

**Avian malaria transmission dynamics in New
Zealand: investigating host and vector
relationships along an elevational gradient**

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This thesis is for the birds...

“Examine each question in terms of what is ethically and aesthetically right, as well as what is economically expedient. A thing is right when it tends to preserve the integrity, stability, and beauty of the biotic community. It is wrong when it tends otherwise.”

~ Aldo Leopold

ABSTRACT

Avian malaria (*Plasmodium* spp.) may be of concern to the New Zealand avifauna, considered to be the most extinction-prone in the world. Although avian malaria has impacted both captive populations and wild individuals in New Zealand, whether or not it is a cause of concern for native bird populations in the wild is unknown. This research provides insight into the general ecology and epidemiology of avian malaria, highlighting patterns of infection risk as well as offering recommendations to improve detection via molecular techniques. Factors potentially influencing transmission dynamics in New Zealand were explored, such as elevation, vector abundance, and native or non-native status.

A survey was conducted in Nelson Lakes National Park (NLNP) to assess the prevalence of avian malaria infection across a broad elevational range (650 – 1400 m), at a site experiencing native bird declines in which malaria has been suggested as playing a role. Results from blood samples collected and analysed over three seasons (n = 436) support the potential for an inverse relationship between force of infection and elevation. This research was the first investigation of avian malaria presence across an elevational gradient in New Zealand. Results also show an overall higher prevalence of malaria in non-native (14.1%) versus native birds (1.7%) living in a shared space, suggesting the possibility of a differential impact on host species that show dissimilar reservoir competence. These findings provide evidence that native New Zealand avifauna may be impacted by avian malaria at a population scale, and a differential impact on host species is occurring due to specific *Plasmodium* lineages. If this is indeed occurring, it would carry with it a whole new complexity to the conservation of native wildlife in New Zealand.

Mosquitoes were also sampled to help characterise the surrounding vector community at the field site at NLNP. Sampling results of the native *Culex pervigilans* (n = 81) were also used to create a model to estimate and make preliminary predictions of mosquito abundance based on actual and projected temperatures. The model shows that temperature influences on mosquito population dynamics in New Zealand likely drives annual, seasonal and elevational differences in mean densities and maximums. The model was used to estimate mosquito abundance at the time and place of known bird mortality events, allowing for speculation into the possible role of vector-borne

diseases at each of these independent events. In some cases patterns are observed that support the hypothesis that a mosquito-borne disease played a role in the mortality events, but not in other cases.

Molecular diagnostic techniques, such as PCR, have played an important role in the advancement and understanding of avian malaria ecology. However problems arise when parasites in the blood cannot be amplified, resulting in false negatives. In an attempt to improve the accuracy of diagnostics by PCR, I evaluated the performance of a commercial DNA extraction kit by modifying the protocol with four elution volume alternatives. The results suggest that the best template is the DNA extract obtained from the second eluate of a first 50 μ L elution step. In one case, the only band visible was from this second eluate, and thus may not have been identified as positive for *Plasmodium* spp. if a different elution protocol had been followed. A low ratio of parasite to host DNA is a major concern in detecting chronic infections in which birds typically carry low levels of parasitemia. Results from this study show that the modification of the elution step of a silica-membrane-based extraction protocol can be an effective approach to decreasing the occurrence of false negatives.

A comparative analysis, using a data set compiled from the literature, was also performed to identify large-scale predictors of malaria parasite prevalence in avian populations based on geographic and host characteristics from the Australasia-Pacific region. Results indicate that a negative correlation exists between avian malaria prevalence and elevation, latitude, and length of incubation. Results also show that a positive correlation exists between avian malaria prevalence and body size. Differences in patterns of infection were also seen across locations, with an interesting inverse trend seen for prevalence in non-native and native hosts between New Zealand and Hawaii, leading to questions related to resistance to infection in different regions.

Overall, this thesis presents evidence that avian malaria is potentially involved in native bird population declines in New Zealand, with non-native birds acting as reservoirs. Further information into native bird susceptibility to infection are needed to definitively confirm these findings, which could be obtained through experimental infection studies. Supporting evidence for such disease impacts will enable appropriate management to be developed and put in place, while a lack of evidence will enable resources to remain focused on the other issues facing New Zealand's native species.

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PERMITTING

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Chapter One

General Introduction

BACKGROUND

Emerging infectious diseases

Emerging infectious diseases (EIDs) are defined as disease-causing agents that rapidly increase in geographic range, host range, or prevalence (Dobson & Foufopoulos 2001). In the last few decades, EIDs have negatively impacted the health of both individuals and populations of wildlife, domestic animals, and humans, and pose a serious threat to the conservation of global diversity (Harvell *et al.* 1999; Daszak *et al.* 2000). Often, it is a change in the ecology of the host, pathogen, and/or vector (in the case of vector-borne diseases) that contributes to the emergence of a particular disease (Schrag & Wiener 1995). For example, over the last few decades the emergence of Nipah virus, lethal to humans, has been attributed to an increase in the number of bats acting as reservoirs near pig farms in Malaysia (Daszak *et al.* 2013). Lyme disease, the most common vector-borne disease in North America, has increased in prevalence due to changes in habitat fragmentation and urbanisation, increasing the number of competent rodent reservoirs (LoGiudice *et al.* 2003). The arrival of West Nile virus (WNV) to North America in 1999 has led to an emergence of the disease following the adaptation of the virus to local mosquito vectors. As a result, WNV has rapidly spread across North America, and has not only caused the deaths of over 1000 people, but that of millions of birds as well (Kilpatrick 2011). As is the case with WNV in North America, often it is native wildlife species that are threatened by non-native parasites they have not evolved with or adapted to (Tompkins & Poulin 2006; Tompkins *et al.* 2015). Avian malaria is another example, and a disease that might be of concern to New Zealand avifauna, considered to be the most extinction-prone in the world (Sekercioğlu *et al.* 2004; Tompkins & Poulin 2006). Here I discuss avian malaria in detail, including information on taxonomy, life cycle, host susceptibility, and population impacts, both globally and in New Zealand.

Avian malaria parasites

The order Haemosporida (Phylum: Apicomplexa) consists of single celled, protozoan parasites that rely on haematophagous invertebrates, typically dipterans (Insecta: Diptera) to complete their life cycle. Haemosporidians infect the blood and tissue cells of vertebrate hosts, with those that infect avian hosts belonging to several

genera, particularly *Haemoproteus*, *Leucocytozoon*, and *Plasmodium*. These three genera of parasites are typically transmitted by biting midges and louse flies (Ceratopogonidae and Hippoboscidae), blackflies (Simuliidae), and mosquitoes (Culicidae), respectively. The term “avian malaria” refers to clinical infections of the avian host by *Plasmodium* parasites (Valkiunas 2005). There is some debate on whether or not to include related genera, especially *Haemoproteus*, in the group of “avian malaria parasites”, leading to an inconsistency in the literature as to what parasite groups are included in studies involving “avian malaria” (Pérez-Tris *et al.* 2005; Valkiunas & Anwar 2005; Martinsen *et al.* 2008). In some cases the term “avian *Plasmodium*” is used to distinguish *Plasmodium* parasites that are found in avian hosts from those found in other hosts (e.g. mammals) (LaPointe *et al.* 2012). Also the term “haemosporidiosis” has been used to describe the clinical infections by *Haemoproteus* and *Leucocytozoon*, while more genus-specific terms have been proposed, such as “haemoproteosis” and “leucocytozoonosis”, respectively (Valkiunas 2005; Valkiunas & Anwar 2005). Regardless of the phrasing, all three of these genera are agents of avian disease.

Plasmodium life cycle

Plasmodium parasites are cosmopolitan in range (except Antarctica; Grimaldi *et al.* 2015) and can infect a broad range of bird hosts causing a range of impacts (Atkinson *et al.* 1995; Beadell *et al.* 2004). *Plasmodium* parasites have been reported in over 400 bird species in 70 different families, but are known to primarily affect passerines (Valkiunas 2005). These parasites are transferred among bird hosts by female mosquitoes feeding on blood. The life cycle of *Plasmodium* parasites consists of reproductive stages in both the mosquito vector and avian host (Fig. 1.1). Since the parasite undergoes sexual reproduction in the mosquito only, the mosquito is considered the definitive host with the bird acting as the intermediate host. The most common vectors of avian *Plasmodium* are mosquitoes (Diptera: Culicidae) of the genus *Culex*, although other genera such as *Aedes* and *Culiseta* have also been identified (Valkiunas 2005; LaPointe *et al.* 2012).

Here I present a generalised life cycle for avian *Plasmodium* (Fig. 1.1) (Atkinson & Van Riper 1991; Atkinson 1999; Valkiunas 2005). The life cycle begins with infective stages (sporozoites) of the parasite found in the salivary glands of the

mosquito vector. During the feeding process, the mosquito pierces the skin of the host to take a blood meal, allowing the sporozoites to invade the host blood or tissue. It is common for mosquitoes to feed on areas of exposed flesh on the host such as around the eyes, beak, and legs. Once in the host, the sporozoites begin to reproduce asexually (schizogony) as schizonts for one or more generations, producing merozoites. These merozoites then invade the red blood cells (erythrocytes) of the host and mature into trophozoites; some of which develop into a gametocyte (a sexually reproductive stage) and the others into an erythrocytic schizont. This additional stage of asexual reproduction within the erythrocytes is unique to *Plasmodium* parasites. The number of merozoites present in a schizont is used as a diagnostic characteristic for *Plasmodium* species identification (Valkiunas 2005). The gametocytes within an infected bird host are then concomitantly ingested by another mosquito during a blood meal. Within the midgut of the mosquito, the gametocytes mature into male and female gametes which undergo sexual reproduction. The resulting diploid zygotes then burrow into the lining of the mosquito midgut and become encapsulated, forming oocysts. After a certain amount of time, depending on the species, these oocysts rupture, releasing sporozoites that ultimately migrate to and invade the salivary glands of the mosquito, completing the cycle.

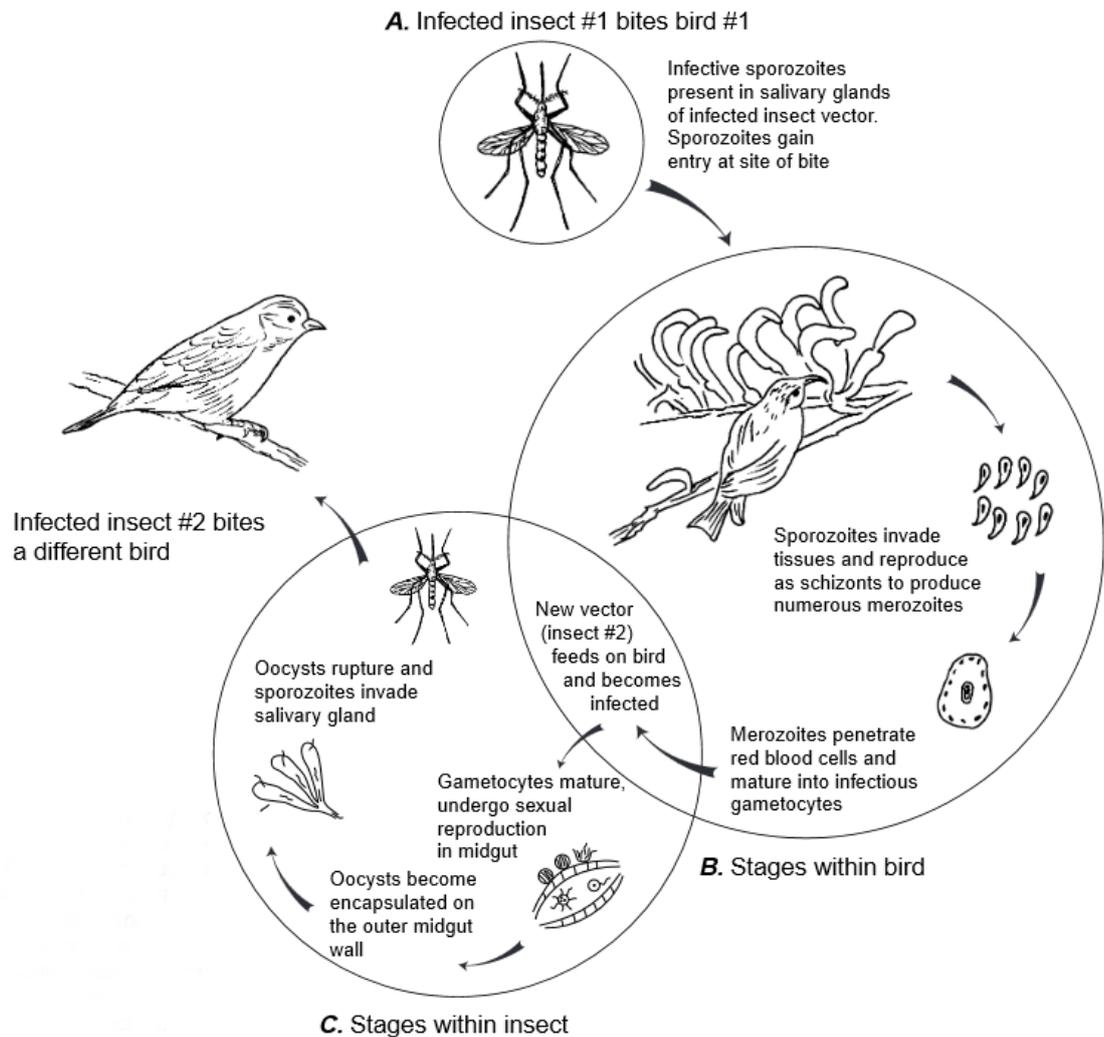


Figure 1.1. The general haemosporidian life cycle consisting of sexual and asexual reproduction in the mosquito vector (the definitive host) and asexual reproduction in the avian host (the intermediate host); taken from Atkinson (1999). The life cycle begins (A), an infected insect biting a susceptible bird. Separate infectious and developmental stages occur in (B), the bird host, and (C), the insect vectors. Note: An additional stage of asexual reproduction within the erythrocytes (red blood cells) of the bird host occurs with *Plasmodium* parasites, which is not seen here.

HOST IMPACTS OF AVIAN MALARIA

Clinical signs

The impact on avian hosts infected with *Plasmodium* parasites varies depending on the host species and parasite type (i.e. genus, species, or lineage). The majority of information available on pathogenic effects of avian malaria comes from

experimental infections studies (Atkinson & Van Riper 1991; Valkiunas 2005). Infection by malaria parasites consists of two phases, acute and chronic. The acute stage occurs immediately after infection, followed by the chronic stage with some seasonal relapses (LaPointe *et al.* 2012). The acute phase is characterised by a high level of parasitemia (the amount of parasites present in host blood) and hosts can show clinical signs such as lethargy, vomiting, pulmonary oedema, reduced weight gain, and sometimes death (Valkiunas 2005; Williams 2005). Individuals experiencing acute illness may also have difficulty foraging or escaping predators (Yorinks & Atkinson 2000). During the chronic phase, hosts that have not died as a result of the acute phase experience a low level of parasitemia. These chronic infections can last for years or for the life of the bird, and in some cases can have a mild impact on individual fitness (Bensch *et al.* 2007). Often, the parasites remain dormant, sometimes in the spleen or liver, and can be found in extremely low numbers, making it difficult to detect their presence from a typical blood sample (Atkinson & Van Riper 1991; Valkiunas 2005). The stage within the *Plasmodium* lifecycle responsible for chronic infections has been insufficiently investigated, however it is suspected that the merozoites are responsible (Valkiunas 2005). Chronically infected hosts play an important role in the overall transmission cycle, acting as reservoirs for subsequent transmission to uninfected hosts. Individuals with chronic infections can also experience relapses of increased infection, often reported in the spring during the breeding seasons, likely occurring if the host becomes immunosuppressed (Atkinson & Van Riper 1991; Schoener *et al.* 2014).

