules

YOY bird population

$$\frac{dSN}{dt} = \frac{A_B}{2 \left(e^{\frac{(t-q_B)^2}{c_B}} \right)} - \left(rb_N \left(\frac{IS}{S} \right) + x + d_1 \right) SN$$

susceptible nude nestlings

$$\frac{dSJ}{dt} = xSN - \left(rb_F \left(\frac{IS}{S} \right) + a + d_2 \right) SJ$$

susceptible feathered YOY

$$\frac{dEJ}{dt} = r \left(\frac{IS}{S} \right) (b_N SN + b_F SJ) - (\lambda_B + d_2) EJ$$

exposed feathered YOY

$$\frac{dIJ}{dt} = \lambda_B EJ - (\delta_A + d_3) IJ$$

acutely infectious YOY

$$\frac{dCIJ}{dt} = \delta_A IJ - d_2 CIJ$$

chronically infectious YOY

Adult bird population

$$\frac{dSA}{dt} = - \left(rb_F \left(\frac{IS}{S} \right) + d_2 \right) SA$$

susceptible feathered adults

$$\frac{dEA}{dt} = rb_F \left(\frac{IS}{S} \right) SA - (\lambda_B + d_2) EA$$

exposed feathered adults

$$\frac{dIA}{dt} = \lambda_B EA - (\delta_A + d_3) IA$$

acutely infectious adults

$$\frac{dCIA}{dt} = \delta_A IA - d_2 CIA$$

chronically infectious adults

Black fly module

$$\frac{dSS}{dt} = \frac{A_s}{\left(e^{\frac{(t-q_s)^2}{c_s}} \right)} - SS \left(\frac{r}{B} (b_A (IA + IJ) + b_R RIA + b_C (CIA + CIJ)) + d_4 \right)$$

susceptible black flies

$$\frac{dES}{dt} = r b_s \left(\frac{BI}{B} \right) SS - (\lambda_s + d_5) ES$$

exposed black flies

$$\frac{dIS}{dt} = \lambda_s ES - d_6 IS$$

infectious black flies

Appendix E

Bird Modules

YOY bird population

$$\frac{dSN}{dt} = \frac{A_B}{2 \left(e^{\frac{(MOD(t,360)-q_B)^2}{c_B}} \right)} - \left(rb_N \left(\frac{IS}{S} \right) + x + d_1 \right) SN \quad \text{susceptible nude nestlings}$$

$$\frac{dSJ}{dt} = xSN - \left(rb_F \left(\frac{IS}{S} \right) + a + d_2 \right) SJ \quad \text{susceptible feathered YOY}$$

$$\frac{dEJ}{dt} = r \left(\frac{IS}{S} \right) (b_N SN + b_F SJ) - (\lambda_B + d_2) EJ \quad \text{exposed feathered YOY}$$

$$\frac{dIJ}{dt} = \lambda_B EJ - (\delta_A + d_3) IJ \quad \text{acutely infectious YOY}$$

$$\frac{dCIJ_1}{dt} = \delta_A IJ - (\sigma_J + d_2) CIJ_1 \quad \text{chronically infectious YOY}$$

$$\frac{dCIJ_{i...4}}{dt} = \sigma_J CIJ_1 - (\sigma_J + d_2) CIJ_{i...4}$$

Adult bird population

$$\frac{dSA}{dt} = (SJ + SA) output - \left(rb_F \left(\frac{IS}{S} \right) + a + d_2 \right) SA \quad \text{susceptible feathered adults}$$

$$\frac{dEA}{dt} = rb_F \left(\frac{IS}{S} \right) SA - (\lambda_B + d_2) EA \quad \text{exposed feathered adults}$$

$$\frac{dIA}{dt} = \lambda_B EA - (\delta_A + d_3) IA \quad \text{acutely infectious adults}$$

$$\frac{dCIA_1}{dt} = \delta_A IA - (\sigma_A + d_2) CIA_1 \quad \text{chronically infectious adults}$$

$$\frac{dCIA_{i...4}}{dt} = \sigma_A CIA_1 - (\sigma_A + d_2) CIA_{i...4}$$

$$\frac{dRIA_1}{dt} = (LIJ + LIA)output - (\delta_R + d_2) RIA_1 \quad \text{relapsing infectious adults}$$

$$\frac{dRIA_{i...20}}{dt} = \delta_R RIA_1 - (\delta_R + d_2) RIA_{i...20}$$

Black fly module

$$\frac{dSS}{dt} = \frac{A_s}{\left(e^{\frac{(MOD(t,360)-q_s)^2}{c_s}} \right)} - SS \left(\frac{r}{B} (b_A (IA + IJ) + b_R RIA + b_C (CIA + CIJ)) + d_4 \right) \quad \text{susceptible black flies}$$

$$\frac{dES}{dt} = r b_s \left(\frac{BI}{B} \right) SS - (\lambda_s + d_5) ES \quad \text{exposed black flies}$$

$$\frac{dIS}{dt} = \lambda_s ES - d_6 IS \quad \text{infectious black flies}$$

Ovens and the overwintering bird population

Overwintering susceptible YOY and adult birds

$$\begin{aligned} SJinput &= \gamma SJ & \text{susceptible YOY oven} \\ SJoven &= oven(SJinput, 180, 180) \\ SJoutput &= outflow(SJoven) \end{aligned}$$

$$\frac{dSJ}{dt} = SJinput - SJoutput \quad \text{susceptible YOY}$$

$$\begin{aligned} SAinput &= \gamma SA & \text{susceptible adult oven} \\ SAoven &= oven(SAinput, 180, 180) \\ SAoutput &= outflow(SAoven) \end{aligned}$$

$$\frac{dSA}{dt} = SA_{input} - SA_{output}$$

susceptible adults

Overwintering, latently infected YOY and adult birds

$$LIJ_{input} = \sigma_J CIJ_4$$

latently infected YOY oven

$$LIJ_{oven} = oven(LIJ_{input}, 160, 200)$$

$$LIJ_{output} = outflow(LIJ_{oven})$$

$$\frac{dLIJ}{dt} = LIJ_{input} - LIJ_{output}$$

latently infected YOY

$$LIA_{input} = \sigma_A CIJ_4$$

latently infected adult oven

$$LIA_{oven} = oven(LIA_{input}, 160, 200)$$

$$LIA_{output} = outflow(LIA_{oven})$$

$$\frac{dLIA}{dt} = LIA_{input} - LIA_{output}$$

latently infected adults

Appendix F

Avian and black fly transmission parameters

Values for the contact rate (r) between host and vector in this system (the number of bites a black fly makes $\text{bird}^{-1} \text{day}^{-1}$), and the probabilities of transmission from an infectious black fly to a nude nestling (b_N) and a feathered YOY or adult bird (b_F), were selected based on the performance of the model and how comparable model outputs were to empirical data. We assumed that because nestlings are altricial, without feathers for a period of six to seven days, and lack anti-vector behaviors such as preening, they would be easier for biting dipterans to feed off on. Thus, the probability of transmission from an infected vector to a nude nestling was set higher than the probability of transmission to a feathered bird. We selected values for r of two bites $\text{blackfly}^{-1} \text{bird}^{-1} \text{day}^{-1}$, for b_N of 0.10, and for b_F of 0.05 because the model generated somewhat realistic prevalences of infection in the bird (70%) and black fly population (23%).

Empirical data collected during the summers of 2003, 2004, and 2005 indicate that the mean prevalence of sparrows infected with *L. fringillinarum* on these sites is on average 50% (Murdock unpublished). However, if our field sample contains some chronically infectious birds across each summer, the empirical data may underestimate prevalence; our visual scoring method can miss infections with low parasitemia (1-3 gametocytes per 10,000 red blood cells), resulting in false negatives. Additionally, in Algonquin, Ontario, 90% to 100% of ornithophilic black flies had *Leucocytozoon* sporozoites present in their salivary glands (Adler et al. 2004). Hellgren et al. (2008)

found 62% of blood-fed black flies ($n = 38$) were positive for *Leucocytozoon* spp. infections. However, the prevalence of infectious black flies infected with a particular species of *Leucocytozoon* may be much lower, because both of these studies examined multiple black fly and *Leucocytozoon* species (also see Chapter three).

The probabilities of transmission from an acutely (b_A), relapsing (b_R), and chronically infectious (b_C) birds to a feeding, susceptible black fly were also somewhat inferred. *Leucocytozoon* parasitemia is highest, intermediate, and lowest in acute, relapsing, and chronic infections, respectively (Khan and Fallis 1970, Allan and Mahrt 1989). Further, increases in parasitemia correspond to an increase in infectivity to biting vectors (Mackinnon and Read 1999). Based on these results, we have assigned transmission probabilities associated with the distinct states of infectiousness to reflect these differences in infectivity to the vector.

Avian and black fly compartmental transition parameters and death rates

We estimated the rate nestlings acquire feathers based on reproductive data from this population (Foufopoulos unpublished) and a population breeding in Tioga Pass, CA (Morton 2002). Nestlings are fully feathered in down feathers from day six to seven in these populations. Thus, the feather rate (F) is simply the inverse of this time interval. To calculate the rate an exposed bird becomes infectious (λ_B) and the rate an exposed black fly becomes infectious (λ_S), we took the inverse of the prepatent period of *L. fringillinarum* in the avian and black fly host. For *L. fringillinarum*, the first appearance of gametocytes in the blood of the bird after being bitten by an infectious vector is five days (Valkiunas 2005a), and the first mature oocysts appear in the midgut lining of the black fly approximately four days after biting an infectious bird, and we assume

sporozoites appear on day five (Adler et al. 2004, Valkiunas 2005a). However, these estimates are based on parasite development times assessed under laboratory conditions at room temperature; the prepatent period in the vector may be of longer duration in an alpine system, which experiences wide fluctuations in daily temperatures (CM per obs.).

We estimated the rate an acutely infectious bird transitions into the chronically infectious stage (δ_A) by determining the interval of time it takes a bird to reach peak parasitemia. Peak parasitemia in primary infections occurs five to 12 days after gametocytes first appear in the blood stream and then shortly thereafter decrease to chronic levels (Valkiunas 2005a). In our model, we assumed that peak parasitemia occurred at day eight and birds entered the chronic phase on day 11. Thus, the rate an acutely infectious bird becomes chronically infectious (δ_A) is equivalent to 11^{-1} days⁻¹.

We then assumed that chronically infectious birds remain in this state until the end of the breeding season when they enter the latently infected, overwinter stage where they are no longer infectious to vectors. The rates that a chronically infectious YOY or adult bird transition into the latently infected, overwinter stage (σ_J and σ_A , respectively), and the rate that susceptible YOY and adult birds transition into the susceptible overwinter stage were set to their current values to ensure all birds transitioned into the associated overwinter stages by the appropriate time of season (day 200). Because the duration of relapse parasitemia can last for months (Valkiunas 2005a), we assumed that relapsing birds transitioned directly into the latently infected, overwinter stage at the end of the season (day 200). Thus, we also determined the rate of this transition (δ_R) by selecting a value for this parameter that ensured all relapsing birds entered the overwinter stage by the end of the season (day 200).

We calculated the daily natural mortality rates for the sparrow population from a life table on the Tioga Pass sparrow population (Morton 2002). For adult birds, approximately 50% survive and return to breed in subsequent years. This annual mortality rate was then adjusted to reflect the daily mortality rate (d_2) of 0.002 birds day⁻¹. We assumed that nestlings suffered a slightly higher daily mortality rate (d_1), which was set to 0.004 birds day⁻¹. However, because roughly 30% of YOY birds survive overwinter, we removed 70% of the returning YOY birds (latently infected and susceptible) before they entered the following breeding season to account for overwinter mortality. Further, because not all adults survive overwinter, we removed 5% of the overwintering adults (latently infected and susceptible) before they entered the subsequent breeding season. The natural death rate of black flies (d_4) was estimated by taking the inverse of the mean of black fly longevity (22.5 days) for *S. silvestre / craigi*.