A number of factors can influence the susceptibility of avian hosts to malaria infection. Some studies report a higher prevalence of infection in males compared with females (Atkinson *et al.* 1995; Wood *et al.* 2007), whereas others report juvenile individuals as more susceptible to infection than adults (Atkinson & Van Riper 1991). Increased levels of parasitemia have also been reported in bird parents with larger clutch sizes (Knowles *et al.* 2011). However these findings vary across studies and may be host or location specific (Ricklefs *et al.* 2005). In some cases, individuals that are not normally exposed to malaria parasites or their vectors might be suddenly exposed to high levels of both, experiencing high levels of infection and susceptibility. Penguin species transferred from the wild to captive environments such as zoos, are a good example. Often these individuals, originally from ambient temperatures not supporting high abundances of mosquitoes, experience high levels of mortality upon transfer,

likely due to a less competent immune system (Grim *et al.* 2003; Vanstreels *et al.* 2015). Therefore host populations that are less exposed to malaria parasites over time, become both evolutionarily and physiologically naïve to infection.

Population impacts

Few studies report mortality due to avian malaria on a population scale. These reports are often from scenarios that involve new host-parasite associations such as captive individuals in zoos or birds from isolated islands (LaPointe *et al.* 2012). However it is likely that population-level surveys often underreport avian malaria prevalence. For instance, mist-netting has an inherent sampling bias, as sick or weak birds may not be caught due to their inactivity, and only birds below a certain level of parasitemia tend to be active (Valkiunas 2005). Additionally, birds with higher parasitemia levels are at a greater risk of predation (Møller & Nielsen 2007), further biasing reported malaria prevalence in wild populations.

Although the impacts of malaria on wild populations of continental species are largely unknown (Wood & Cosgrove 2006; LaPointe *et al.* 2012), the arrival of avian malaria to the isolated islands of Hawaii resulted in a significant impact to many endemic forest bird species (Atkinson *et al.* 1995; Atkinson & Dusek 2000). These population impacts are the most dramatic documented to date, and are a result of the arrival of *Plasmodium relictum* and the mosquito *Culex quinquefasciatus* Say in the early 20th century (Atkinson & LaPointe 2009). Following these introductions, a wave of native bird extinctions occurred a century later, with avian malaria suspected of playing a key role (van Riper *et al.* 1986). Currently, avian malaria is considered a major factor in the population declines and limited distribution of native Hawaiian forest bird species (Atkinson & Samuel 2010). The patterns of distribution seen in Hawaiian forests correlate with high malaria transmission at low elevations, intermittent transmission at middle elevations, and little to no transmission at high elevations, in direct correlation with mosquito density (Atkinson & Samuel 2010; LaPointe *et al.* 2012).

AVIAN MALARIA IN NEW ZEALAND

In addition to native species, the New Zealand avifauna now includes multiple non-native species, mostly of European origin. At least 137 non-native bird species

were introduced by the early 1900s, with the majority of introductions occurring in the mid to late 1800s for ornamental reasons from acclimatisation societies (McDowall 1994; Veltman *et al.* 1996; Green 1997). Of these species, only 28 have naturalised and are resident today (Veltman *et al.* 1996). These include the Eurasian blackbird (*Turdus merula*), song thrush (*T. philomelos*), house sparrow (*Passer domesticus*), and starling (*Sturnus vulgaris*), which are abundant throughout the country (Green 1997; Heather & Robertson 2000). The direct impacts of these non-native species on native species have been documented, such as competition for resources (Williams & Karl 1996; Williams 2006). However, indirect interactions mediated by shared parasites have not been investigated in great detail, and the present thesis seeks to further existing knowledge on this topic.

The presence of malaria parasites has been confirmed in native New Zealand avifauna and has been linked to mortality in multiple species, including the mohua (*Mohoua ochrocephala*), hibi (*Notiomystis cincta*), kereru (*Hemiphaga novaeseelandiae*), great spotted and brown kiwi (*Apteryx haastii* and *A. australis*), New Zealand dotterel (*Charadrius obscurus*), and South Island saddleback (*Philesturnus carunculatus*) (Alley *et al.* 2008; Derraik *et al.* 2008; Howe *et al.* 2012). The presence of malaria parasites has also been confirmed in multiple non-native species in New Zealand (Tompkins & Gleeson 2006; Sturrock & Tompkins 2008; Ewen *et al.* 2012; Howe *et al.* 2012; Sijbranda *et al.* 2016), including the blackbird, song thrush, house sparrow, and starling. High prevalence reported in these non-native birds has led to suggestions that they may be acting as reservoir species, from which spillover to native species may occur (Tompkins & Gleeson 2006). Overall, *Plasmodium* parasites have been reported in 35 bird species in New Zealand to date (Schoener *et al.* 2014).

The exact number of *Plasmodium* lineages in New Zealand is unclear, but numbers as high as 17 have been reported based on genetic analyses, with four generally accepted non-native lineage clusters (Ewen *et al.* 2012; Howe *et al.* 2012; Schoener *et al.* 2014). These globally widespread lineages are *Plasmodium* sp. lineage LINN1, *P. (Novyella) vaughani* lineage SYAT05, *P. (Huffia) elongatum* lineage GRW06, and *P. (Haemamoeba) relictum* (lineages GRW4 and SGS1). Additional unresolved lineages have been reported, including some speculated as being native to New Zealand (“lineage ANME01”; Baillie *et al.* 2012).

Of the 15 species of mosquitoes in New Zealand, two are suspected of being vectors of avian malaria, the introduced *Cx. quinquefasciatus* and the endemic *Cx. pervigilans* Bergroth (Holder *et al.* 1999; Tompkins & Gleeson 2006; Massey *et al.* 2007; Derraik *et al.* 2008). The invasive *Cx. quinquefasciatus* was introduced into New Zealand in the early 19th century (LaPointe *et al.* 2012) but in recent decades has expanded its range from its introduction sites in the northern portion of the North Island to as far south as Christchurch on the South Island (Holder *et al.* 1999). The native *Cx. pervigilans*, however, has a wide tolerance of environmental conditions and is the most prevalent and widespread mosquito species in New Zealand, distributed throughout the length of the country (Belkin 1968; Cane & Disbury 2010). An inverse relationship between avian malaria prevalence (i.e. the proportion of infected hosts) and latitude has been reported in New Zealand, likely due to the correlation between mosquito density and temperature (Tompkins & Gleeson 2006; Baillie & Brunton 2011); however elevational relationships, like those seen in Hawaii (van Riper *et al.* 1986; Ahumada *et al.* 2004), have yet to be investigated.

AIMS OF THE THESIS

The overall aim of this thesis is to investigate the effects of avian malaria on the avifauna of New Zealand and explore the potential effects of biotic and abiotic factors on transmission dynamics. Factors such as elevation, vector abundance, and native or non-native status of avian hosts were taken into consideration. By conducting surveys I sought to assess the presence and prevalence of avian malaria infection across a broad elevational range, at a site experiencing native bird declines in which malaria has been suggested as playing a role (Elliott *et al.* 2010; Tompkins & Jakob-Hoff 2011). I also sought to identify and better understand parasite-host associations by identifying differences in infection among parasite lineages and host species by collecting and analysing host blood samples. In addition, by sampling for mosquitoes, I aimed to help characterise the surrounding vector community. Using these mosquito data, I then sought to create a model to estimate and make preliminary predictions of mosquito abundance under various temperature regimes. The questions addressed in this thesis are intended to help close the knowledge gap of the avian malaria system in New Zealand, getting us one step closer to understanding the effects on the native avifauna at a population scale. On a broader scale, this study aimed to provide insight into the

general ecology and epidemiology of avian malaria, highlighting differences in patterns of prevalence between New Zealand and other locations and providing recommendations to improve detection via molecular techniques.

This thesis has the following specific research objectives:

1. Identify large-scale predictors of malaria parasite prevalence in avian populations based on geographic and host characteristics from the Australasia-Pacific region (Chapter 2).
2. Improve the protocol for detection of avian malaria from host blood (Chapter 3).
3. Identify patterns of avian malaria in wild birds in Nelson Lakes National Park, New Zealand and test predictors of malaria prevalence, such as elevation and native or non-native status (Chapter 4).
4. Estimate mosquito abundance based on daily temperature data and to make preliminary predictions of mosquito distribution (Chapter 5).
5. Explore the potential for avian malaria to be playing a role in population-level declines in the New Zealand avifauna (Chapters 4 and 5).

STRUCTURE OF THE THESIS

Objective 1 was achieved by performing a comparative analysis using a data set compiled from the literature (see Chapter 2). Objective 2 was achieved by investigating the performance of a modified DNA extraction technique for detecting avian malaria parasites from bird blood via PCR by reducing the potential for the reporting of false negatives (see Chapter 3). To achieve Objective 3, I conducted a survey to identify patterns of avian malaria in wild birds in Nelson Lakes National Park (see Chapter 4). Objective 4 was achieved by constructing a realistic model for *Culex* mosquitoes in New Zealand (see Chapter 5). Chapters 4 and 5 were also used to achieve Objective 5, and begin to explore the potential for avian malaria to be playing a role in population-level declines in the New Zealand avifauna.

Chapters two, three, four, and five were initially written in manuscript format for submission and publication in scientific journals and were subsequently modified for inclusion into this thesis. Consequently, there may be minor elements of repetition

between chapters, however an attempt was made to minimise this. I will be the first author of all intended publications, which means I am solely responsible for all research, sampling, and data analysis. The intended manuscript co-authors listed below provided constructive criticism during the experimental design process as well as comments on earlier drafts of the text (R. Poulin – all chapters; D. Tompkins – all chapters; I. Blasco-Costa – chapter 3). Appendix Two is a poster summarising research from my PhD Study and presented at the 64th Annual International Conference of the Wildlife Disease Association in Queensland, Australia, July 2015.

Chapter One: General Introduction

Chapter Two: Drivers of malaria prevalence in avian populations in the Australasia-Pacific region. To be submitted as: C.N. Niebuhr, D.M. Tompkins, and R. Poulin

Chapter Three: Improving detection of avian malaria from host blood: a step towards a standardised protocol for diagnostics. To be submitted as: C.N. Niebuhr and I. Blasco-Costa

Chapter Four: Avian malaria prevalence in native and non-native New Zealand birds along an elevational gradient. To be submitted as: C.N. Niebuhr, R. Poulin, and D.M. Tompkins

Chapter Five: Population dynamics of an endemic New Zealand mosquito, *Culex pervigilans*: Modeling a suspected disease vector. To be submitted as: C.N. Niebuhr, R. Poulin, and D.M. Tompkins

Chapter Six: General Discussion

Chapter Two

Drivers of malaria prevalence in avian populations in the Australasia-Pacific region

INTRODUCTION

The transmission of parasites to their hosts can be influenced by a number of biotic and abiotic factors, which can affect unique components or stages within the transmission cycle (Valkiunas 2005; Tompkins & Poulin 2006; Hellgren *et al.* 2008). Factors such as temperature, host susceptibility, and level of exposure to vectors help determine patterns of parasite infection (Atkinson & Dusek 2000; Poulin 2006b; Hellgren *et al.* 2008). The proportion of infected individuals in a host population, the prevalence of infection, can be influenced by properties of either the local environment or of the host species itself. For instance, among intertidal gastropods infected by contact-acquired trematodes, host species living on soft-sediment shores consistently experience higher prevalence of infections than those on rocky shores (Poulin & Mouritsen 2003), whereas among different mammal species exposed to direct life-cycle nematodes acquired via ingestion, host population density correlates positively with prevalence (Arneberg 2001). The impact from these factors on animal populations can differ in respect to parasite (Poulin 2006a), vector (Hamer *et al.* 2010), or host species (Atkinson & LaPointe 2009) as well as geographic location (Ricklefs & Fallon 2002). To explore this in more detail, let us consider the avian malaria system.

Avian malaria parasites (of the genus *Plasmodium*) and related haemosporidians (*Haemoproteus* and *Leucocytozoon*) are globally distributed (except Antarctica; Grimaldi *et al.* 2015) and present in a majority of avian families (Valkiunas 2005). Prevalence of infection by these disease agents in a focal host population often varies in regards to location or species composition of the avian community (Tompkins & Gleeson 2006; Valkiunas *et al.* 2009; Samuel *et al.* 2015). This is not surprising when we look at the multitude of potential sources of variation within the avian malaria system itself. For example, avian *Plasmodium* parasites are transmitted primarily by mosquitoes (Diptera: Culicidae) and infect a broad range of avian hosts, while avian *Haemoproteus* parasites are transmitted primarily by biting midges (Ceratopogonidae) and louse flies (Hippoboscidae) and tend to be relatively host-specific (Beadell *et al.* 2004; Valkiunas 2005). When comparing prevalence of infection between avian individuals or populations, the type of parasite present must thus be taken into account.

Another example of potential sources of variation in avian malaria infections can be found when comparing data between different geographic locations. Hawaii,

unlike many mainland locations, has no native mosquitoes, with a competent vector species for avian malaria not being introduced until the early 19th century (Hardy 1960). Different levels of exposure to malaria parasites over time has led to high mortality in endemic, therefore naïve, birds (van Riper *et al.* 1986; Samuel *et al.* 2011), with less impact on species introduced from mainland locations (Valkiunas 2005). Differences in parasite infection can also be found within locations, as opposed to between. An inverse relationship has been reported between avian malaria prevalence and both elevation and latitude, in Hawaii and New Zealand, respectively (van Riper *et al.* 1986; Tompkins & Gleeson 2006), since mosquito densities are highly dependent on temperature (Ahumada *et al.* 2004).

Various host life-history traits might also influence the probability of malaria infection. For example, a positive association between malaria prevalence and host body size has been reported, explained by a larger host having a greater surface area for potential mosquito feeding (Atkinson & Van Riper 1991; Valkiunas 2005) and exuding a higher quantity of olfactory cues, such as carbon dioxide (Takken & Verhulst 2013). Another factor reported to increase the probability of infection is the fledging age of the host species, which is likely due to nestlings, as compared to fledglings or adults, being more easily available and attractive to vectors (Valkiunas 2005). The longer the nestling remains in the nest, the greater risk of exposure to vectors it will have. Similarly, as fledging age is associated with the duration of exposure of nestlings to potential vectors, the length of incubation can be associated with the duration of exposure of adults (parents) to potential vectors. In other words, it may be reasonable to assume that, like nestlings, incubating parents have a greater risk of exposure to vectors than birds that are more mobile. Another life-history trait associated with malaria prevalence is clutch size, with previous research reporting a positive correlation with malaria prevalence (Oppliger *et al.* 1997), and a higher malaria infection intensity in wild bird parents with larger clutch sizes (Knowles *et al.* 2011). This relationship could be explained by an increase in reproductive effort in parents for larger clutch sizes such as increased flight distances between nest and foraging site (Stauss *et al.* 2005), resulting in a decrease in immune function (Knowles *et al.* 2011). Such patterns could have implications for avian populations of conservation concern, since parents with the increased foraging effort required to sustain larger clutches are more prone to predation (Lima 1987) and experience a high infection intensity (Møller & Nielsen 2007).

In order to better understand and predict patterns of parasite infection, it is important to identify the biotic and abiotic factors involved and the influences they may have on the disease system. The aim of this study was to identify large-scale patterns of malaria parasite prevalence in avian populations in the Australasia-Pacific region, according to geographic and host characteristics, using a comparative analysis across avian host species. I compiled data on *Plasmodium* prevalence from a variety of host species from a number of studies conducted in the Australasia-Pacific region.

METHODS

Search parameters

To find studies that incorporated prevalence data for malaria parasites and related haemosporidians in avian hosts, I searched Web of Science (and the references cited within) with the following Boolean search parameters: “TI=((malaria OR Plasmodium OR Haemoproteus OR Leucocytozoon OR haematozoa*) AND (bird* OR passeri* OR avian)) AND TS=(Prevalence)”. After an initial screening for irrelevant hits by reviewing abstracts, the search yielded a total of 82 studies. Prevalence data from Chapter 4 was also included as an additional study (cited as “Niebuhr, C. N., Poulin, R., and Tompkins, D. M., unpublished data”). From these 83 total studies, 19 met the specific inclusion criteria (summarised below). See Appendix 1 for summary of studies used in the analysis.

Search criteria

I narrowed the initial search results to studies that reported data from the Australasia and Pacific regions, excluding those from mainland Asia or the Americas. Further criteria limited the data set to only studies that reported sample size, allowing overall prevalence data to be based on a known number of blood samples. Although many studies presented results grouped by host or parasite, I included only those studies that reported prevalence data that included host taxonomy down to species level and parasite down to genus level. Due to the higher proportion of studies investigating *Plasmodium* prevalence as compared to *Haemoproteus* and *Leucocytozoon*, and the frequent grouping of prevalence data over multiple parasite genera, I ultimately limited the data set to hosts infected with *Plasmodium* parasites only. Finally, only data from

individual samples from each study that had no missing values in the data set, i.e. for which I could obtain values for each predictor variable, were used in the analysis.

Model variables

Each study used in the analysis incorporates multiple individual samples into the data set, each representing a unique sampling event from an individual avian host, with malaria infection identified as either present or absent. In total, data for one dependent variable (disease presence) and eleven independent variables were recorded for each sample included in the dataset. These included three geographic variables (latitude, elevation, and location), six host variables (native versus non-native classification, adult body mass, fledging age, incubation time, clutch size, host family, and host genus), and one additional variable (individual study) (Table 2.1). Often much of the predictor data was not directly given in the studies and had to be acquired from other sources (see below). Also through these sources, I was able to update the taxonomic names provided for many of the host species used in the data set. Latitude data was recorded as absolute latitude, or degrees from the equator, and elevation was recorded as metres above sea level. In cases where samples were grouped or when specific latitude and elevation were not provided, accurate as possible maximum and minimum values were assigned to each sample in the data set. In these cases, mean values of maximum and minimum were used in the analysis. In the case of elevation, if a sampling site was not specified, resulting in an extreme elevational range (e.g. data from three undisclosed sites in the North Island of New Zealand; Baron *et al.* 2014) no value was entered into the data set. Locations were classified by the political region in which the sampling took place (e.g. Fiji, Hawaii, and New Zealand).

Table 2.1. Abbreviations and definitions of variables used in the comparative analysis of *Plasmodium* prevalence in the Australasia-Pacific region.

Abbreviation	Definition	Dimension
Sample	Independent sampling event	-
Disease	Malaria infection detected	yes / no
Latitude	Absolute latitude from equator	degrees
Elevation	Elevation above sea level	metres
Location	Country or region of study	-
Class	Classification at sampling location	non-native / native
ABM	Mean adult body mass	grams
Fledge	Mean fledging age	days
Incubation	Mean incubation length	days
Clutch	Mean size of clutch	# of eggs
Study	Individual study providing data to data set	-
Family	Taxonomic family of avian host	-
Genus	Taxonomic genus of avian host (nested within Family)	-

Host species were assigned a classification (Class), either non-native or native (which included endemic) according to the location of sampling (Table 2.1). Host life-history data was recorded by species and included adult body mass (ABM; mean body mass of adult males and females in grams), fledging age (Fledge; mean age at which a bird is capable of flight, in days), incubation time (Incubation; mean length of time between egg oviposition and hatching; in days), and clutch size (Clutch; mean number of eggs per clutch). For Fledge and Incubation variables, if only maximum and minimum data was available, the mean was recorded in the data set. The majority of the life-history data was obtained from a single consolidated database (Myhrvold *et al.* 2015), with additional supplemental data from multiple sources based on specific gaps in data (Miskelly 2013; “Birds in Backyards” 2015, “Coraciiformes Taxon Advisory Group”, “Finch Information Center”; Rodewald 2015). To avoid phylogenetic independent contrasts, I factored in the phylogenetic relationship among host species by including both family (Family) and genus (Genus) of the host. To account for other variation that may help explain patterns in prevalence observed in the data set (e.g.

season or method of sampling), an additional variable was included (Study), which incorporated each individual study that contributed data to the data set.

Data analysis

A binomial generalized linear mixed model (GLMM) was used to analyse the data set to explore predictors of malaria prevalence of infection in birds. I used binary logistic regression with a logit link to model disease presence (infected = 1, not infected = 0) against eight fixed effects (Latitude, Elevation, Location, Class, ABM, Fledge, Incubation, Clutch) and three random effect (Study, Family, and Genus). For the analysis, the random effect of Genus was nested within Family. The analysis was performed using the statistical software IBM SPSS Statistics version 20.0 (SPSS Inc. Chicago, IL, USA). A significance level of 0.05 was used.

RESULTS

A total of 19 studies published between 2003 and 2016 met the criteria and were included in the data set, with sample sizes ranging from 21 to 1277 birds (Table 2.2.) Samples used in the data set were collected from a total of six regions, American Samoa, Australia, Cook Islands, Fiji, Hawaii, and New Zealand (Table 2.2, Fig. 2.1), and consisted of 8256 blood samples from 58 non-migratory host species in 34 families and 9 orders (Table 2.3). Samples were grouped by host classification as non-native (n = 2203) and native (n = 6053) (Table 2.3). Across samples, I observed a broad range of values for the variables of elevation and latitude, along with host life-history data (ABM, Fledge, Incubation, and Clutch) (Table 2.4).

Table 2.2. Summary of categorical variables used in the comparative analysis of *Plasmodium* prevalence in the Australasia-Pacific region (see Table 2.1 for definitions of variables. See Appendix 1 for summary of studies).

Variable		# of samples	Percent of samples in data set
Disease	Not present	7094	85.9%
	Present	1162	14.1%
	Total	8256	100.0%
Study	1	235	2.8%
	2	1277	15.5%
	3	1094	13.3%
	4	438	5.3%
	5	362	4.4%
	6	1005	12.2%
	7	131	1.6%
	8	100	1.2%
	9	363	4.4%
	10	1014	12.3%
	11	774	9.4%
	12	55	0.7%
	13	89	1.1%
	14	99	1.2%
	15	21	0.3%
	16	436	5.3%
	17	222	2.7%
	18	109	1.3%
	19	432	5.2%
	Total	8256	100%
Location	American Samoa	21	0.3%
	Australia	2045	24.8%
	Cook Islands	22	0.3%
	Fiji	10	0.1%
	Hawaii	3044	36.9%
	New Zealand	3114	37.7%
	Total	8256	100%
Class	Non-native	2203	26.7%
	Native	6053	73.3%
	Total	8256	100%

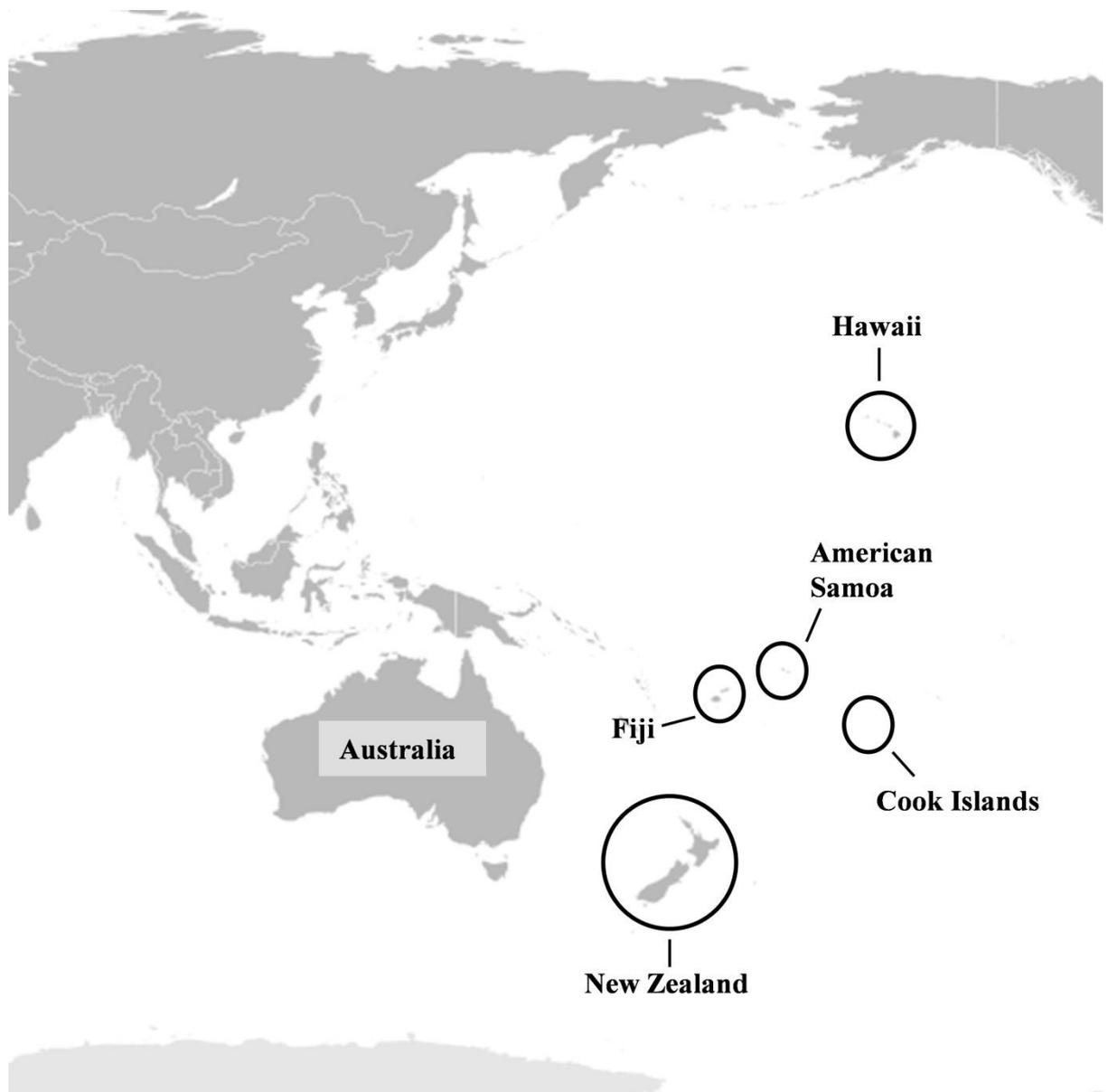


Figure 2.1. Locations sampled in studies to construct the data set, which include American Samoa, Australia, Cook Islands, Fiji, Hawaii, and New Zealand.

Table 2.3. List of avian host species (n = 58) from 19 studies used to identify factors predicting *Plasmodium* infection in wild birds in the Australasia-Pacific region and accompanying host life-history data.