All other death rates were inferred. We assumed that exposed, chronically infectious, and relapsing infectious birds did not experience a decrease in survivorship due to infection and were subjected to natural mortality only (d_2). We also assumed that exposed black flies would be resting after taking a successful blood meal and would experience a slightly lower mortality rate (d_5) than the natural death rate. Finally, we assumed acutely infectious birds and host-seeking infectious black flies would experience higher death rates (d_3 and d_6 , respectively) than natural mortality due to parasite pathology.

Parameters associated with nestling hatch and black fly emergence functions

The parameters associated with the time dependent nestling hatch and black fly emergence functions were estimated from empirical data collected from the White-

crowned Sparrow and *S. silvestre / craigi* populations living on our field sites. We compiled nest monitoring data from 2003 and 2004 to determine the number of nestlings hatched per female per day. This generated a curve with the peak number of nestlings hatching female⁻¹ day⁻¹ falling approximately on day 57 (June 26th) of each breeding season. We used the following function to approximate the curve generated from the empirical data,

$$\frac{dSN}{dt} = \frac{A_B}{2 \left(e^{\frac{(t-q_B)^2}{c_B}} \right)} - \left(rb_N \left(\frac{IS}{S} \right) + x + d_1 \right) SN$$

The height of the nestling hatch curve, the peak number of nestlings that hatch female⁻¹ day⁻¹ (A_B) was determined by taking the maximum number of nestlings hatching daily (nine day⁻¹) and dividing by the total number of breeding females (54). We determined the parameter for the spread of the nestling hatch function (c_S) and the day the peak number of nestlings that hatch female⁻¹ day⁻¹ by matching the spread and peak day of the model generated curve to the spread and peak day of the empirical data curve (Figure 2).

We used the same time-dependent function to control how and when black flies emerge throughout the summer season and estimated parameters of this function from trapping data collected throughout the summers of 2005 and 2007.

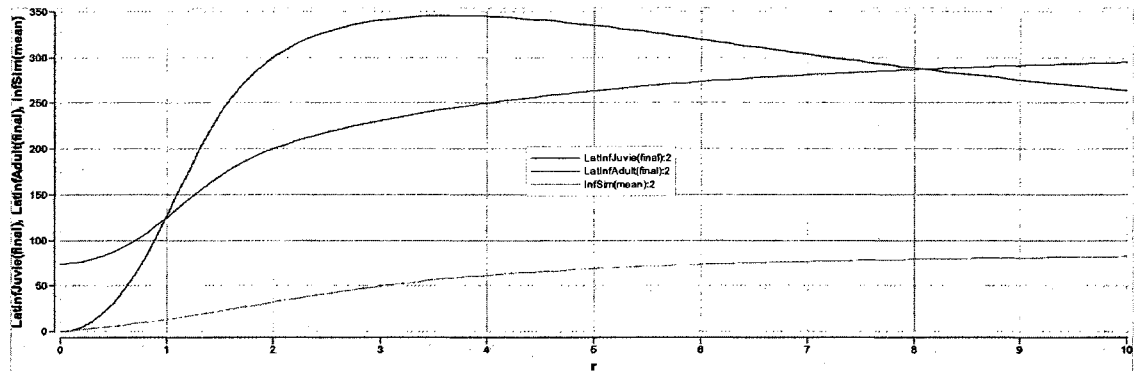
$$\frac{dSS}{dt} = \frac{A_S}{\left(e^{\frac{(t-q_S)^2}{c_S}} \right)} - SS \left(\frac{r}{B} (b_A (IA + IJ) + b_R RIA + b_C (CIA + CIJ)) + d_4 \right)$$

We determined an empirical curve from the number of *S. silvestre / craigi* captured in CDC light traps on a daily basis. The height of the black fly emergence function (A_S), the spread of the emergence function (c_S), and the day when peak number of black flies

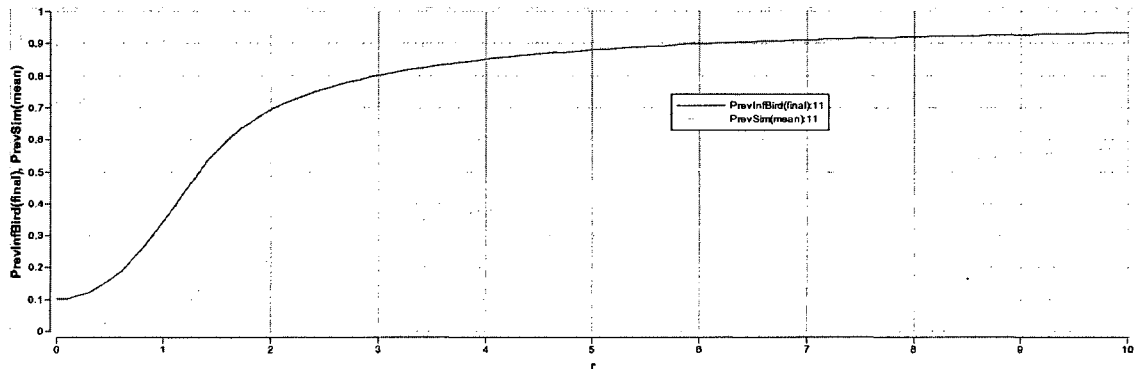
emerge (q_s) were determined by matching the model curve to the empirical curve for *S. silvestre / craigi* daily abundance (Figure 2).

Appendix G

$r = 2$, the number of black fly bites per bird per day (0-10)

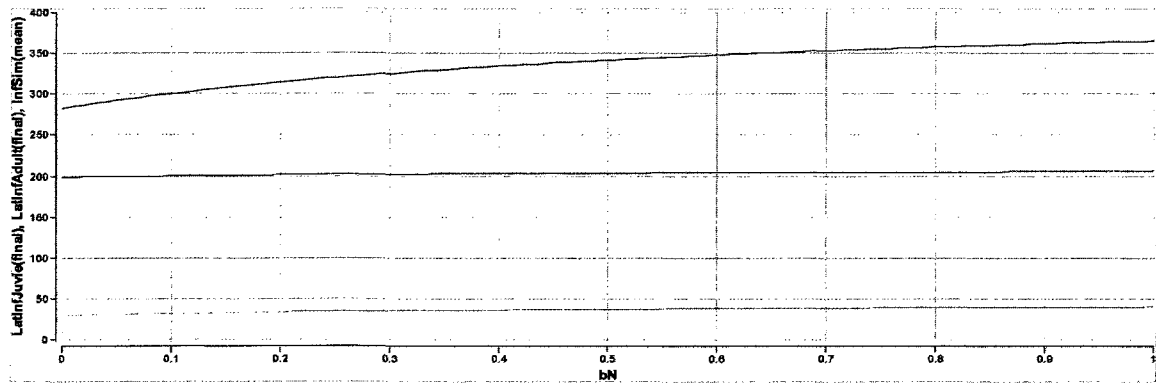


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious adults

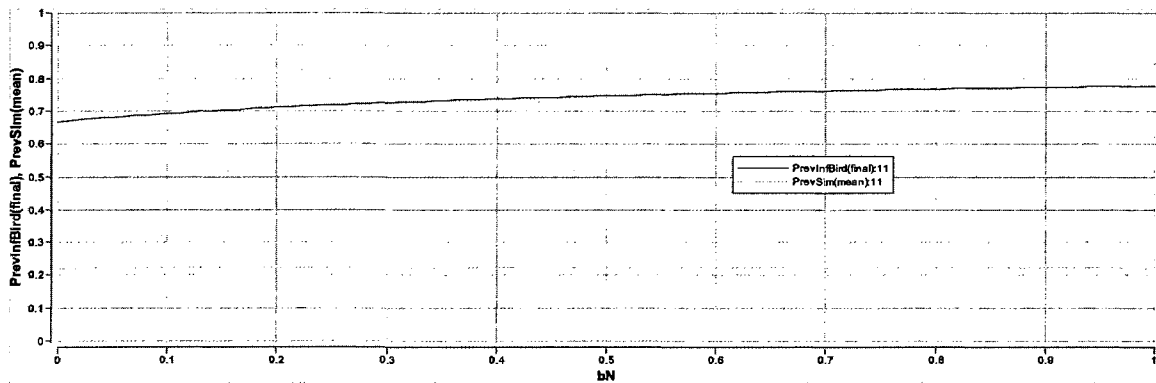


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$b_N = 0.10$, the probability that an infectious vector will successfully transmit to a nude nestling

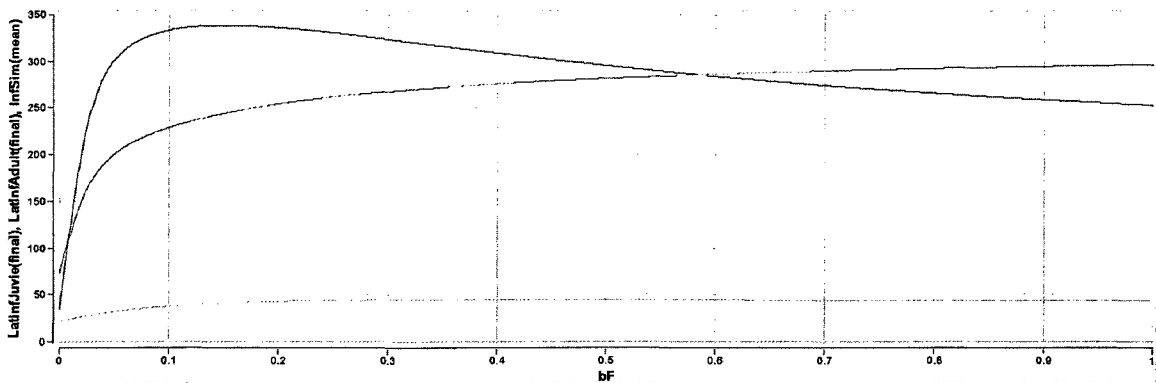


red = final number of latently infected adults, black = final number of latently infected juveniles, green = mean number of infectious black flies

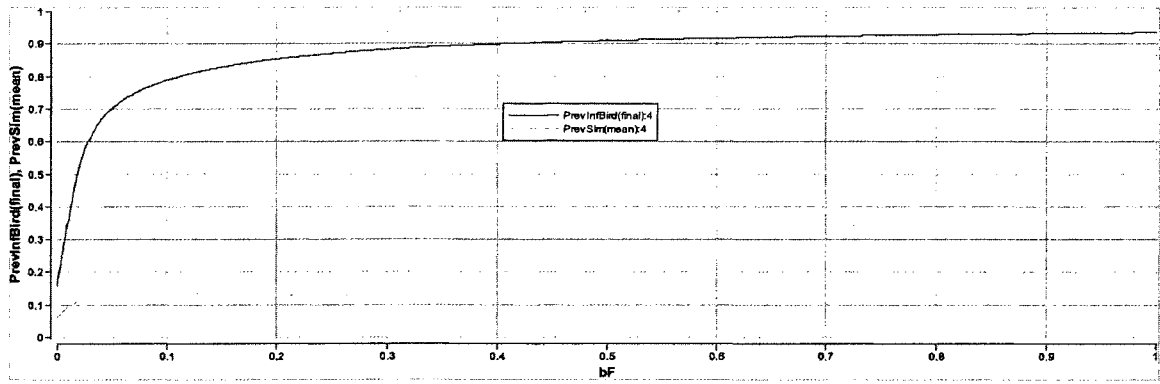


blue = final prevalence of infected birds, yellow = mean prevalence of infectious black flies

$b_F = 0.05$, the probability that an infectious vector with successfully transmit to a feathered bird (YOY or adult)