Order	Family	Scientific Name	Common Name	Adult body mass (g)	Fledging age (d)	Incubation (d)	Clutch size (# eggs)
Anseriformes	Anatidae	<i>Hymenolaimus malacorhynchos</i>	Blue Duck	832.67	75	34	5.47
Charadriiformes	Charadriidae	<i>Chasiempis sandwichensis</i>	Hawaii Elepaio	14.03	16	18	2
		<i>Chasiempis sclateri</i>	Kauai Elepaio	14.03	16	18	2
Columbiformes	Columbidae	<i>Columba livia</i>	Rock Pigeon	320	31	17.5	2
		<i>Hemiphaga novaeseelandiae</i>	Kereru	652.5	37.5*	28.5	1
Coraciiformes	Alcedinidae	<i>Todiramphus chloris</i>	Collared Kingfisher	66.25	44	18*	3.89
	Halcyonidae	<i>Todiramphus sanctus</i>	Sacred Kingfisher	53.95	26	20.5*	4.64
Passeriformes	Acanthisittidae	<i>Acanthisitta chloris</i>	Rifleman	6.53	24	19.5	4
		<i>Acridotheres tristis</i>	Common Myna	113.5	23	17.5	4.5
	Acanthizidae	<i>Gerygone igata</i>	Grey Warbler	6.4	17.2	19.5	3.35
	Artamidae	<i>Gymnorhina tibicen</i>	Australian magpie	290.5	28	20.5	4
	Callaeidae	<i>Callaeas wilsoni</i>	Kokako	225.5	27.5	21.5	2.5
	Climacteridae	<i>Philesturnus rufusater</i>	NI Saddleback	70	24.5*	18	2.5
		<i>Climacteris picumnus</i>	Brown Treecreeper	29.4	26	17	3
		<i>Pardalotus punctatus</i>	Spotted Pardalote	8.88	21	19	4.2
	Emberizidae	<i>Emberiza citrinella</i>	Yellowhammer	29.7	12.55	13	3.9
	Estrildidae	<i>Lonchura malacca</i>	Chestnut Mannikin	12.6	25	12.5	5.5
		<i>Lonchura punctulata</i>	Nutmeg Mannikin	14	18	16	5.5
		<i>Acanthis flammea</i>	Common Redpoll	13	12	10.5	5
		<i>Cardinalis cardinalis</i>	Northern Cardinal	43.05	14.25	12.5	3.12
		<i>Carduelis carduelis</i>	European Goldfinch	16	13.5	13	4.9
		<i>Chloris chloris</i>	Greenfinch	27.65	13.5	13	4.9
		<i>Chlorodrepanis stejnegeri</i>	Kauai Amakihi	16.9	18.8	14	3
		<i>Chlorodrepanis virens</i>	Hawaii Amakihi	13.35	16.8	14	2.5
		<i>Haemorhous mexicanus</i>	House Finch	21.4	15.05	14	4.31
		<i>Himatione sanguinea</i>	Apapane	14.4	16	13	3
		<i>Loxops caeruleirostris</i>	Akekee	10.95	18†	15*†	2
		<i>Magumma parva</i>	Anianiau	9.75	18	14	3
		<i>Paroreomyza montana</i>	Maui Alauahio	8.88	18	16.5	4.2
		<i>Vestiaria coccinea</i>	Iiwi	17.5	21.5	14	2
		<i>Fringilla coelebs</i>	Chaffinch	22.63	13.5	12.5	4.5
	Hirundinidae	<i>Hirundo neoxena</i>	Pacific Swallow	15	20.5	16	3.5
	Meliphagidae	<i>Anthornis melanura</i>	Bellbird	27.15	19	15	3.11
		<i>Prothemadera novaeseelandiae</i>	Tui	107.25	21	14	3
	Mohouidae	<i>Mohoua albicilla</i>	Whitehead	22.25	17	18	3
	Muscicapidae	<i>Horornis diphone</i>	Japanese Bush Warbler	14.4	13	15	4.35
	Notiomystidae	<i>Notiomystis cincta</i>	Hihi	89.5	27.49	15.72	4
	Pachycephalidae	<i>Mohoua novaeseelandiae</i>	Brown Creeper	12.2	20	19	3
	Passeridae	<i>Passer domesticus</i>	House Sparrow	27.7	15	11.75	4.35
	Petroicidae	<i>Petroica australis</i>	South Island Robin	35	20.8	17.7	2.7
		<i>Petroica longipes</i>	North Island Robin	35	21	19	2.45
		<i>Petroica macrocephala</i>	Tomtit	11.6	18.5	16*	3.95
	Prunellidae	<i>Prunella modularis</i>	Dunnoek	20.2	12.5	12.5	4.9
	Pycnonotidae	<i>Pycnonotus cafer</i>	Red-Vented Bulbul	41.25	12	13	2.75
	Rhipiduridae	<i>Rhipidura fuliginosa</i>	NZ Fantail	7.75	14.2	15.2	2.2
	Sturnidae	<i>Sturnus vulgaris</i>	Common Starling	77.68	21	12	5
	Timaliidae	<i>Garrulax canorus</i>	Melodious Laughing Thrush	65.95	14.5*	14.5*	3.2
		<i>Leiothrix lutea</i>	Red-Billed Leiothrix	21.5	11	12	2.82
	Turdidae	<i>Copsychus malabaricus</i>	White-Rumped Shama	29.68	12.5	14	3.95
		<i>Myadestes obscurus</i>	Omao	50.45	17	16	1.75
		<i>Turdus merula</i>	Eurasian Blackbird	102	13.5	13.5	4
		<i>Turdus philomelos</i>	Song Thrush	68.8	15	11.5	4
	Zosteropidae	<i>Zosterops japonicus</i>	Japanese White-Eye	11.25	10	11	3.15
		<i>Zosterops lateralis</i>	Silvereye	12.40	12	11.5	3.15
Psittaciformes	Nestoridae	<i>Nestor meridionalis</i>	Kaka	429	73	20.4	4.5
Strigiformes	Strigidae	<i>Ninox novaeseelandiae</i>	Morepork	195	40	29	2.5
Struthioniformes	Apterygidae	<i>Apteryx australis</i>	South Island Brown Kiwi	2600	6	79	1.25
		<i>Apteryx mantelli</i>	North Island Brown Kiwi	2631	6	79	1.45

*Indicates the data is the mean of available maximum and minimum values.

†No information but likely similar to closely related species Akepa (Rodewald 2015); see data from Lepson & Freed (1997).

Table 2.4. Summary of continuous variables used in the comparative analysis of *Plasmodium* prevalence in the Australasia-Pacific region (see Table 2.1 for definitions of variables).

Predictor Variable	Minimum	Maximum	Mean	Std. Deviation
Latitude	14.3	45.9	29.68	8.78
Elevation	25	1830	620.17	526.37
ABM	6.4	2631	81.50	362.02
Fledge	6	75	17.06	6.03
Incubation	10.5	79	15.44	9.37
Clutch	1	5.5	3.21	0.71

Five of the eight fixed-effect variables contributed to the variation in the model ($p < 0.05$; Table 2.5). For the random effects, the Study variable had an estimate of variance of 2.550, which explained approximately 49% of the random effect variance ($P = 0.028$; Table 2.6). This is the best estimate given the data, however due to the wide 95% confidence intervals, it is difficult to accurately estimate the proportion of the fixed-effect variance accounted for by the Study variable. The values of variances for the Family and Genus variables were 1.448 and 1.194, respectively, and in total explained approximately 51% of the random effect variance; however these values were not significant ($P > 0.05$; Table 2.6). For the fixed effects, negative correlations with presence of infection were observed for latitude, elevation, and incubation length while a positive correlation was observed for ABM (Table 2.7). *Plasmodium* prevalence was also shown to vary by location, but not host classification (Table 2.8).

Table 2.5. Factors predicting *Plasmodium* infection in wild birds in the Australasia-Pacific region.

Predictor	df	F	p-Value
Location	5	2.695	0.019
Latitude	1	16.204	< 0.001
Elevation	1	26.342	< 0.001
Class	1	2.654	0.103
ABM	1	4.055	0.044
Fledge	1	1.062	0.303
Incubation	1	4.65	0.031
Clutch	1	1.898	0.168

Table 2.6. Estimate of variance associated with random effects in the model (see Table 2.1 for definitions of variables) used to identify factors predicting *Plasmodium* infection in wild birds in the Australasia-Pacific region.

Random Effect	Estimate of Variance	Std. Error	p-Value	95% Confidence Interval	
				Lower	Upper
Study	2.550	1.164	0.028	1.043	6.237
Family	1.448	1.136	0.203	0.311	6.741
Genus	1.194	0.712	0.094	0.371	3.843

Table 2.7. Correlation coefficients and 95% confidence intervals for continuous variables used to identify factors predicting *Plasmodium* infection in wild birds in the Australasia-Pacific region.

	Coefficient	Std. Error	p-Value	95% Confidence Interval	
				Lower	Upper
				Latitude	-0.140
Elevation	-0.002	0.000	< 0.001	-0.002	-0.001
ABM	0.006	0.003	0.044	0.000	0.011
Fledge	-0.031	0.030	0.303	-0.091	0.028
Incubation	-0.234	0.109	0.031	-0.447	-0.021
Clutch	0.392	0.284	0.168	-0.166	0.950

Table 2.8. *Plasmodium* presence in birds sampled in 19 studies from the Australasia-Pacific region. The table includes data organised by (a) non-native and native hosts, (b) location of sampling, and (c) by non-native and native hosts within each location.

(a) Disease presence of non-native and native host

		Non-native	Native	TOTAL
Disease	not present	1850	5244	7094
	present	353	809	1162
TOTAL		2203	6053	8256
Prevalence		16.0%	13.4%	14.1%

(b) Disease presence from six different regions

		American Samoa	Australia	Cook Islands	Fiji	Hawaii	New Zealand	TOTAL
Disease	not present	14	2016	13	10	2497	2544	7094
	present	7	29	9	0	547	570	1162
TOTAL		21	2045	22	10	3044	3114	8256
Prevalence		33.3%	1.4%	40.9%	0%	18.0%	18.3%	14.1%

(c) Combined: Disease presence of non-native and native host from six different regions

	Disease	American Samoa		Australia		Cook Islands		Fiji		Hawaii		New Zealand		TOTAL
		Non-native	Native	Non-native	Native	Non-native	Native	Non-native	Native	Non-native	Native	Non-native	Native	
	not present	3	11	14	2002	13	0	10	0	1074	1423	736	1808	7094
	present	0	7	12	17	9	0	0	0	66	481	266	304	1162
	TOTAL	3	18	26	2019	22	0	10	0	1140	1904	1002	2112	8256
	Prevalence	0%	38.9%	46.2%	0.8%	40.9%	-	0%	-	5.8%	25.3%	26.5%	14.4%	14.1%

DISCUSSION

The ability of parasites to infect host populations depends on a number of factors, leading to differences in prevalence of infection between and within animal populations. Many of these differences in prevalence have been associated with the exposure of the host to vectors and susceptibility of the host to infection (Atkinson & Dusek 2000; Hellgren *et al.* 2008; Samuel *et al.* 2011). Results from my analysis showed that a large host body size helps drive malaria prevalence, supporting previous findings suggesting a larger host body increases potential vector exposure (Atkinson & Van Riper 1991; Valkiunas 2005). This relationship may be important to captive facilities used to translocate, breed, or maintain species or individuals of concern, since multiple instances of deaths due to avian malaria in captive birds have been documented (Grim *et al.* 2003; Alley *et al.* 2008; Banda *et al.* 2013). For example, in the case of limited resources, emphasis on vector avoidance (e.g. location of open air housing in relation to standing water) or vector control (e.g. removal of standing water or the application of larvicide) could be prioritised towards facilities or exhibits incorporating larger bird species. However to prioritise species of similar body size, further research would be needed on the efficacy of vector avoidance or control strategies on individual species.

In my analysis, fledging age was not a significant source of variation in malaria prevalence, although this relationship has been reported in the literature (Valkiunas 2005). One possible explanation may be that the exposure of avian hosts to potential vectors at certain points in the hosts' lifespan varies by species. For example, previous research on the common chaffinch (*Fringilla coelebs*) shows that infection in young birds can occur both before and after they leave the nest, emphasising that the nestling stage is not crucial to infection (Valkiunas 2005). A bias may also exist in the results due to the inclusion of samples taken from birds of various ages. Older adult individuals would have a much greater amount of exposure to infection in their lifetime than recently fledged individuals. To more accurately assess the effect of fledging age on parasite prevalence, it may be more appropriate to collect samples from all individuals immediately prior to fledging. A negative correlation between length of incubation and malaria prevalence was observed. If the assumption is made that the duration a parent remains on the nest during incubation influences vector exposure similarly to that of

nestlings remaining in the nest before fledging, we would expect the same pattern between incubation length and prevalence as seen with fledging age, however this was not the case. Again, the results may be biased due to differences between the roles of the sexes, in this instance not accounting for any variation between males and females in respect to their incubation roles (i.e. time spent incubating eggs). Clearly more information is needed regarding vector exposure to individuals in the nest, as other factors may be involved such as type and location of the nest. The analysis did not indicate clutch size to have an influence on malaria prevalence, although this relationship has been demonstrated in previous research that showed trade-offs between reproduction and immune defence investments (Oppliger *et al.* 1997; Knowles *et al.* 2011). Differences in reproductive efforts between or within populations could be helping to bias the results. Further investigation into such differences may be needed before understanding the effects of clutch size or other host susceptibility factors on malaria prevalence.

The largest sources of variation in *Plasmodium* prevalence were due to elevation and latitude. These results support previous findings that malaria prevalence of infection decreases with an increase in both elevation and latitude, likely due to the lower mosquito densities at colder temperatures (van Riper *et al.* 1986; Tompkins & Gleeson 2006). These patterns of infection correlate with the hypothesis that the elevational and latitudinal distributions of native bird species can be negatively impacted due to avian malaria.

the negative impact of avian malaria on the population dynamics of native bird species is influenced by , influencing their elevational abundance and distribution (van Riper *et al.* 1986; LaPointe *et al.* 2010; Samuel *et al.* 2015). Understanding the elevational and latitudinal distributions of malaria infections has conservation and management implications for the future, since climate change could intensify the negative impacts of malaria to bird populations (Benning *et al.* 2002).

Malaria prevalence was influenced by location, but not host classification (Table 2.8). Prevalence reported for American Samoa, Cook Islands, and Fiji may be less accurate due to low sample sizes. This is partly due to the lack of studies in these regions, and partly due to much of the data not meeting the inclusion criteria due to gaps in the host life-history data. In addition, for these locations as well as Australia, a

disproportionate number of native hosts were sampled in comparison to non-native ones. However, a very low prevalence was seen in native birds of Australia (0.8%) (Table 2.8). Although there are only three native species represented in this category, perhaps either malaria parasites have not infiltrated these populations, or individuals of these species are highly susceptible and not active enough to be caught for sampling. More research into malaria prevalence and host associations in Australia may help to provide answers. Interestingly, while the number of samples and overall *Plasmodium* prevalence from Hawaiian and New Zealand studies were similar, an inverse trend was seen for prevalence of non-native and native hosts between the two locations (Table 2.8, Fig. 2.2). One explanation could be that these patterns are associated with the development of some resistance in native species in some regions more than others, as suggested for Hawaii compared to New Zealand (Chapter 4). Further research is needed to explore the potential differences in prevalence patterns between these two locations.

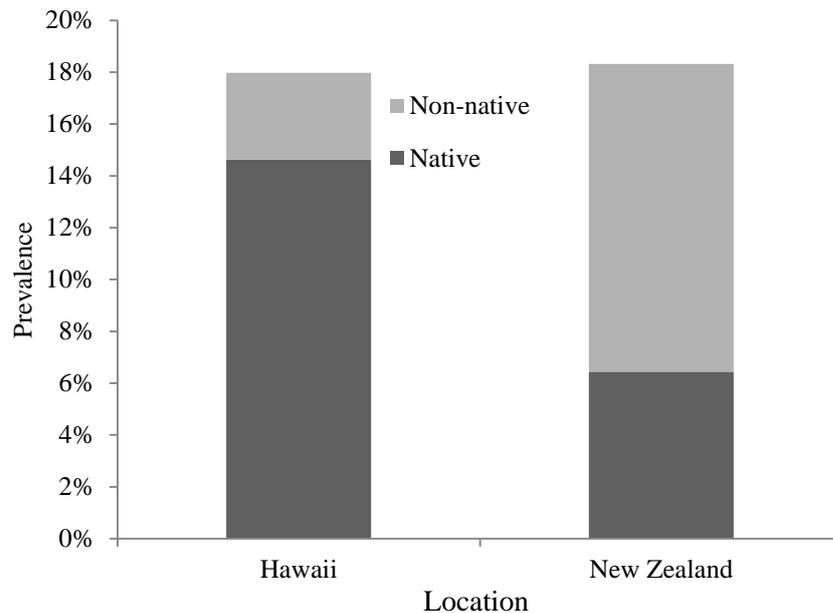


Figure 2.2. Overall *Plasmodium* prevalence in non-native and native bird hosts in Hawaii (18.0%, n = 3044) and New Zealand (18.3%, n = 3114).

Conclusion

Although I have achieved my initial goal of identifying and discussing patterns of malaria parasite prevalence in avian populations, deeper analyses are necessary to fully understand the factors driving these patterns. I suggest that further research is

needed to investigate other factors that may be driving parasite prevalence, such as nest type (i.e. within tree cavity or out in the open), height above ground of nest, coastal versus inland populations, and colonial versus solitary species. Additionally, I recommend exploring drivers of malaria infections beyond the Australasia-Pacific region, as well as expanding the focus beyond *Plasmodium* to related parasites, such as *Haemoproteus* and *Leucocytozoon*. It is important to note that not every sample from each study was included in the analysis due to missing data, such as the lack of elevational data in a large number of avian malaria surveys, the reporting of prevalence without sample size, the lumping of samples by host group (thus preventing accurate life-history data for hosts) and parasite group (e.g. reporting of combined *Plasmodium* and *Haemoproteus* prevalence; Jones *et al.* 2013). To minimise the potential for missing data in analyses such as this, future studies should present prevalence data along with corresponding sample sizes and refrain from lumping data by groups (e.g. hosts and/or parasites) if possible.

Since the analysis method requires no missing data for each variable, effort should be made to obtain as much information as possible, thus preventing bias towards information from studies and species that are more easily obtained. Potential biases for for this method may be due to the Ultimately, by beginning to identify likely drivers of malaria prevalence, we are able to better understand large-scale patterns across multiple avian populations.

Chapter Three

Improving detection of avian malaria from host blood: a step towards a standardised protocol for diagnostics

INTRODUCTION

Avian malaria due to *Plasmodium* spp. has caused significant declines of native birds in Hawaii (van Riper et al. 1986, Atkinson and Samuel 2010) and has caused deaths in multiple endangered bird species in New Zealand (Alley *et al.* 2008, 2010; Howe *et al.* 2012). Infections by avian malaria result in the parasitisation of the red blood cells of the host. Molecular diagnostic techniques, such as polymerase chain reaction (PCR), have played an important role in the advancement and understanding of avian malaria taxonomy and ecology (Ricklefs & Fallon 2002; Hellgren *et al.* 2004). For example, PCR is capable of detecting any stage of *Plasmodium*, dead or alive, although alive at the time of sampling is easier, (Freed & Cann 2006), as well as both parasite and host blood meal from a single mosquito (Massey *et al.* 2007). Problems arise when parasitemia levels are at a level too low to be detected or unable to be amplified by PCR, resulting in false negatives (Freed & Cann 2006).

Also contributing to potential false-negative results is PCR inhibition, which is the most common reason for the failure of DNA amplification during PCR, assuming sufficient copies of DNA are present, and can (Alaeddini 2012). PCR inhibitors can be found in a variety of types of samples, including blood (Akane *et al.* 1994), tissue (Bélec *et al.* 1998), urine (Khan *et al.* 1991), and soil (Tsai & Olson 1992). Other sources of inhibitors can be found in compounds that come into contact with samples during preservation and analysis, such as NaCl, sodium dodecyl sulfate (SDS), and even glove powder (Kreader 1996; Wilson 1997). To minimise the potential for false-negative PCR results, a sufficient amount of inhibitors must be removed from a sample. Due to the highly variable nature of PCR inhibitors, no one DNA extraction and purification procedure exists that is ideal for all scenarios (Zhang *et al.* 1995; Fredricks & Relman 1998; Al-soud & Rådström 2001).

Although both human and avian malaria studies must account for false negatives, these are more common in avian malaria diagnostics. Much of this bias is attributed to the blood of the bird host which, due to nucleated red blood cells, has over 350 times the DNA than that of mammalian blood (Freed & Cann 2006). Thus avian malaria diagnostics must detect parasite DNA from a sample containing more than two orders of magnitude more host DNA than from a mammalian sample with a similar level of parasitemia. To further complicate the process, the average bird genome size is

1300 megabase pairs (Mbp), while the genome size of *Plasmodium* parasites is approximately 25 Mbp, restricting the total yield of parasite DNA to less than 3% regardless of the level of parasitemia (Palinauskas *et al.* 2010). The problems of PCR diagnostics for malaria samples from birds, as opposed to humans or other mammals, have been compared to problems found when working with forensic and ancient DNA samples, highlighting the need for malaria PCR protocols to be more exacting for birds than humans (Freed & Cann 2006).

The use of commercially available DNA purification kits can provide an effective strategy to eliminate PCR inhibition, obtain high quality DNA, and ultimately reduce the potential for false negatives (Yang *et al.* 1997). Many commercial kits incorporate silica-membrane-based nucleic acid purification in the form of a spin column containing a silica-gel membrane. The membrane allows DNA fragments to selectively bind to the membrane while contaminants, such as nucleotides, proteins, and salts, are able to pass through. Washing then helps remove the remaining contaminants and PCR inhibitors that may be still present. The final step consists of an elution, the extraction of one material from another by washing with a solvent. In this case water or a buffer is used to unbind the DNA from the membrane. Another strategy for overcoming inhibition issues is to dilute the DNA extract, effectively diluting any PCR inhibitors down to a sufficient level to minimise or eliminate the inhibition effect (Regan *et al.* 2012). However this option may not be feasible for highly degraded templates of DNA (Ye *et al.* 2004).

Some studies, as part of their optimisation strategy for maximising DNA yield, have altered the elution step of the extraction protocol. In some cases multiple elutions are performed and pooled together (Desneux & Pourcher 2014), whereas in other cases multiple elutions were performed but kept separate from each other (Ohmori *et al.* 2013). Although one study reports that the reasoning for the modification of the elution step was to produce less degraded DNA (Toader *et al.* 2009), in many studies the reason is not stated. For example, no explanation was provided for the use of un-pooled second eluates for PCR amplifications for zooxanthellae (Ulstrup & Van Oppen 2003), coral (Concepcion *et al.* 2006), oak tree (Toader *et al.* 2009), and sea slug samples (Goodheart & Valdés 2012), as well as for a study amplifying virus DNA from leaf samples (Gillen *et al.* 2009). Of note, all five of the previous mentioned studies used

DNeasy kits (QIAGEN, Valencia, California, USA), which are common commercially available silica-membrane-based DNA extraction and purification kits.

Avian malaria studies have used a variety of extraction methods to successfully extract parasite DNA from avian blood samples (Ricklefs *et al.* 2005; Tompkins & Gleeson 2006; Valkiunas *et al.* 2007), including silica-based DNA purification. The DNeasy Blood & Tissue Kit (QIAGEN) has been used for samples from a variety of malaria and avian species throughout the world (Wood *et al.* 2007; Castro *et al.* 2011; Atkinson *et al.* 2014). To my knowledge, no avian malaria study has reported using a non-pooled second eluate sample for PCR, however it is possible that this protocol modification was simply not mentioned and assumed by the authors to fall under the “following the manufacturer’s protocol” umbrella. The manufacturer’s extraction protocol for the DNeasy Blood & Tissue Kit (QIAGEN DNeasy Blood & Tissue Handbook, 07/2006, pg. 27) does give mention of an optional additional step of repeating the first elution (i.e. adding a second elution step). The protocol for this additional step suggests either using a different microcentrifuge tube for the second elution step to prevent the dilution of the first eluate, or to combine both eluates. However the explanation provided considering this additional step (regardless of the option of combining both eluates) is simply to increase the overall DNA yield. In the case of blood samples for avian malaria analysis, which include both parasite and host DNA, an increase in DNA yield does not necessarily have an effect on ratio of parasite to host DNA. The protocol indicates the use of 200 μL of elution buffer for the elution steps, but also mentions the option to decrease the amount of elution buffer to 100 μL . The idea behind the decrease in buffer used is to increase the resulting DNA concentration of the sample, but as a result, a decrease in DNA yield will occur. As with the DNA yield, any increase in DNA concentration in samples tested for avian malaria will occur for both host and parasite DNA.

The purpose of this study was to investigate the performance of a modified DNA extraction technique for detecting *Plasmodium* spp. from bird blood via PCR; specifically exploring a two-step elution modification of a standard DNA extraction protocol. I propose a standardised protocol for extraction of *Plasmodium* DNA from avian hosts that reduces the likelihood of false negatives, allowing for more accurate diagnostics and comparisons of *Plasmodium* prevalence across studies.

METHODS

Blood samples used in this study were collected from passerines at Nelson Lakes National Park, South Island, New Zealand during 2013-2015. Samples were collected via brachial venipuncture and stored, unfrozen, in a lysis buffer (0.1 M NaCl, 1 mM EDTA, 0.1 M Tris-HCl pH 8.0) for one to five months before processing. As a part of my molecular optimisation process, I identified the implementation of a second elution step as a possible addition to the extraction protocol. Of note, during this optimisation process, the dilution of genomic DNA and/or the addition of bovine serum albumin (BSA) did not appear to enhance PCR amplification yield, and thus were not used in the protocol. The following methods were then conducted on six samples that were previously identified by gel electrophoresis to contain malaria parasites that were later confirmed to be *Plasmodium* spp. by DNA sequencing (Chapter 4). These samples consisted of blood from four Eurasian blackbirds (*Turdus merula*), one song thrush (*T. philomelos*), and one house sparrow (*Passer domesticus*).

DNA for these samples was extracted using a DNeasy Blood & Tissue Kit following the manufacturer's protocol (Table 3.1), with the following modifications. Steps 1-3 were conducted twice for each sample. Also, the initial incubation time with proteinase K from step 2 was extended overnight to increase DNA yield (Atkinson *et al.* 2014). Prior to step 4, paired samples were pooled and mixed thoroughly by vortexing. Pooled samples were then split into paired samples and run individually for the remainder of the procedure. For the first elution step (step 7), one sample in a pair was eluted with 50 μ L while the other sample in that pair was eluted with 200 μ L of Buffer AE (provided in the kit). These resulting eluates are herein referred to as the 'first eluates'. Using the same spin column, but with a new sample tube, all twelve spin columns were eluted a second time with 100 μ L of Buffer AE. These resulting eluates are herein referred to as the 'second eluates'. To assess integrity, 10 μ L genomic DNA was run on 0.5% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen, Carlsbad CA, USA) for eight minutes and visualised under UV light.

Table 3.1. Manufacturer's protocol for DNeasy Blood & Tissue Kit (QIAGEN), a silica-membrane-based DNA extraction kit (QIAGEN DNeasy Blood & Tissue Handbook, 07/2006, pg. 27).

Step	Summary
1	Addition of proteinase K and PBS to sample (5-10 μ L anticoagulated blood)
2	Incubation step
3	Addition of ethanol
4	Introduce sample to spin column
5	Wash step 1
6	Wash step 2
7	Elution step 1
8	Optional elution step 2

Step	Detailed instructions
1	Nucleated: Pipet 20 μ L proteinase K into a 1.5 ml or 2 ml microcentrifuge tube (not provided). Add 5–10 μ L anticoagulated blood. Adjust the volume to 220 μ L with PBS. Continue with step 2.
2	Add 200 μ L Buffer AL (without added ethanol). Mix thoroughly by vortexing, and incubate at 56°C for 10 min.
3	Add 200 μ L ethanol (96–100%) to the sample, and mix thoroughly by vortexing.
4	Pipet the mixture from step 3 into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at $\geq 6000 \times g$ (8000 rpm) for 1 min. Discard flow-through and collection tube.
5	Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 μ L Buffer AW1, and centrifuge for 1 min at $\geq 6000 \times g$ (8000 rpm). Discard flow-through and collection tube.
6	Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 μ L Buffer AW2, and centrifuge for 3 min at 20,000 $\times g$ (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
7	Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 μ L Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at $\geq 6000 \times g$ (8000 rpm) to elute. <i>Elution with 100 μL (instead of 200 μL) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield.</i>
8	Recommended: For maximum DNA yield, repeat elution once as described in step 7. <i>This step leads to increased overall DNA yield. A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 7 can be reused for the second elution step. Note: Do not elute more than 200 μL into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.</i>

Samples were screened for the presence of *Plasmodium* spp. using nested PCR to amplify a 478 bp fragment of the mitochondrial cytochrome *b* gene. I followed the method of Hellgren et al. (2004), with slight modifications. The first-round primers were HaemNFI (5'-CATATATTAAGAGAAITATGGAG-3') (I = inosine, a universal base) and HaemNR3 (5'-ATAGAAAGATAAGAAATACCATTC-3'). The first PCR was performed in volumes of 10 μ L, which included 2 μ L of genomic DNA, 0.125 mM each dNTP, 1.5 mM MgCl₂, 0.6 mM each primer, and 0.5 units *Taq* DNA polymerase (MyTaq™; Bioline Ltd., London, United Kingdom). The thermal profile consisted of a 3-min 94°C denaturalization step, followed by 20 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 45 s, ending with an elongation step of 72°C for 10 min. The second-round primers were HaemF (5'-ATGGTGCTTTCGATATATGCATG-3') and HaemR2 (5'-GCATTATCTGGATGTGATAATGGT-3'). The composition of the second round PCR reactions was as above, except 1 μ L of PCR product from the first round was used as template instead of genomic DNA. The thermal profile for the second round PCR was the same as that for the first, except the number of cycles was increased from 20 to 35. To check for PCR amplification, 3 μ L of PCR products from the second round were analysed using 2% agarose gel stained with SYBR (*get info) and visualised under UV light.

RESULTS

The DNA concentration of the extracted genomic DNA was measured by spectrophotometry (NanoDrop 1000, Thermo Scientific) and estimated at 2-10 ng/ μ L for all eluates. Although the DNA yield (consisting of both bird and malaria DNA) was slightly higher in the second eluate for five of the six samples as compared to the first, overall the yield was very similar (Table 3.2). By visual inspection of the agarose gel, however, the second eluate appeared to show less degradation as compared to the first eluate (Fig. 3.1).

Table 3.2. Genomic DNA concentrations after two different elution steps using two protocol options of the DNeasy Blood & Tissue Kit (QIAGEN). Sample letters represent *Plasmodium* spp. positive blood samples from six individual birds (A-D, *Turdus merula*; E, *Passer domesticus*; F, *Turdus philomelos*).