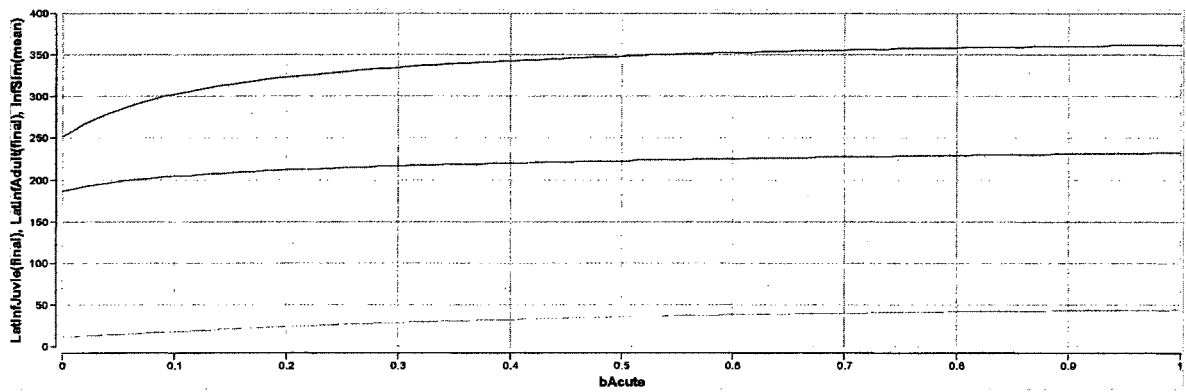


red = final number of latently infected adults, black = final number of latently infected juveniles, green = mean number of infectious black flies

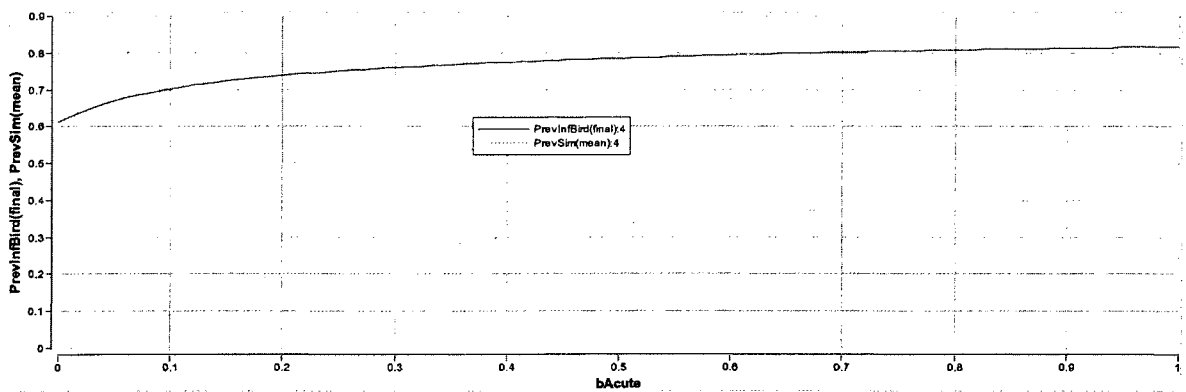


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$b_A = 0.10$, the probability that a vector with become successfully infected after feeding off of an acutely infectious bird.

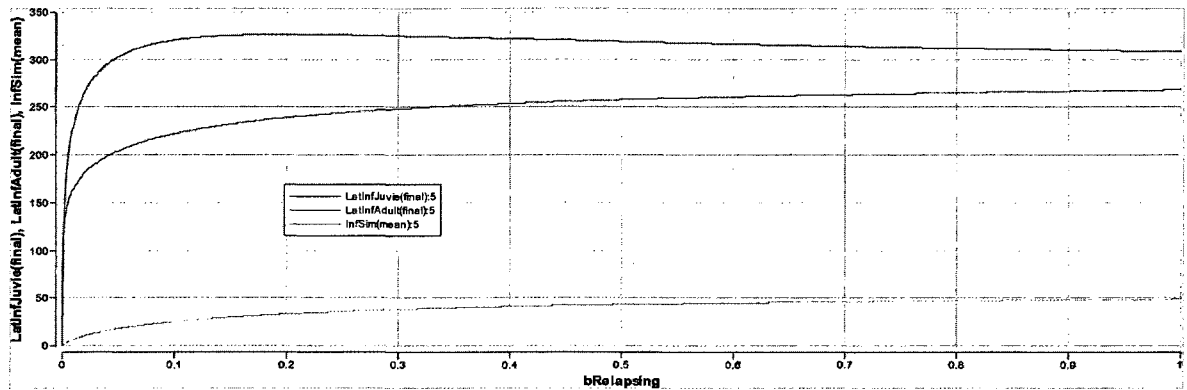


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious black flies

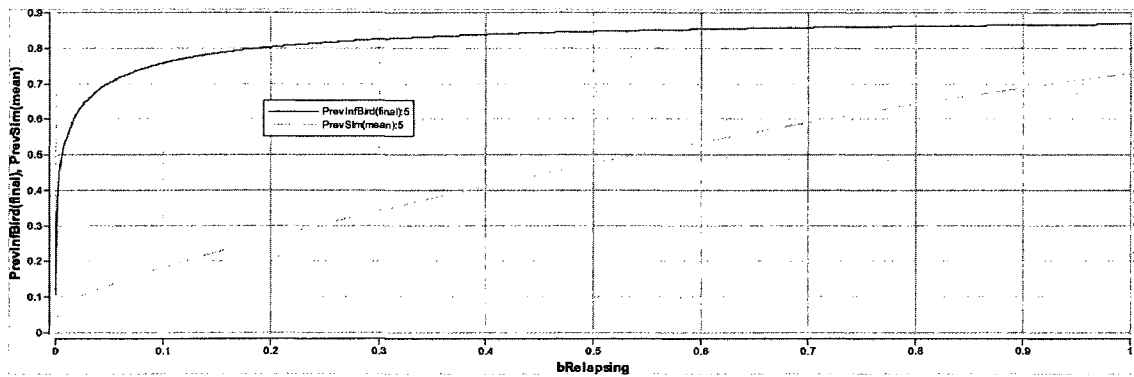


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$b_R = 0.05$, the probability that a simuliid will be successfully infected after feeding off of a relapsing infectious bird.

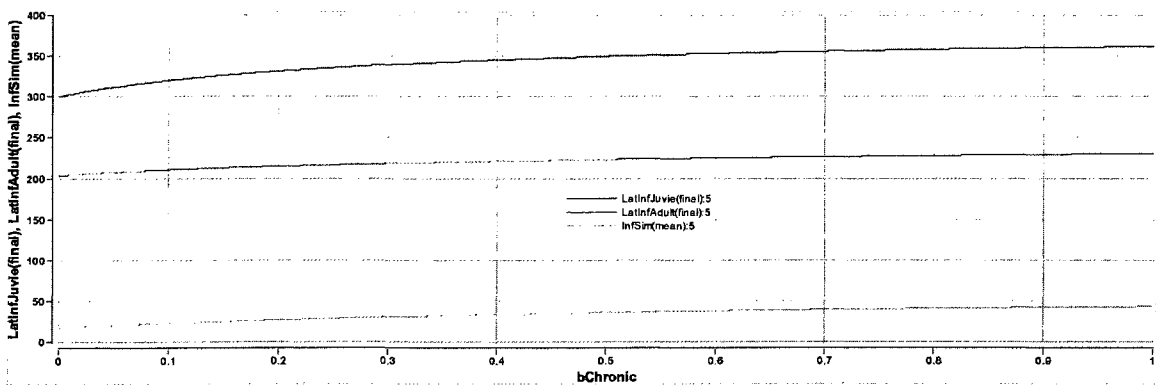


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious black flies

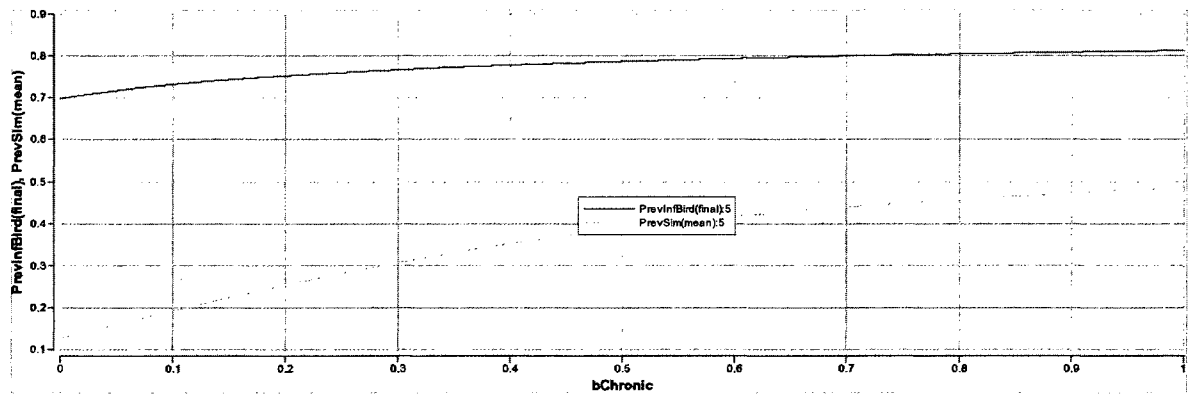


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$b_C = 0.01$, the probability that a simuliid vector will become successfully infected after feeding off of a chronically infectious bird

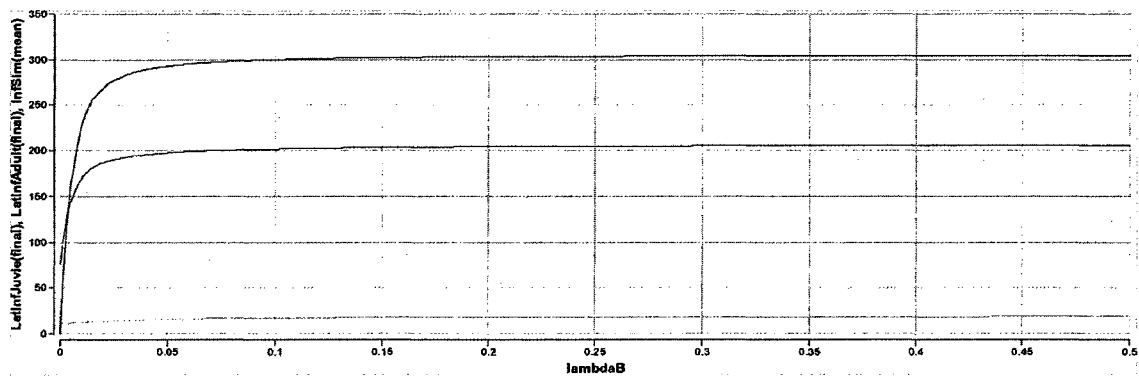


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious black flies

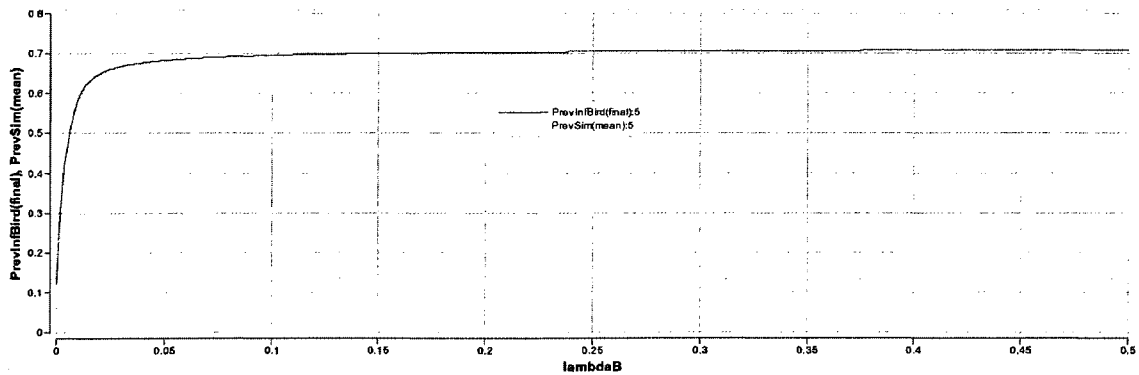


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$\lambda_B = 0.2$, the rate exposed birds become infectious

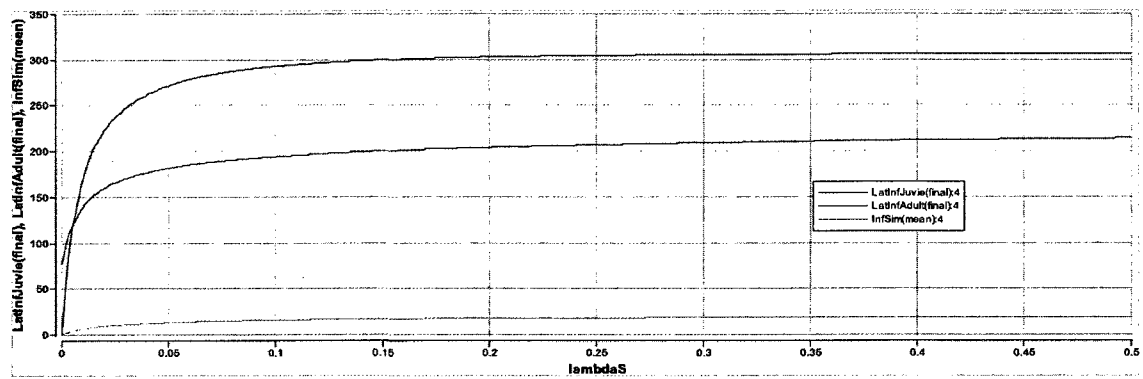


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious black flies

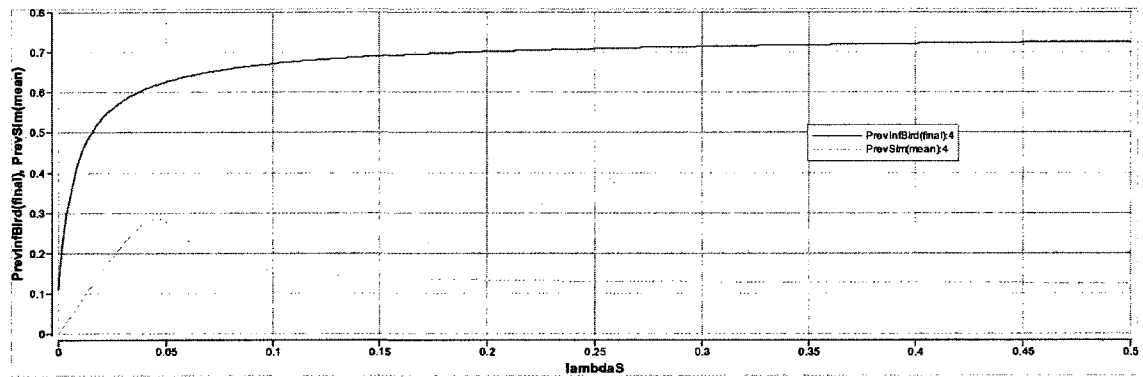


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$\lambda_S = 0.20$, the rate exposed black flies become infectious

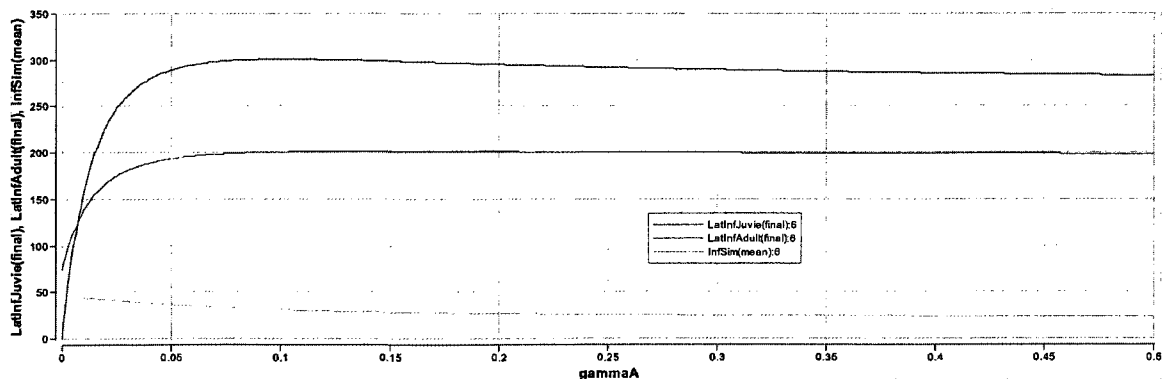


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious black flies

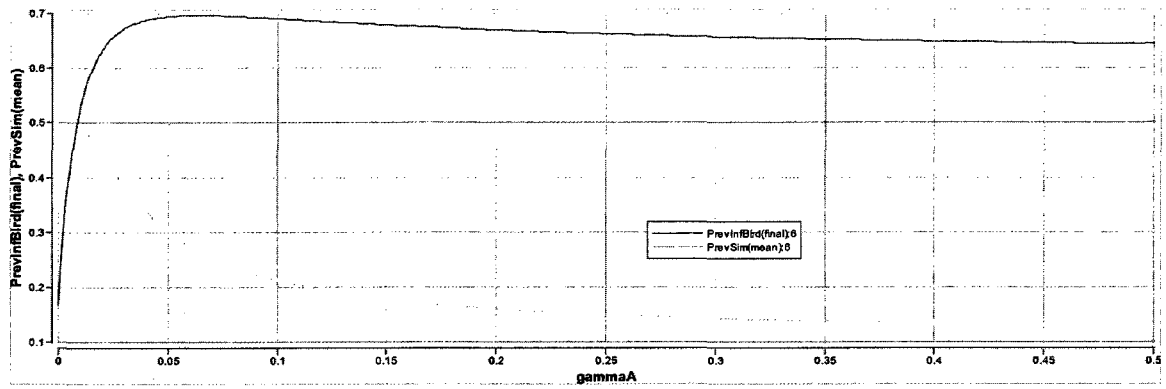


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

γ_A (in the program) = 0.09, the rate at which acutely infectious birds transition into the chronically infectious stage (corresponds to δ_A).

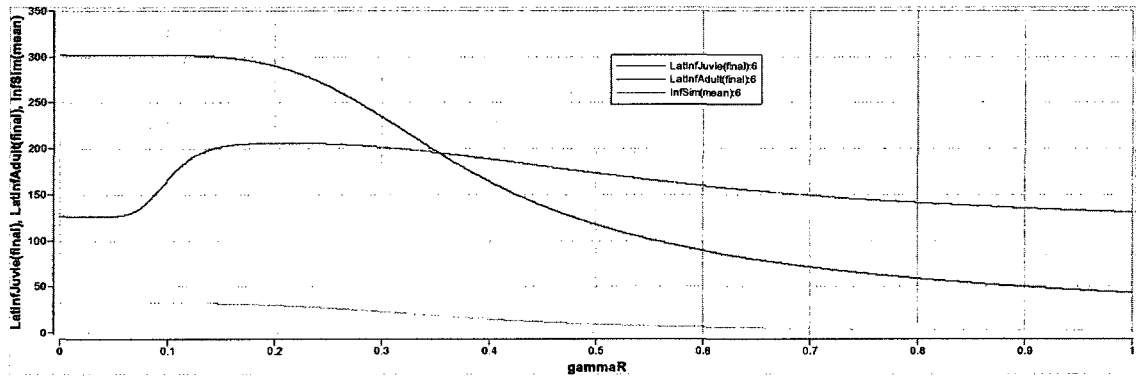


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean infectious adults

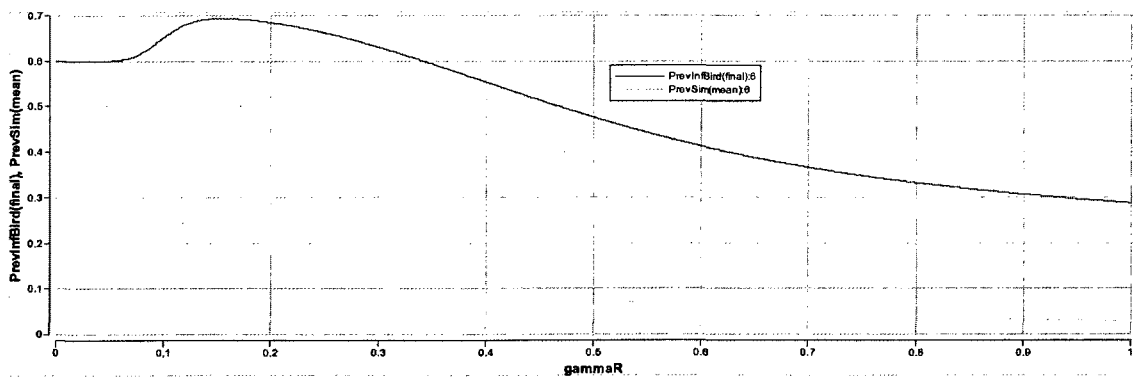


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

gammaR (in the program) = 0.14, the rate at which relapsing infectious adults transition through each relapsing compartment, and finally into the latent oven (corresponds with δ_R in the chapter)

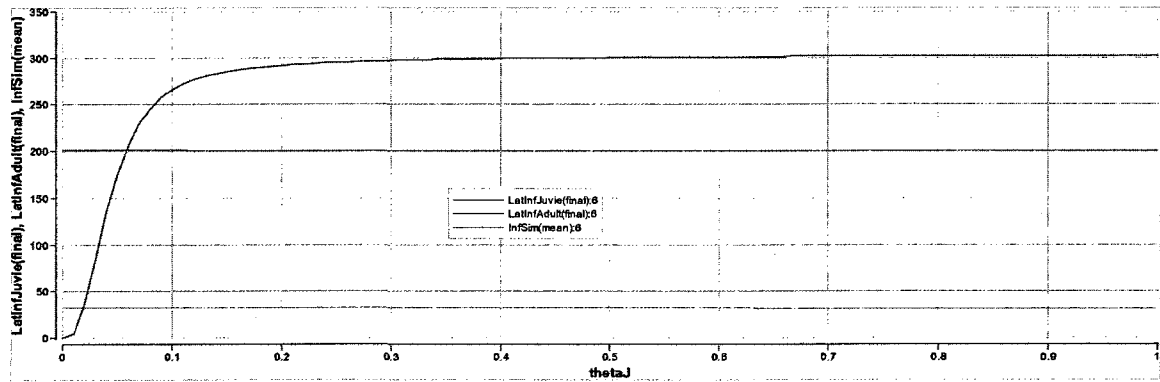


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious black flies

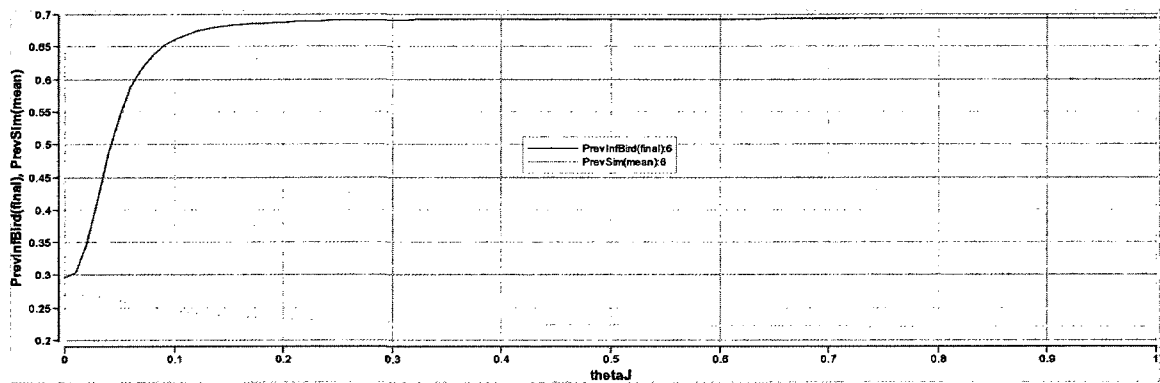


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

thetaJ (in the program) = 0.50, the rate chronically infectious juveniles transition into the latently infected stage (corresponds with σ_J in the chapter)