		DNA yield (ng/ μ L)	
		1st eluate (50 μ L)	2nd eluate (100 μ L)
Protocol Option 1	Sample		
	A	7.1	7.1
	B	5.8	7.1
	C	6.4	8.4
	D	9.2	7.5
	E	7.2	8.1
F	6.8	7.2	
		1st eluate (200 μ L)	2nd eluate (100 μ L)
Protocol Option 2	Sample		
	A	3.7	5.8
	B	4.0	6.7
	C	5.2	5.5
	D	4.4	7.5
	E	2.8	5.7
F	3.4	6.4	

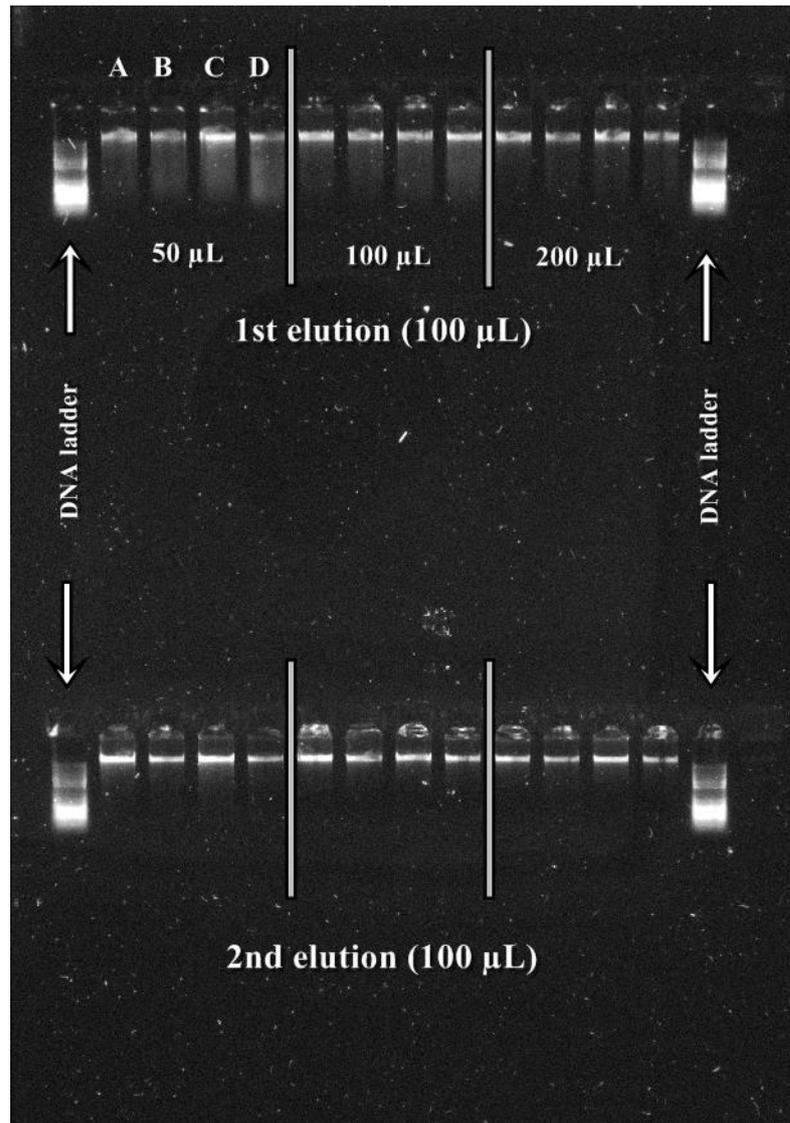


Figure 3.1. Agarose gel electrophoresis of genomic DNA isolated from four *Plasmodium* positive Eurasian blackbird (*Turdus merula*) individuals after two different elution steps using different protocol options of the DNeasy Blood & Tissue Kit (QIAGEN).

After PCR, the agarose gel showed *Plasmodium* spp. DNA fragments of 478 bp for some, but not all of the various elution combinations (Fig. 3.2). Of the bands that were present, some appeared very bright while others were weak and barely visible, including some that were difficult to see before adjusting the image digitally. All bands were easily visible for the second eluate of the 50 μ L first elution from all six blood samples. These bands appeared as bright or brighter when compared to any other elution combination for each individual sample. For one sample (sample D, Fig. 3.2), the only band visible was from the second eluate of the 50 μ L first elution, and thus

may not have been identified as positive for *Plasmodium* spp. if a different extraction protocol option was followed.

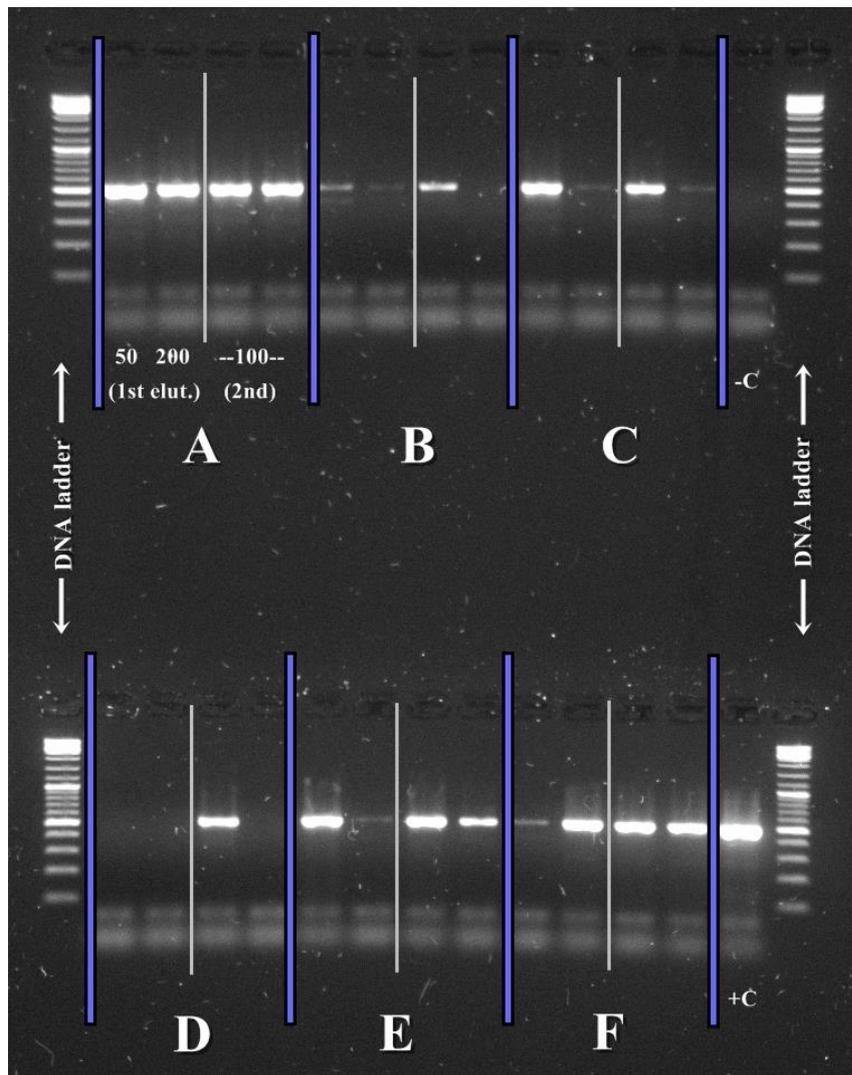


Figure 3.2. Agarose gel showing results from elution modifications in the extraction protocol. The first two wells within each letter represent paired elutions from protocol option 1 (50 and 100 μ L of buffer), while the last two wells within each letter represent protocol option 2 (200 and 100 μ L of buffer). Bands represent 478 bp DNA fragments of *Plasmodium* parasites. Sample letters represent six individual birds (A-D, *Turdus merula*; E, *Passer domesticus*; F, *Turdus philomelos*). Note: +C and -C represent positive and negative controls.

DISCUSSION

The results show that modifying the elution step of a silica-membrane-based extraction protocol can be an effective approach to increasing sensitivity and/or

decreasing the occurrence of false negatives when attempting to detect *Plasmodium* spp. from bird blood via PCR. Specifically, samples produced from unpooled second elutions with 100 μ L of elution buffer from an original first elution with 50 μ L of elution buffer, allowed for brighter and more visible bands when viewed on an agarose gel. Also in one case, this elution protocol allowed for the detection of a malaria positive sample that may have otherwise appeared as a false negative if a different protocol option was followed.

I suspect that during the elution process, the bird DNA (being larger, therefore heavier, than the parasite DNA) is eluted first through the silica-gel membrane. This concept is similar to the concept of size exclusion chromatography (or gel chromatography), which relates DNA molecule size to pore size of the silica gel (Hilario 2004; Wang *et al.* 2010). In theory, particles of different sizes will elute at different rates, with small particles (e.g. parasite DNA) eluting late, after penetrating all sizes of pores, and larger molecules (e.g. bird DNA), unable to penetrate smaller pores, pass by the smaller pores and are eluted earlier within the liquid (e.g. elution buffer) as it passes through the column. In other words, the smaller molecules are better attached to the membrane and thus more resistant to passing through.

In this study, what is likely happening is that the 50 μ L first eluate (from protocol option 1) consists of a very low ratio of parasite to host DNA, whereas the 200 μ L first eluate (from protocol option 2) consists of an average to low ratio (Fig. 3.3). In the second elution step, the same spin column membranes previously eluted with buffer will contain a higher ratio of parasite to host DNA than prior to the first elution. Therefore the second eluates will have a higher parasite to host DNA ratio than their respective first eluates. Additionally, the second eluate from protocol option 1 will have a higher parasite to host ratio than the second eluate from protocol option 2 (Fig. 3.3). It is also possible that by adding an unpooled second elution step, less total PCR inhibitors (from those that remained on the silica-gel membrane after the washing step) were collected into the second eluate; however further research is needed to properly test this possibility. This concept is similar to the optimisation approach of diluting the DNA extract as mentioned previously.

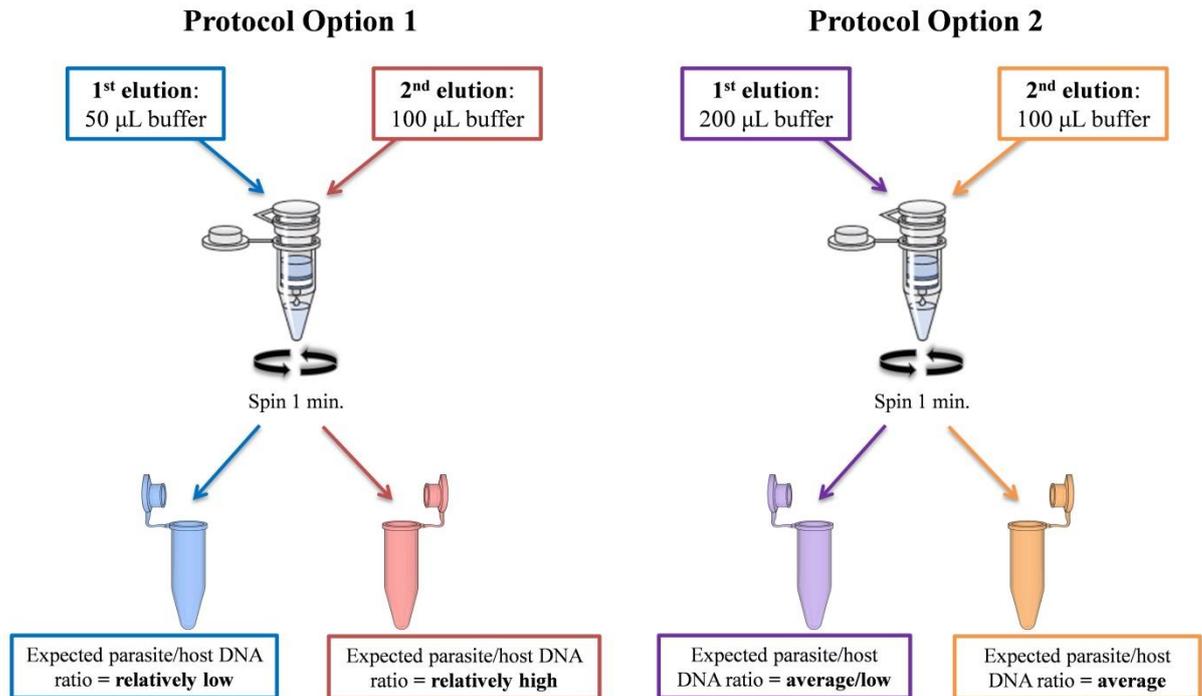


Figure 3.3. Diagram describing the expected parasite to host DNA ratios using four possible unpooled elution-step combinations of a spin column-based commercial DNA extraction kit. I recommend using the eluate from the second elution step of protocol option 1 when attempting to extract and amplify avian malaria parasites from bird blood.

Conclusion

The low ratio of parasite to host DNA may be a concern when analysing bird blood samples for malaria parasites. This may especially be the case in situations of chronic infections, typically associated with low levels of parasitemia (Atkinson & Van Riper 1991; Woodworth *et al.* 2005). Since chronically infected birds can serve as reservoirs of disease, leading to possible spillback events (LaPointe *et al.* 2012), it is important to make as accurate a diagnosis as possible by minimising false negatives. Currently, the avian malaria prevalence in potential reservoir species may be underreported in the literature, in part due to the DNA extraction protocol used. It is unlikely that false negatives can be eliminated completely when working with avian malaria (Freed & Cann 2006), however, any increase in the sensitivity of detection by PCR could prove extremely beneficial in the diagnosis or necropsy of high priority individuals as well as during large-scale surveys, when numerous samples must be screened quickly and on a limited budget. Therefore, when attempting to detect malaria

from bird blood via PCR, I recommend following an elution protocol such as that proposed here.

Chapter Four

Avian malaria prevalence in native and non-native New Zealand birds along an elevational gradient

INTRODUCTION

Emerging infectious diseases, defined as disease-causing agents that rapidly increase in geographic range, host range, or prevalence, pose a serious threat to the conservation of global diversity (Daszak *et al.* 2000). Often it is native wildlife species that are threatened by non-native parasites they have not evolved with or adapted to (Tompkins & Poulin 2006; Tompkins *et al.* 2015). Avian malaria is one such disease that may be of concern to the New Zealand avifauna, considered to be the most extinction-prone in the world (Sekercioglu *et al.* 2004; Tompkins & Poulin 2006).

Avian malaria is a vector-borne disease caused by protozoan parasites of the genus *Plasmodium* (Valkiunas 2005). Avian *Plasmodium* spp. are cosmopolitan in range (except Antarctica; Grimaldi *et al.* 2015) and can infect a broad range of bird hosts causing a range of impacts (Atkinson *et al.* 1995; Beadell *et al.* 2004). Infection by malaria parasites consists of two phases, acute and chronic. The acute stage occurs immediately after infection, followed by the chronic stage with some seasonal relapses (LaPointe *et al.* 2012). The acute phase is characterised by a high level of parasitemia and hosts can show clinical signs such as lethargy, reduced weight gain, and sometimes death (Valkiunas 2005; Williams 2005). Individuals experiencing acute illness may have difficulty foraging or escaping predators (Yorinks & Atkinson 2000). During the chronic phase, hosts that do not die as a result of the acute phase experience a low level of parasitemia. These chronic infections can last for years or for the life of the bird, and in some cases can have a mild impact on individual fitness (Bensch *et al.* 2007).

The most dramatic impact of avian malaria documented resulted from the arrival of *Plasmodium relictum* to Hawaii in the early 20th century (Atkinson & LaPointe 2009). In Hawaii, avian malaria is vectored by the non-native mosquito *Culex quinquefasciatus* and is considered a major factor in the population declines and restricted distribution of native forest bird species (Atkinson & Samuel 2010). The transmission dynamics of avian malaria varies greatly across elevational gradients in Hawaii forests, with patterns showing high malaria transmission at low elevations, intermittent transmission at middle elevations, and little to no transmission at high elevations, in direct correlation with mosquito density (Atkinson & Samuel 2010; LaPointe *et al.* 2012).

The presence of *Plasmodium* parasites has been confirmed in native New Zealand avifauna and has been linked to mortality in multiple species, including the mohua (*Mohoua ochrocephala*), hihi (*Notiomystis cincta*), kereru (*Hemiphaga novaeseelandiae*), great spotted and brown kiwi (*Apteryx haastii* and *A. australis*), New Zealand dotterel (*Charadrius obscurus*), and South Island saddleback (*Philesturnus carunculatus*) (Alley *et al.* 2008; Derraik *et al.* 2008; Howe *et al.* 2012). *Plasmodium* parasites have also been confirmed in multiple non-native species in New Zealand (Tompkins & Gleeson 2006; Sturrock & Tompkins 2008; Ewen *et al.* 2012; Howe *et al.* 2012; Sijbranda *et al.* 2016), including the Eurasian blackbird (*Turdus merula*), song thrush (*T. philomelos*), house sparrow (*Passer domesticus*), and starling (*Sturnus vulgaris*), leading to suggestions that they may be acting as reservoir species, from which spillover to native species may occur (Tompkins & Gleeson 2006). The exact number of *Plasmodium* lineages in New Zealand is unclear, but as many as 17 have been reported, with four generally accepted non-native lineage clusters (Ewen *et al.* 2012; Howe *et al.* 2012; Schoener *et al.* 2014). Additional unresolved lineages have been reported, including some speculated as native to New Zealand (Baillie & Brunton 2011).

Although avian malaria has impacted both captive populations and wild individuals in New Zealand, whether or not wild populations have been impacted is unknown. Additionally, while studies exist that have investigated latitudinal relationships of avian malaria in New Zealand (Tompkins & Gleeson 2006; Baillie & Brunton 2011), no such studies have explored elevational patterns. Previous research conducted in Nelson Lakes National Park (NLNP), New Zealand, indicates a general declines in bird abundance during the last 30 years, most notably in five native bird species: bellbird (*Anthornis melanura*), rifleman (*Acanthisitta chloris*), grey warbler (*Gerygone igata*), New Zealand tomtit (*Petroica macrocephala*), and tui (*Prosthemadera novaeseelandiae*) (Elliott *et al.* 2010). For many of the species that showed a change in abundance, there was a greater change at lower elevations with an apparent threshold around 1000 m. The authors suggested that the greater population declines at low elevations were due to predation, however it also may indicate that malaria, as a vector-borne disease, could be involved (Tompkins & Jakob-Hoff 2011). Since mosquito densities are highly dependent on temperature, transmission rates would vary correspondingly by elevation (Ahumada *et al.* 2004).

We conducted a survey to identify patterns of avian malaria in wild birds in NLNP. The overall aim was to test the potential for avian malaria to have played a role in population-level declines in native birds at the study site. Since one of the components that determines the rate at which the host population acquires the infection (known as the force of infection; McCallum 2005) is the prevalence, i.e. the proportion of infected individuals, the study was designed to test predictors of malaria prevalence in native bird populations, specifically elevation and host species. I hypothesized that prevalence of infection is positively associated with (i) a decrease in elevation and (ii) non-native versus native host species.

METHODS

Study site and sampling

Sampling was conducted along the St. Arnaud Range in Nelson Lakes National Park, South Island of New Zealand. This area of the St. Arnaud Range is included within the Rotoiti Nature Recovery Project (RNRP), one of six mainland ecological restoration projects ('mainland islands') in New Zealand (Harper & Brown 2014). These mainland islands are sites for various ecological restoration efforts and wildlife management. The RNRP consists of a diverse avifauna, including many threatened or declining species such as South Island robin (*Petroica australis*), kaka (*Nestor meridionalis*), and rifleman. Additionally, the RNRP is being used for great spotted kiwi translocations. The St. Arnaud range consists of native southern beech (*Nothofagus* spp.) rainforest, ranging in elevation from 620 m to an abrupt tree line at approximately 1400 m. A domestic sheep pasture and the village of St. Arnaud (population approximately 500) border the St. Arnaud range at approximately 800 and 650 m in elevation, respectively. Sampling took place at four elevational steps (650, 800, 1200, and 1400 m), located above and below the reported bird decline threshold of 1000 m elevation (Elliott *et al.* 2010). Each of the four elevational steps included a sampling site in native forest habitat, with the addition of one sampling site in urban (village of St. Arnaud) and one in edge (fence line between native forest and sheep pasture) habitats (Fig. 4.1). In total, samples were collected from six different sites at four elevations: 650 m (urban: 41°48'16"S, 172°50'35"E; native forest: 41°48'42"S, 172°51'3"E), 800 m (edge: 41°48'33"S, 172°52'12"E; forest: 41°48'44"S, 172°52'5"E), 1200 m (forest: 41°48'15"E, 172°53'55"E) and 1400 m (forest: 41°49'22", 172°52'46")

(Fig. 4.1). Birds were captured by mist netting between sunrise (0500 hr) and an hour before sunset (2100 hr) during the warmer months for three consecutive years, 2012-13 (March-April), 2013-14 (November-March), and 2014-15 (January-April). Due to the various sampling locations and uneven capture rates, mist-netting effort was standardised by calculating the total number of birds caught per 100 net hours. All birds were weighed to the nearest 0.5 g and banded with numbered metal leg bands to prevent re-sampling. Age class (juvenile and adult) and sex (male and female) of each bird was recorded when possible. After swabbing with 70% ethanol, 50-100 μ l of blood was collected by capillary tubes via brachial venipuncture with 27 gauge needles, and stored unfrozen in a Queen's lysis buffer (Seutin *et al.* 1991) until processed for molecular analysis.

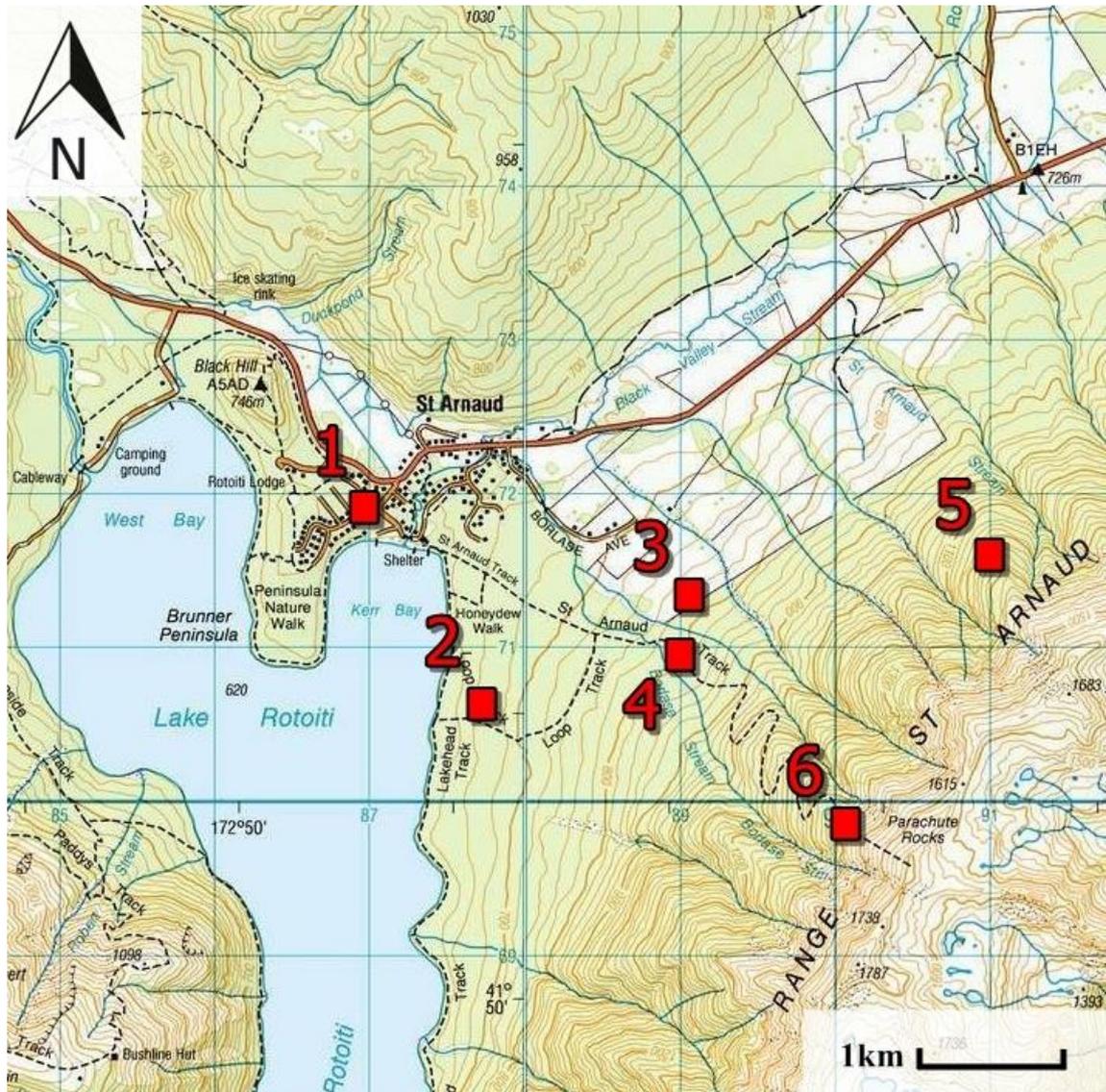


Figure 4.1. Sampling sites along the St. Arnaud range in Nelson Lakes National Park, New Zealand. Sampling took place in native forest habitat at four elevations (650, 800, 1200, and 1400 m), in urban habitat at 650 m, and in pasture-forest edge habitat at 800 m. Each numbered square represents a sampling site (1 = urban / 650 m; 2 = native forest / 650 m; 3 = edge / 800 m; 4 = native forest / 800 m; 5 = native forest / 1200 m; 6 = native forest / 1400 m).

Parasite detection

DNA was extracted from blood samples using a DNeasy Blood & Tissue Kit (QIAGEN, Valencia, California, USA) with the some modifications to the manufacturer's protocol. Following the addition of proteinase K, samples were incubated overnight. The first elution was collected using 50 μ L of Buffer AE. In a new

sample tube, a second elution was collected using 100 μ L of Buffer AE. In all cases, the second elution was used for subsequent polymerase chain reaction (PCR), since this was found to be more reliable (see Chapter 3). Samples were screened for the presence of *Plasmodium* spp. (and *Haemoproteus* spp., a related parasite) using nested PCR to amplify a 478 bp fragment of the mitochondrial cytochrome *b* gene following the method of Hellgren et al. (2004) with slight modifications. The first-round primers used were HaemNFI (5'-CATATATTAAGAGAAITATGGAG-3') (I = inosine, a universal base) and HaemNR3 (5'-ATAGAAAGATAAGAAATACCATTC-3'). The first PCR was performed in volumes of 10 μ L, which included 2 μ L of DNA, 0.125 mM dNTP, 1.5 mM MgCl₂, 0.6 mM each primer, and 0.5 units *Taq* DNA polymerase (MyTaq™; Bioline Ltd., London, United Kingdom). The thermal profile consisted of a 3-min 94°C enzyme activation step, followed by 20 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 45 s, ending with an elongation step of 72°C for 10 min. The second-round primers HaemF (5'-ATGGTGCTTTCGATATATGCATG-3') and HaemR2 (5'-GCATTATCTGGATGTGATAATGGT-3') were used. The composition of the second round PCR reaction mix was as above, except 1 μ L of PCR product from the first round was used as template instead of genomic DNA. The thermal profile for the second round PCR was the same as that for the first, except the number of cycles was increased from 20 to 35. To check for PCR amplification, 3 μ L of PCR products from the second round were analysed using 2% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen, Carlsbad CA, USA) and visualised under UV light. Nested PCR products were purified with Exo-SAP (USB, Cleveland, OH, USA) and run on an ABI 3730xl DNA Analyser by the Genetics Analysis Service, University of Otago. *Plasmodium* lineages were identified by performing a BLAST search on the NCBI GenBank nucleotide database and the MalAvi database (Bensch *et al.* 2009).

Data analysis

Due to low sample sizes at each of the higher elevation sites, the sampling sites were combined into two elevational groups (Egroup): “lower” (650 and 800 m) and “higher” elevation (1200 and 1400 m). Although 650 and 800 m elevations might typically be considered “middle” elevation sites, since no elevations existed at this field site below 600 m, thus no true “low” elevation site could be assessed, the designations

of “lower” and “higher elevations” are used to emphasise the elevation of both groups, relative to each other. Bird species were also placed into two different bird groups (Bgroup) by New Zealand classification: non-native and native. For this study, New Zealand native and endemic birds were not treated separately, and were placed together in the “native” group. An assessment of a habitat type effect (habitat) and sampling year effect (year) was also considered in the analysis. Generalised linear models were used to explore the effects of four independent variables on malaria prevalence. I used logistic regression to model malaria prevalence (infected = 1, not infected = 0) against four fixed factors (year, habitat, bird group, and elevation group). A model selection approach was used to identify the simplest best fitting model using Akaike’s Information Criterion (AIC) (Table 4.1), with the lowest AIC value indicating the best fitting model (Burnham *et al.* 2011). Bird age and sex were not included in this analysis because of a large number of missing values (only 190 out of 436 birds sampled had a known age and sex). To determine if any significant relationship was present between malaria prevalence and bird age and sex, independent *t*-tests were performed for each. Chi-square tests and two-tailed Fisher’s exact tests (if the frequency in one or more groups was less than five) were used to compare prevalence between malaria parasite lineages using VassarStats (Lowry 2004). All other analyses were performed using the statistical software IBM SPSS Statistics version 20.0 (SPSS Inc. Chicago, IL, USA). A significance level of 0.05 was used for all analyses.

RESULTS

Summary of data

Over three consecutive field seasons in NLNP, blood samples were collected and analysed from 436 individual birds representing 15 species from 11 families (Table 4.2). A total of 369 samples were collected from lower elevations (650 and 800m) and 67 samples from higher elevations (1200 and 1400m; Table 4.3). More non-native birds were caught at lower than higher elevation, whereas numbers for native birds was similar between elevations (Fig. 4.2). These results were obtained by standardising the capture rates, based on the total number of net hours for lower and higher elevations calculated at 1024 and 327, respectively. *Plasmodium* spp. was detected in a total of 26 blood samples from three of six non-native and two of nine native bird species.

No *Haemoproteus* parasites were detected in this study. An overall malaria prevalence of 6% (n = 436) was observed (Table 4.3).

Table 4.1. Summary of backward model selection of Akaike's information criterion (AIC) for regression models of four independent variables (year, habitat, Bgroup, and Egroup) against the binomial dependent variable of malaria infection (infected = 1, not infected = 0) of wild birds of Nelson Lakes National Park, New Zealand. The independent variables used in model are year (sampling year), habitat (habitat type), Bgroup (non-native or native bird classification), and Egroup (elevational grouping of sample sites). K is the number of parameters.