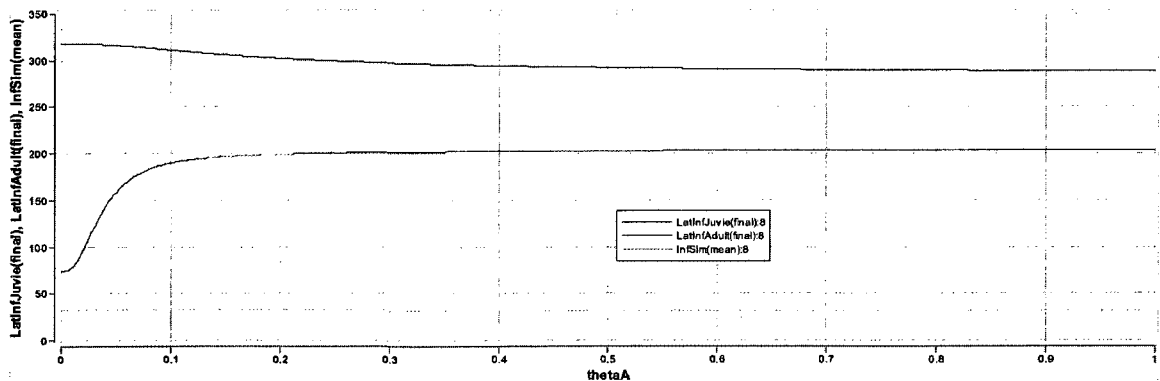


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = number of infectious black flies

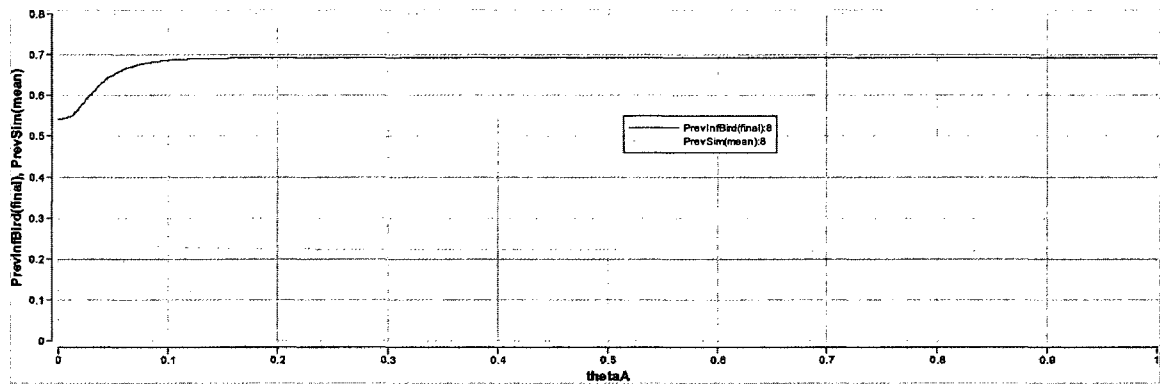


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

thetaA (in the program) = 0.23, the rate chronically infectious adults transition to the latently infected stage (corresponds with σ_A in the chapter)

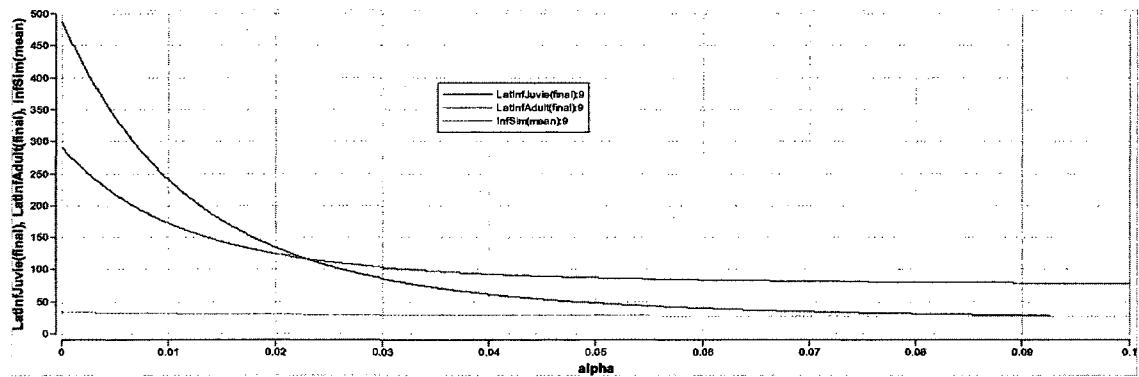


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious black flies

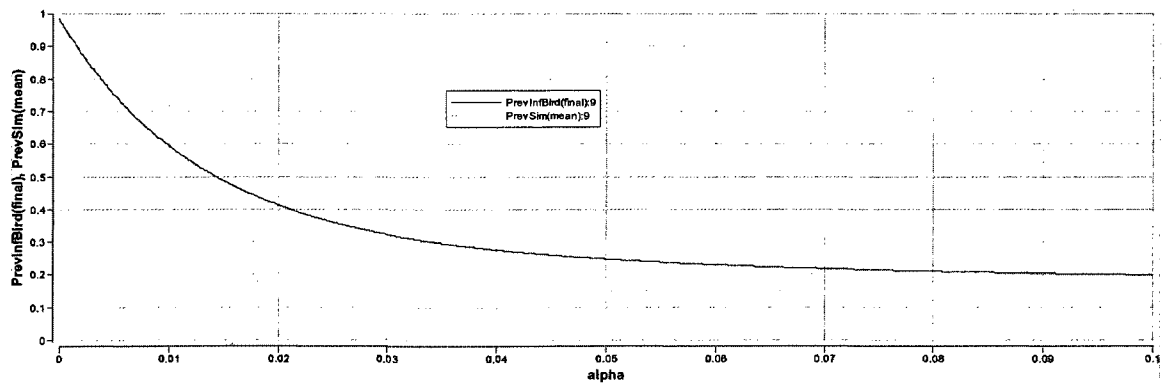


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

alpha (in the program) = 0.0067, the rate that susceptible birds transition into the overwinter compartment (corresponds with γ in the chapter)

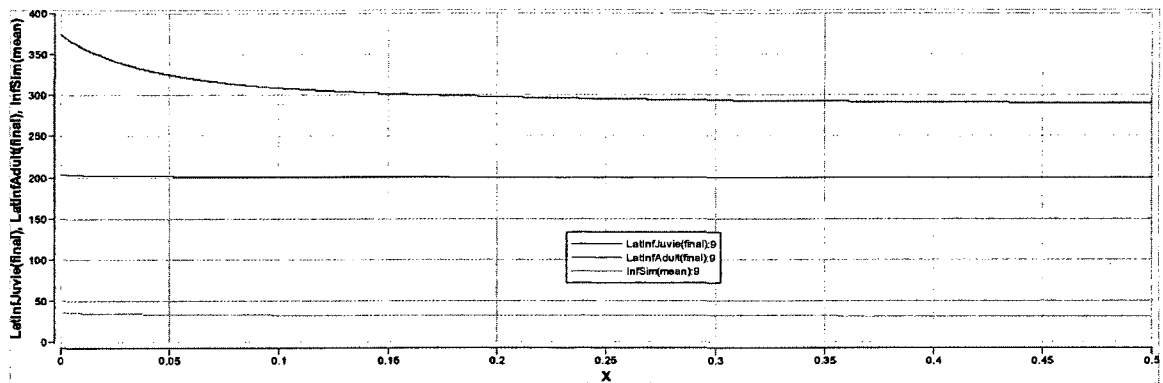


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious black flies

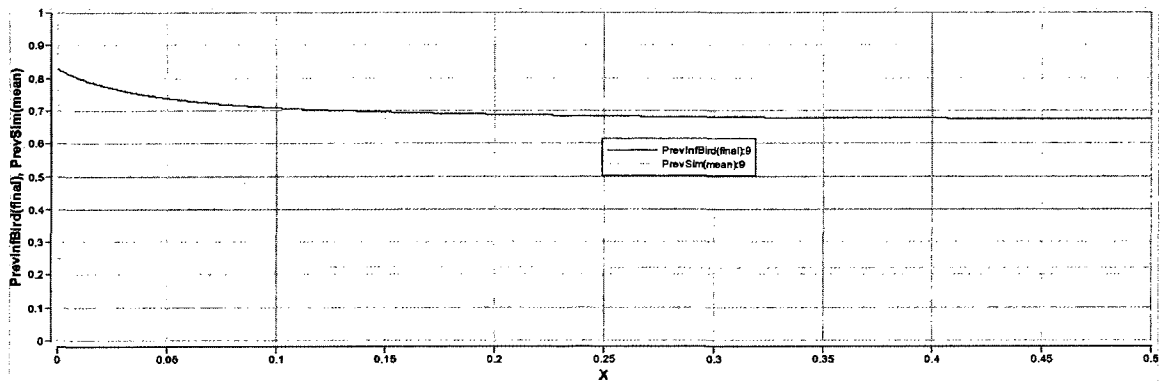


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

F = 0.167, rate at which nude nestlings acquire feathers

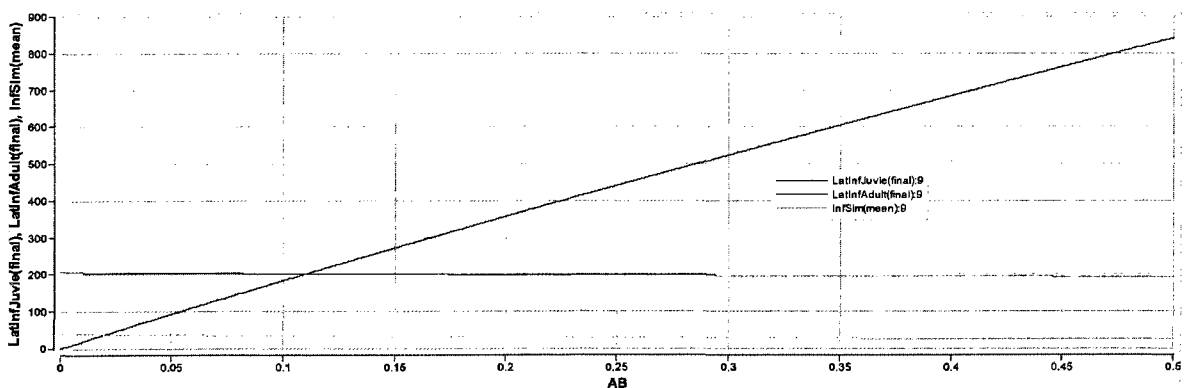


red = final number of latently infected adults, **black** = final number of latently infected YOY, **green** = mean number of infectious black flies

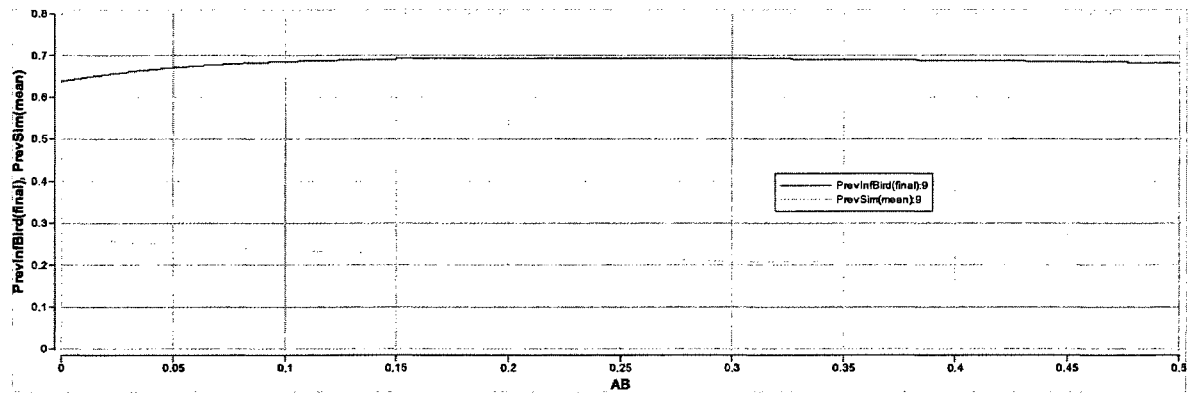


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

AB = 0.167, the mean number of nestlings / female / day in nestling hatch function

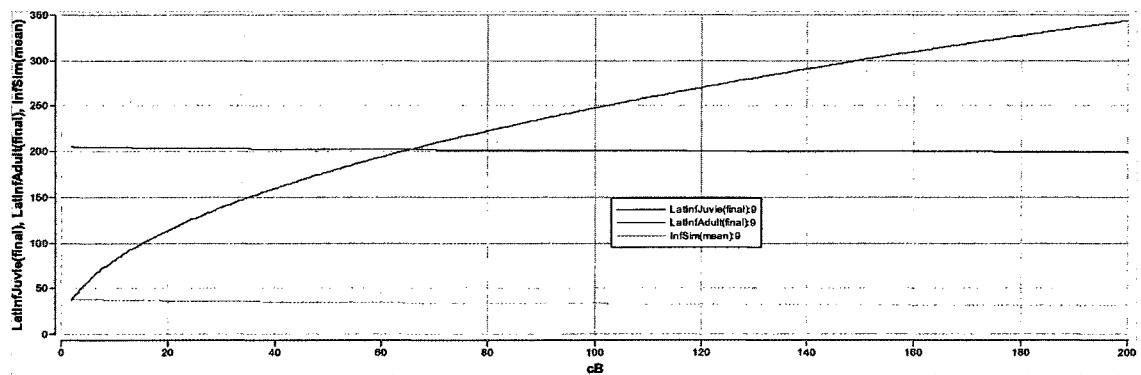


red = final number of latently infected adults, **black** = final number of latently infected YOY, and **green** = mean number of infectious black flies

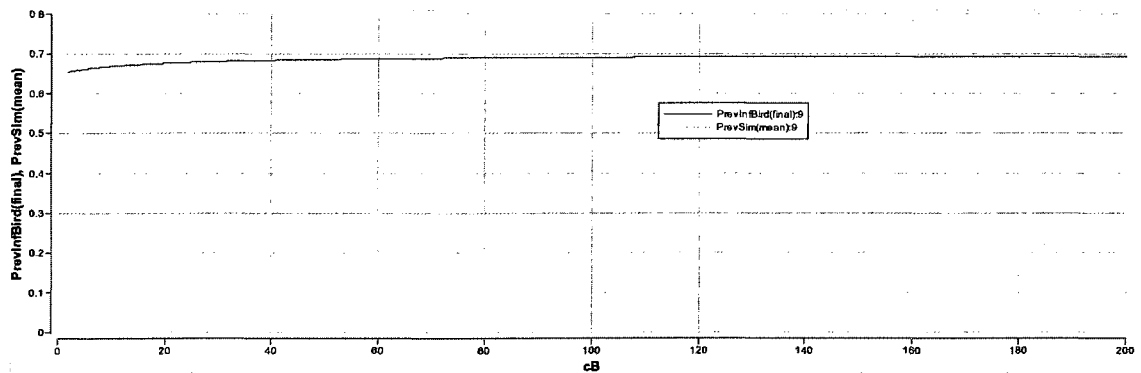


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$c_B = 150$, the spread of the nestling hatch function

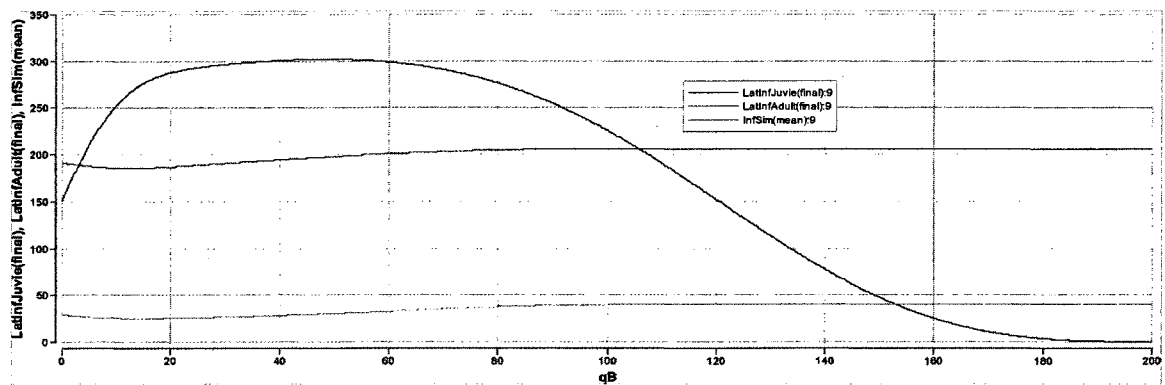


red = final number of latently infected adults, **black** = final number of latently infected YOY, and **green** = mean number of infectious black flies

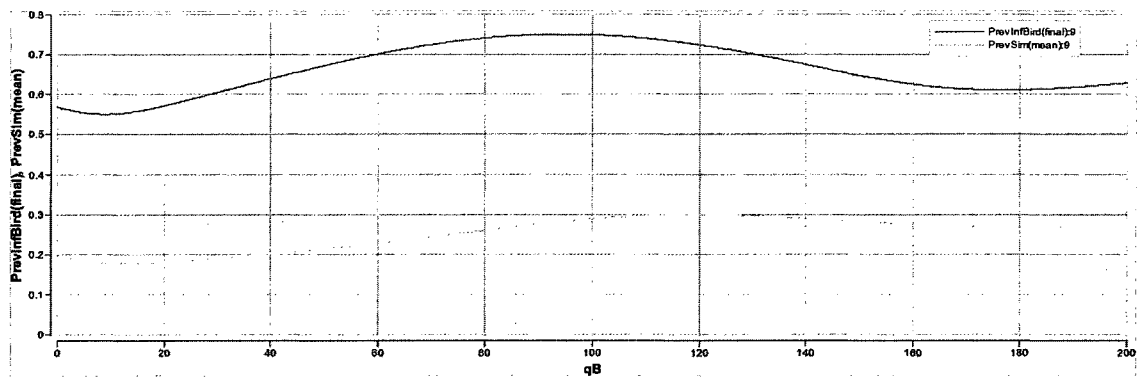


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$q_B = 57$, the date where the mean of the nestling hatch curve centers on

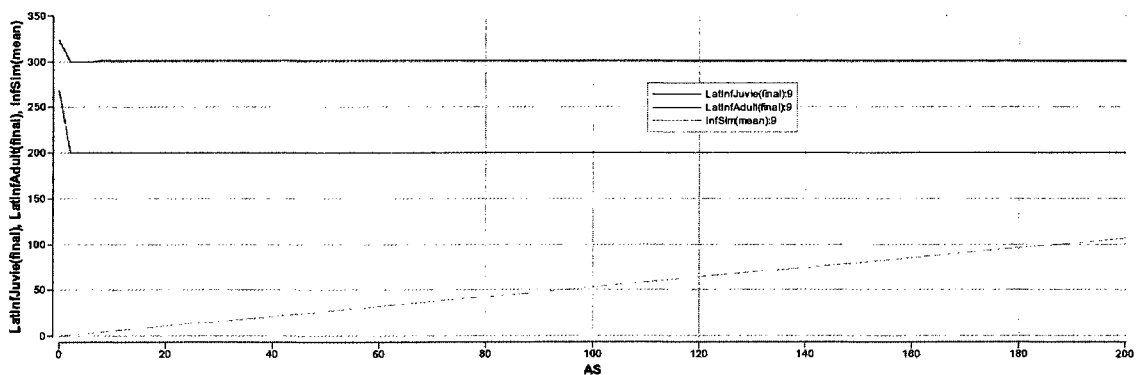


red = final number of latently infected adults, **black** = final number of latently infected juveniles, **green** = mean number of infectious black flies

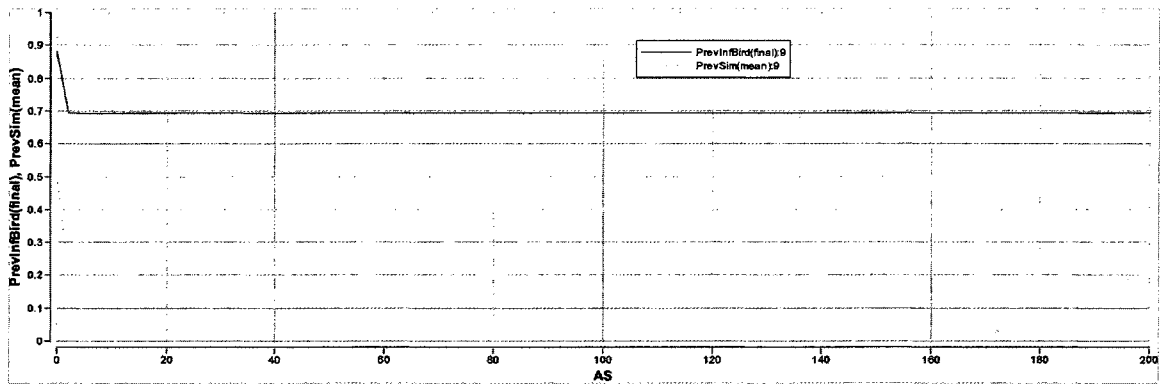


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$A_S = 60$, the mean number of emerging black flies per day

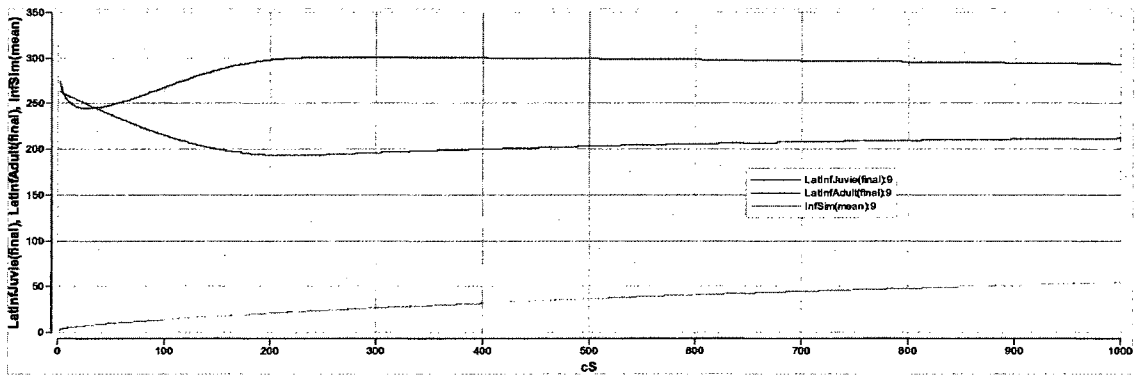


red = final number of latently infected adults, **black** = final number of latently infected YOY, and **green** = mean number of infectious black flies

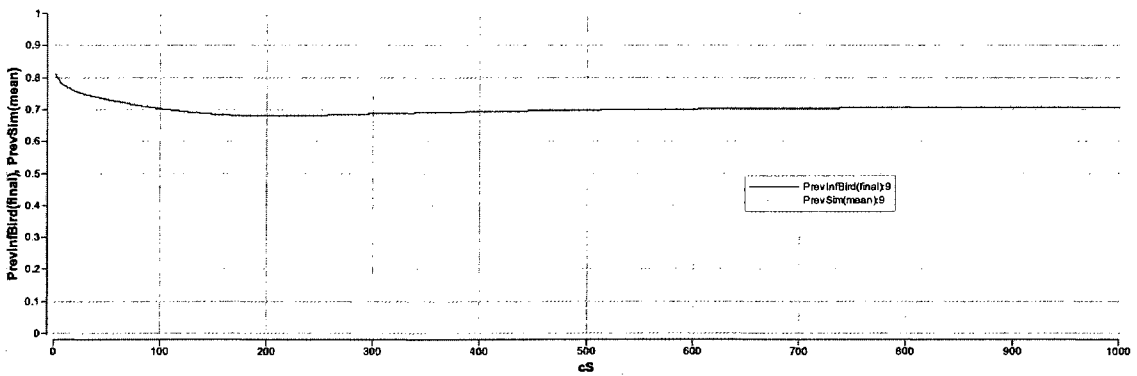


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$c_S = 400$, controls the spread of the black fly emergence function

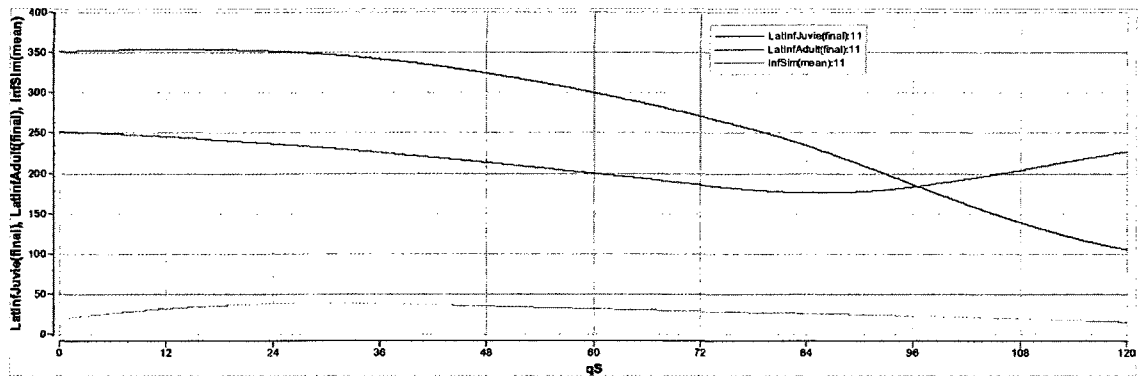


red = final number of latently infected adults, **black** = final number of latently infected YOY, and **green** = mean number of infectious black flies

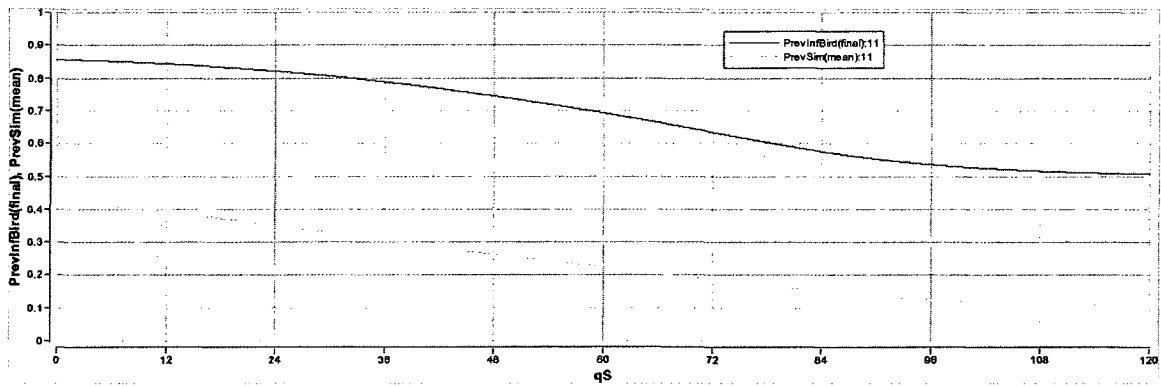


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$q_s = 60$, the date at which the mean of the black fly emergence function is centered on

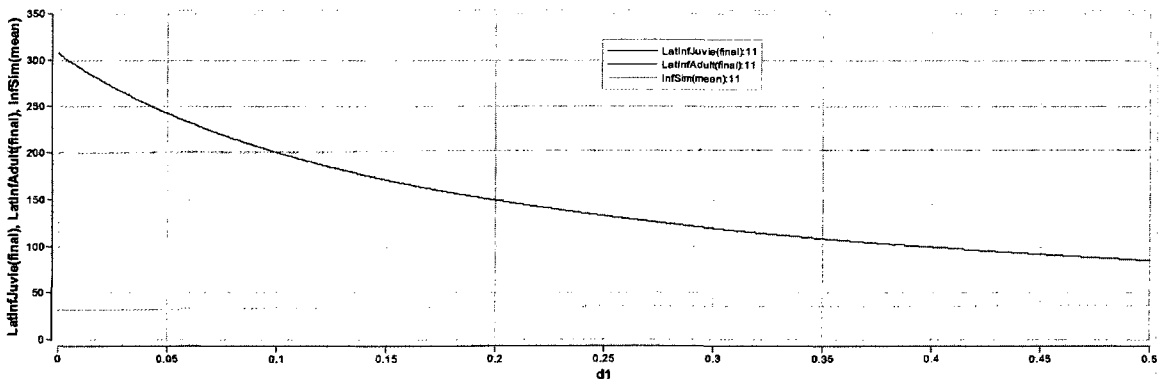


red = final number of latently infected adults, **black** = final number of latently infected YOY, **green** = mean number of infectious black flies

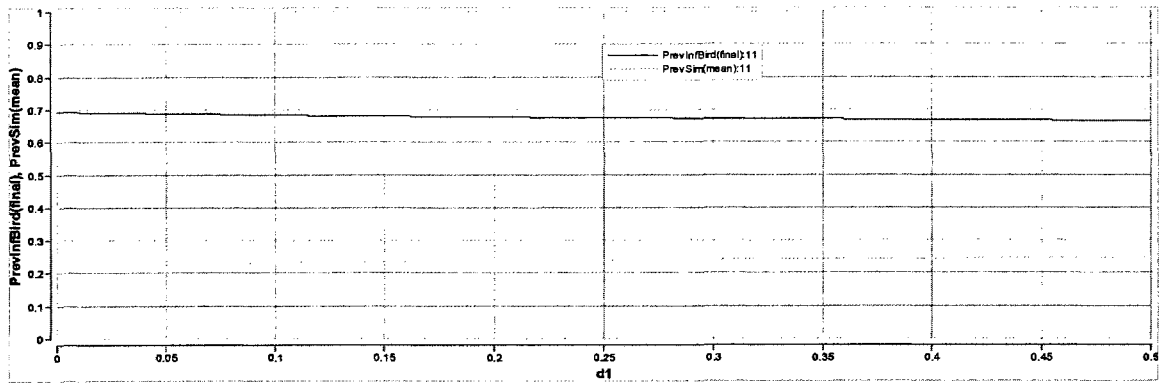


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$d_1 = 0.0041$, the natural death rate of nude nestlings

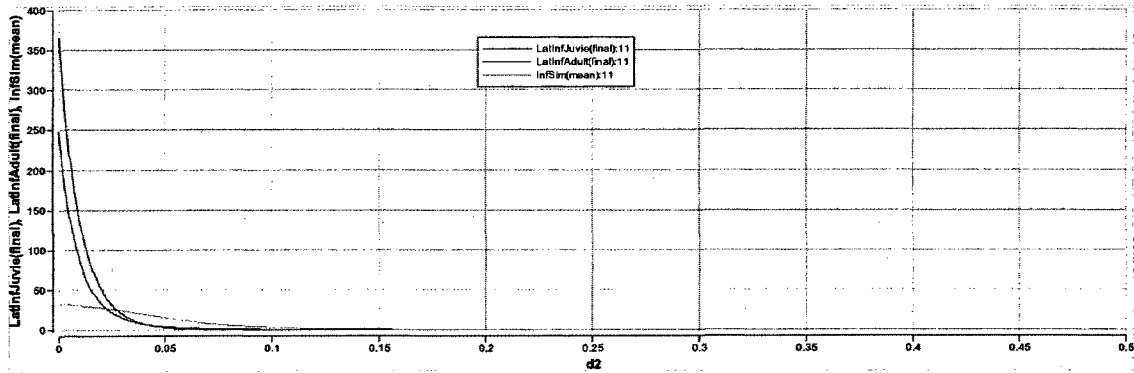


red = final number of latently infected adults, **black** = final number of latently infected YOY, and **green** = mean number of infectious black flies

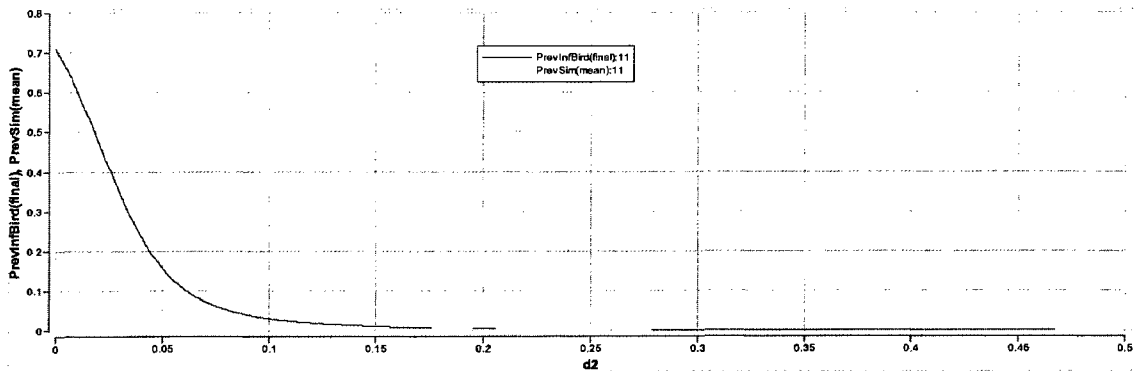


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$d_2 = 0.0018$, the natural death rate of feathered YOY and adults

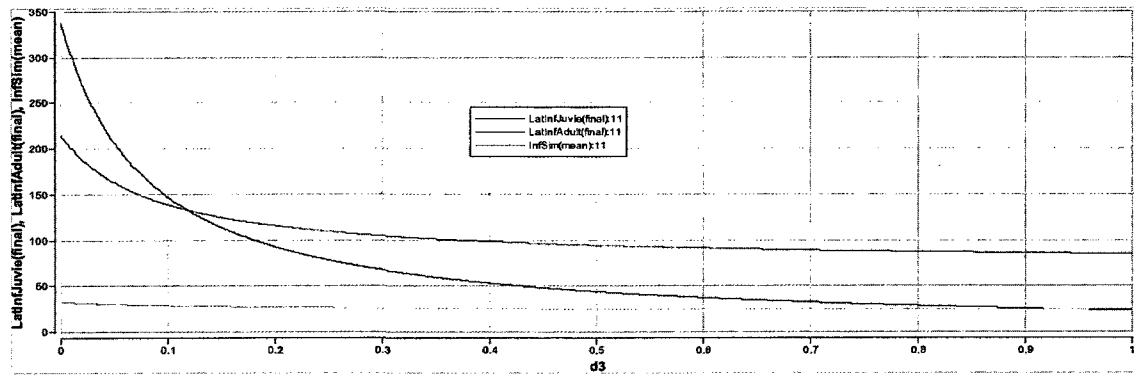


red = final number of latently infected adults, **black** = final number of latently infected YOY, and **green** = mean number of infectious black flies

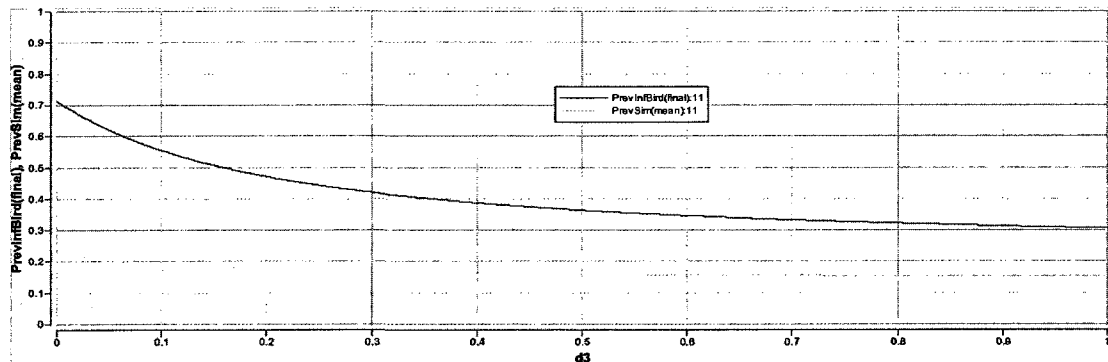


blue = final prevalence of infected adult birds, **yellow** = mean prevalence of infectious black flies

$d_3 = 0.01$, the death rate of acutely infectious birds

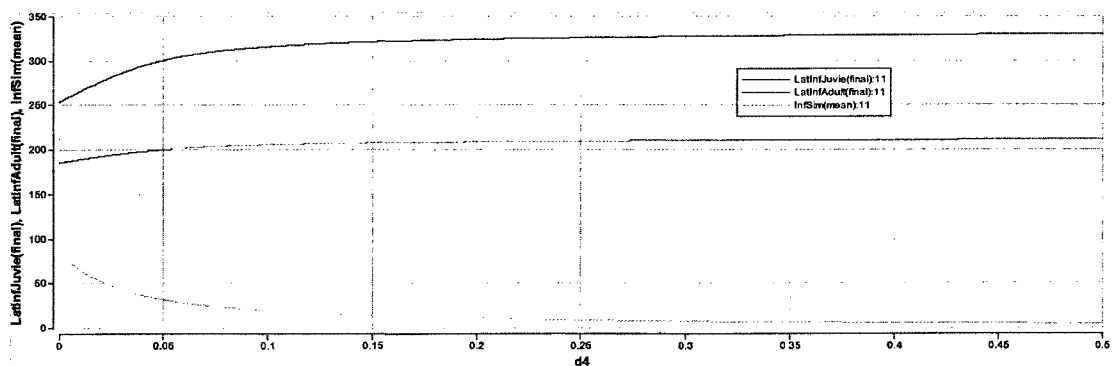


red = final number of latently infected adults, **black** = final number of latently infected YOY, and **green** = mean number of infectious black flies

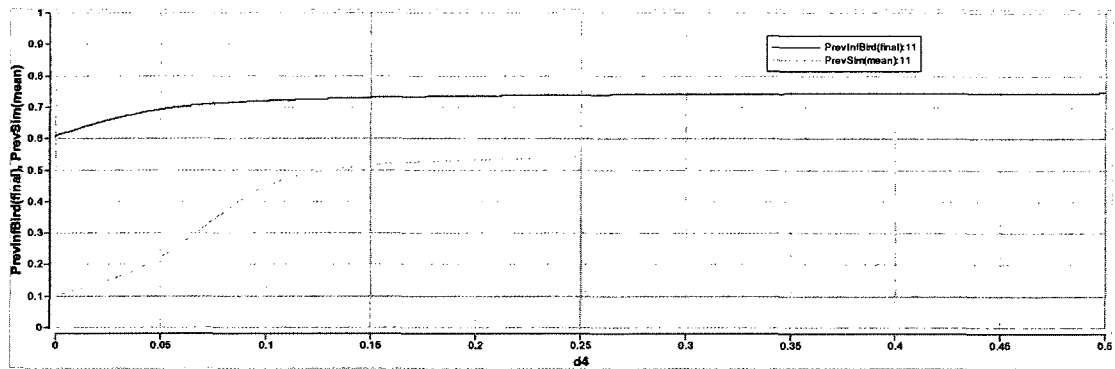


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$d_4 = 0.05$, the natural death rate of host-seeking, susceptible black flies

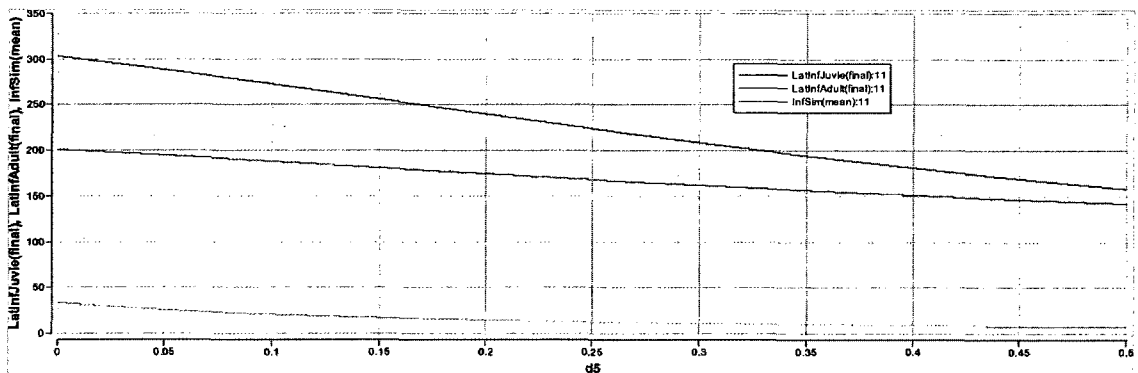


red = final number of latently infected adults, **black** = final number of latently infected YOY, and **green** = mean number of infectious black flies

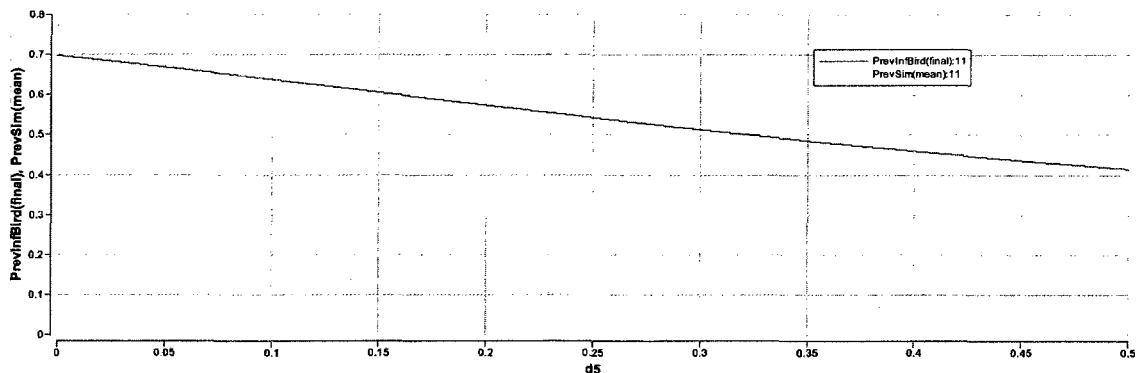


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$d_5 = 0.01$, the death rate of engorged, resting black flies

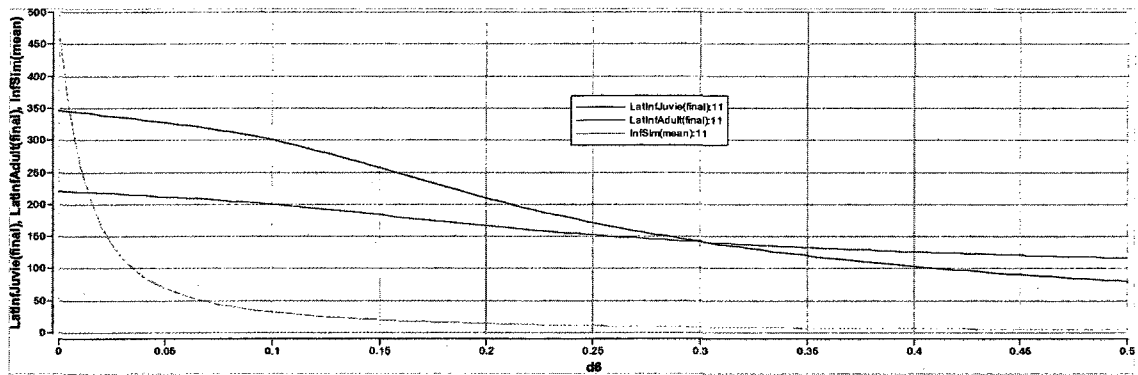


red = final number of latently infected adults, **black** = final number of latently infected YOY, and **green** = mean number of infectious black flies

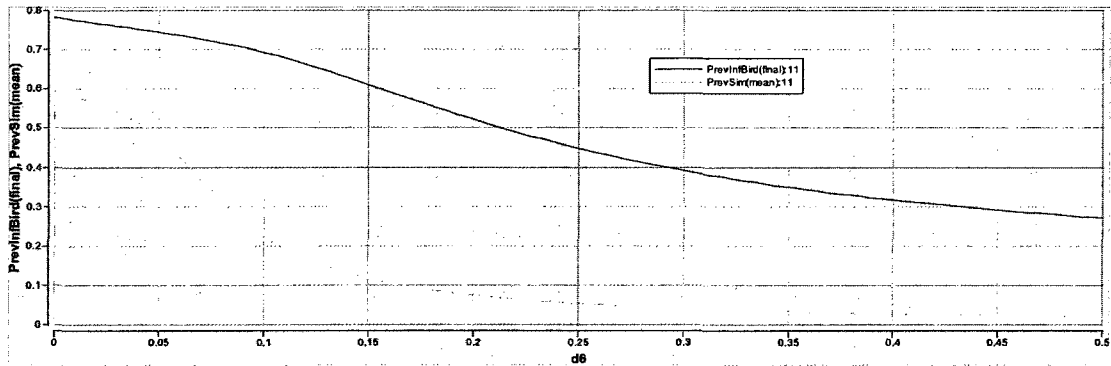


blue = final prevalence of infected birds, **yellow** = mean prevalence of infectious black flies

$d_6 = 0.10$, the death rate of host-seeking, infectious black flies



red = final number of latently infected adults, black = final number of latently infected YOY, and green = mean number of infectious black flies



blue = final prevalence of infected birds, yellow = mean prevalence of infectious black flies

Appendix H

Because of our experimental design, we used a categorical variable, season (*Early* vs. *Late*), and a continuous time parameter, (*Day of Season*), to evaluate the effects of time on stress response (baseline and stress-induced CORT concentrations) and wing web swelling, respectively. We also included log-transformed total blood parasitemia and total amount of drug treatment received across each season as covariates in all models. Total parasitemia was log-transformed because the distribution approximately followed a negative binomial distribution. Body condition (residuals from regression of mass on tarsus) was originally included in the models because past studies have suggested that condition can have a significant impact on baseline and stress-induced CORT concentrations (e.g. Romero and Wikelski 2001, Mullner et al. 2004) as well as wing-web swelling (e.g. O'Brien and Dawson 2008). However, body condition was not related to any of our response variables and was not included in the final models.

All continuous variables were centered before running the LMMs. We fit original models using REML (restricted maximum likelihood) estimation and then reduced the models through backward elimination of non-significant interactions. Non-significant main factors and covariates were not removed from the final models. Comparisons were made between the full and reduced models with likelihood ratio tests based on fitting models with maximum likelihood methods. We used Tukey-adjusted post hoc tests to determine significant differences among the means of response variables in different treatment groups and for other categorical predictors. Relationships between continuous

predictors and the dependent variables were plotted based on the residuals from the LMM, after adjusting for the other predictors in the final model.

Appendix I

Past research indicates that an individual requires sufficient energy resources to mount a strong immune response to a new, antigenic challenge (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Norris and Evans 2000). In House Sparrows (*Passer domesticus*), the total cost of the response to PHA injection is equivalent to 4.20 kJ per day (29% of resting metabolic rate) or half an egg a day (Martin et al. 2003). Thus, breeding, non-drug-treated females subject to chronic hemosporidian parasite burdens may simply not have as many resources available to respond to the administered PHA challenge. Past literature demonstrates that trade-offs do exist not only between mounting an immune response and parental effort (e.g. Deerenberg et al. 1997, Nordling et al. 1998, Moreno et al. 1999), but with other energetic demands such as future adult survival (e.g. Moret and Schmid-Hempel 2000), nestling growth (e.g. Klasing et al. 1987), and maintenance of sexual characteristics (e.g. Saino and Moller 1996, Zuk and Johnsen 2000).

Why does antimalarial drug treatment result in stronger immune responses in female but not male sparrows? Infections with hemosporidian parasites, especially *Haemoproteus*, appear to be more costly for breeding female White-crowned Sparrows because they only impact female reproductive success (Bonier et al. 2007). Additionally, because male parental investment is comparatively minor and occurs late in the season, any trade-offs between immune function and reproduction may be less

pronounced. Consequently, parasite suppression through antimalarial treatment may not affect male responsiveness to the PHA challenge.

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