Model	K	Log-Likelihood	AIC	Δ AIC
1. Year+Habitat+Bgroup+Egroup	4	-18.57	51.14	0
2. Year+Habitat+Bgroup	3	-19.63	51.27	0.13
3. Habitat+Bgroup+Egroup	3	-26.96	63.92	12.78
4. Habitat+Bgroup	2	-28.23	64.46	13.32
5. Bgroup	1	-37.27	78.54	27.40
6. Year+Bgroup	2	-35.61	79.23	28.09
7. Bgroup+Egroup	2	-37.22	80.44	29.30
8. Year+Bgroup+Egroup	3	-35.61	81.23	30.09
9. Null	Intercept	-50.40	102.79	51.65
10. Egroup	1	-49.66	103.32	52.18
11. Habitat+Egroup	2	-47.79	103.58	52.44
12. Habitat	1	-49.14	104.27	53.13
13. Year+Habitat+Egroup	3	-46.80	105.61	54.47
14. Year	1	-49.85	105.71	54.57
15. Year+Habitat	2	-48.14	106.27	55.13
16. Year+Egroup	2	-49.20	106.39	55.25

Table 4.2. List of sampled avian host species and *Plasmodium* lineages identified at four elevational steps at Nelson Lakes National Park. Values indicate the number of infected individuals over the total number examined with prevalence in parentheses. Sampling took place during the warmer months for three consecutive years from 2012–13 to 2014–15 with samples totalling 181, 155, and 100, respectively.

Order	Family	Species	650 m	800 m	1200 m	1400 m	Total	<i>Plasmodium</i> lineages
Passeriformes	Fringillidae	Goldfinch <i>Carduelis carduelis</i>	-	0/3	-	-	0/3 (0%)	
		Chaffinch <i>Fringilla coelebs</i>	0/8	0/23	-	0/1	0/32 (0%)	
Musci	Muscicapidae	Eurasian Blackbird <i>Turdus merula</i>	4/11	8*/15	1/1	0/4	13/31 (42%)	LINN1 (10); LINN1/GRW06 (1)*; SYAT05 (2)
		Song thrush <i>Turdus philomelos</i>	0/1	4/9	-	1/1	5/11 (45%)	LINN1 (5)
Ploceidae	Ploceidae	House sparrow <i>Passer domesticus</i>	3/61	-	-	-	3/61 (5%)	GRW06 (3)
		Dunnoch <i>Prunella modularis</i>	0/5	0/3	-	0/3	0/11 (0%)	
Non-natives combined			7/86 (8%)	12/52 (23%)	1/1 (100%)	1/9 (13%)	21/149 (14%)	
Acanthizidae	Acanthizidae	Grey warbler <i>Gerygone igata</i>	0/1	0/2	-	0/1	0/4 (0%)	
		Tomtit <i>Petroica macrocephala</i>	-	0/28	0/12	0/15	0/55 (0%)	
Eopsaltriidae	Eopsaltriidae	South Island robin <i>Petroica australis</i>	0/3	1/4	0/1	-	1/8 (13%)	SYAT05 (1)†
		Bellbird <i>Anthornis melanura</i>	0/4	0/44	0/5	0/10	0/63 (0%)	
Meliphagidae	Meliphagidae	Tui <i>Prosthemnodon novaezeelandiae</i>	0/3	0/3	-	0/1	0/7 (0%)	
		Fantail <i>Rhipidura fuliginosa</i>	0/1	0/7	0/1	0/1	0/10 (0%)	
Monarchidae	Monarchidae	Brown creeper <i>Mohoua novaezeelandiae</i>	-	-	-	0/2	0/2 (0%)	
		Rifleman <i>Acanthisitta chloris</i>	-	-	0/7	-	0/7 (0%)	
Xenicidae	Xenicidae	Zosteropidae	0/23	4/107	-	0/1	4/131 (3%)	SYAT05 (4)†
		Natives combined	0/35 (0%)	6/196 (3%)	0/26 (0%)	0/31 (0%)	5/287 (2%)	
Total combined			7/121 (6%)	18/248 (8%)	1/27 (4%)	1/40 (3%)	26/436 (6%)	

* Indicates a double infection.

† Indicates a new lineage–host association.

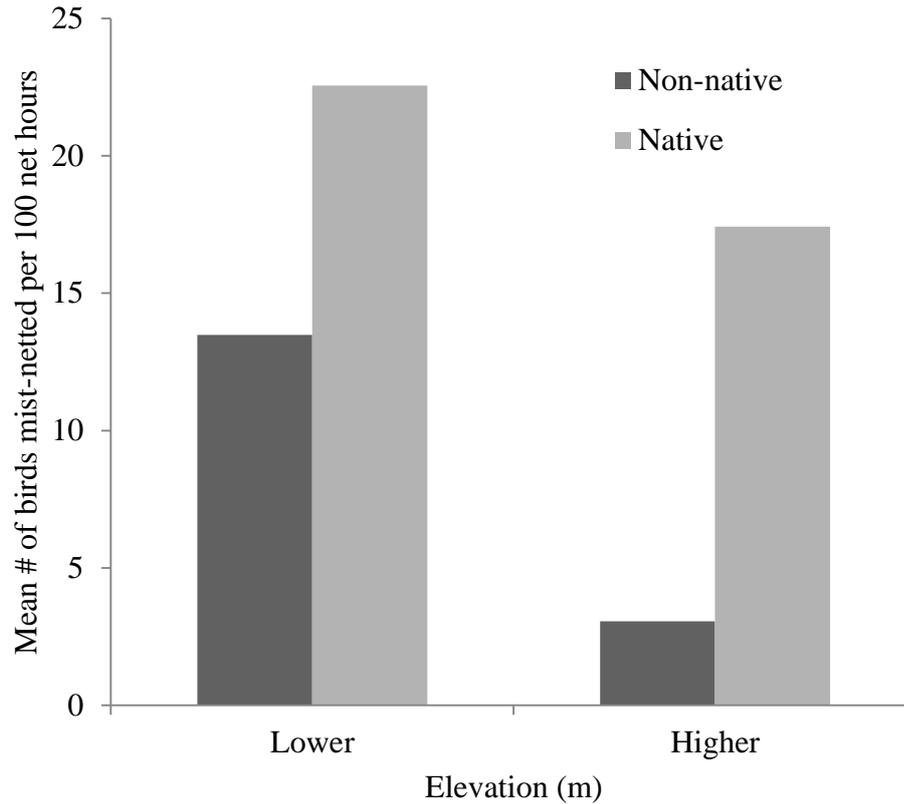


Figure 4.2. Standardised mist-net capture rates of wild birds for three consecutive years (2012-13 to 2014-15) at Nelson Lakes National Park, New Zealand.

Table 4.3. *Plasmodium* prevalence in wild birds of Nelson Lakes National Park, by year, elevation, and bird group. Values indicate the number of infected individuals over the total number examined, with prevalence in parentheses.

		2012-13	2013-14	2014-15	Total
Non-native	Lower elevation	9/56	6/59	4/21	19/138 (14%)
	Higher elevation	1/1	1/8	0/1	2/10 (20%)
		10/57 (18%)	7/67 (10%)	4/22 (18%)	21/148 (14%)
Native	Lower elevation	3/98	0/63	2/72	5/231 (2%)
	Higher elevation	0/26	0/25	0/6	0/57 (0%)
		3/124 (2%)	0/88 (0%)	2/78 (3%)	5/288 (2%)
Total		13/181 (7%)	7/155 (5%)	6/100 (6%)	26/436 (6%)

Model factors influencing malaria prevalence

Model selection resulted in two candidate models ($\Delta\text{AIC} < 2$) that best explain the variation in malaria prevalence in this study (Table 4.1). Values from Model 1, containing all four factors, are used herein. Overall prevalence of infection varied by

year (Wald $\chi^2_2 = 12.384$, $p = 0.002$) (Table 4.4), with prevalence in 2012-13 being 7.2% ($n = 181$), 2013-14 being 4.5% ($n = 155$), and 2014-15 being 6.0% ($n = 100$) (Table 4.3). A significant difference was observed between years 2013-14 and 2014-15 ($P = 0.008$) (Fig. 4.3). Prevalence of infection also varied by habitat type (Wald $\chi^2_2 = 22.987$, $P < 0.0001$) (Table 4.4), with prevalence of 2.2% ($n = 45$), 4.8% ($n = 105$), and 7.7% ($n = 261$) for edge, urban, and forest habitats, respectively (Fig. 4.3). A significant difference was observed between urban and forest habitats ($P = <0.0001$) (Fig. 4.3). Birds sampled at lower and higher elevations showed prevalence of 6.5% ($n = 369$) and 3.0% ($n = 67$), respectively (Table 4.3). Although no difference was found between elevations (Wald $\chi^2_1 = 1.761$, $P = 0.185$), a trend was observed showing an inverse relationship between prevalence of infection and elevation (Fig. 4.3). When bird species were grouped, parasite prevalence was significantly higher in non-native species (14.1%; $n = 148$) than in native species (1.7%; $n = 288$; Wald $\chi^2_1 = 36.366$, $P < 0.0001$) (Table 4.3; Fig. 4.3). Of the five bird species that were positive for malaria parasites, blackbird and song thrush showed the highest overall parasite prevalence; 42% ($n = 31$; CI 26-59) and 45% ($n = 11$; CI 21-72), respectively (Table 4.2). Parasite prevalence for house sparrow, South Island robin, and silvereye (*Zosterops lateralis*) was 5% ($n = 60$; CI 2-14), 13% ($n = 8$; CI 2-47), and 3% ($n = 131$; CI 1-8), respectively. No malaria parasites were detected in ten species, three non-native: goldfinch (*Carduelis carduelis*), chaffinch (*Fringilla coelebs*), dunnoek (*Prunella modularis*), and seven native: grey warbler, tomtit, bellbird, tui, fantail, rifleman, and brown creeper (*Mohoua novaeseelandiae*).

Table 4.4. Factors predicting *Plasmodium* infection in wild birds of Nelson Lakes National Park, New Zealand.

Predictor	df	Wald Chi-Square	p-Value
Year	2	12.384	0.002
Habitat	2	22.987	< 0.001
Bird group	1	36.366	< 0.001
Elevation group	1	1.761	0.185

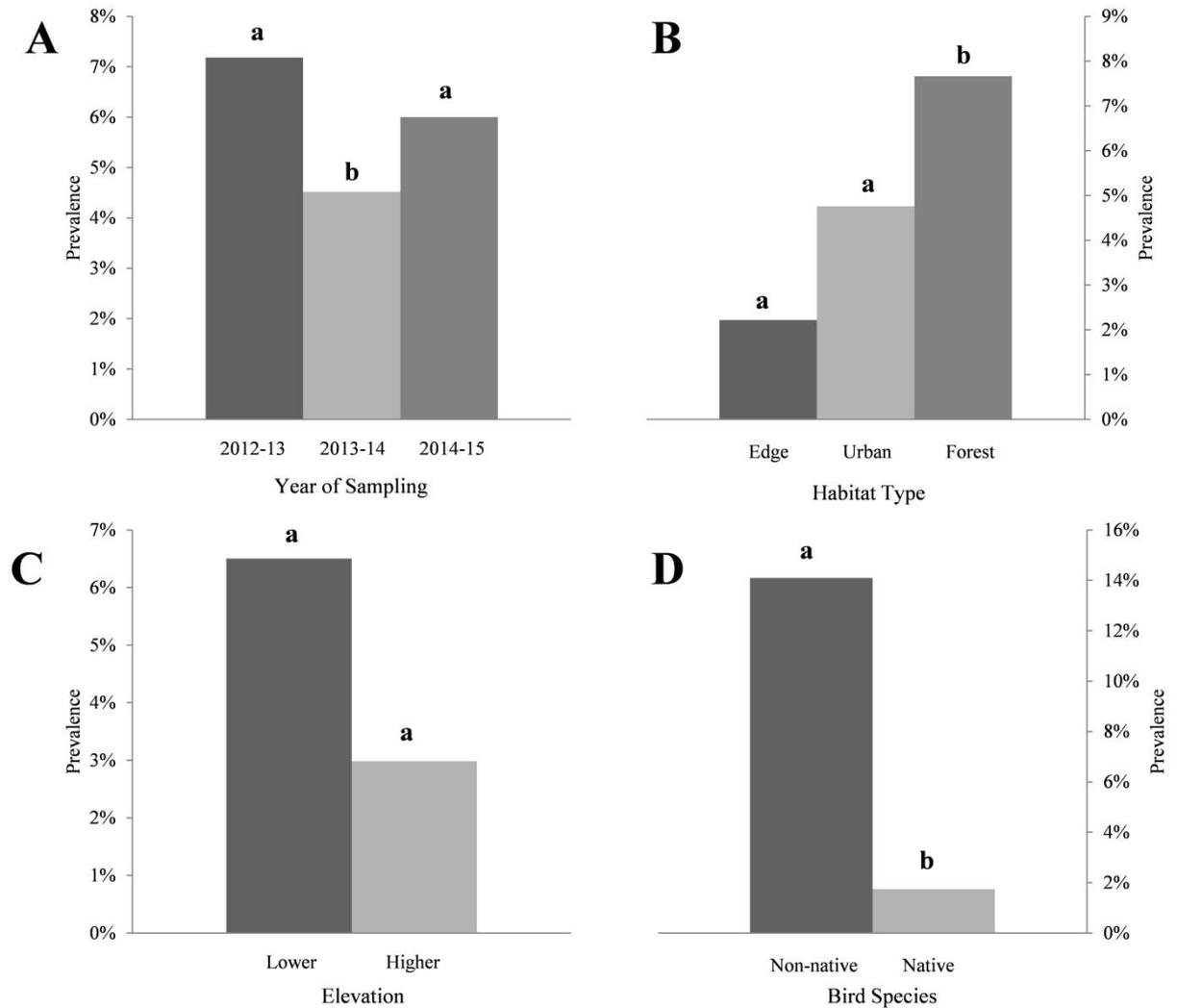


Figure 4.3. Summary of *Plasmodium* prevalence data by year of sampling (A), habitat type (B), elevational group (C), and non-native or native status of bird host (D) at Nelson Lakes National Park, New Zealand. Columns within categorical groups with different letters differ ($P < 0.05$). Note the differences in scale.

Malaria lineage identification

All 27 positive samples were successfully sequenced and three avian malaria lineages were identified (Fig. 4.4). Sequences showed a 99-100% homology to published sequences in GenBank and MalAvi (except for the lineage identified from the South Island robin, which showed a 95% homology using forward sequence only). These were *Plasmodium* sp. lineage LINN1 (GenBank GQ471953, MalAvi LINN1), *P. (Huffia) elongatum* lineage GRW06 (GenBank DQ368381, MalAvi GRW06), and *P.*

(Novyella) vaughani lineage SYAT05 (GenBank DQ847271, MalAvi SYAT05).

Lineage LINN1 was found in 16 birds, including 11 blackbirds and five song thrush, and it was the only lineage found at the higher elevation sites. Lineage GRW06 was found in four birds, including one blackbird and three house sparrows. Lineage SYAT05 was found in seven birds, including two blackbirds, one South Island robin, and four silvereys and was the only lineage found in native bird species. One double infection (lineages LINN1 and GRW06) was found in one blackbird individual at 800 m. Lineage LINN1 prevalence was significantly higher in non-native hosts (11%; $n = 148$; CI 7-17) than in native hosts (0%; $n = 288$; CI 0-1; $\chi^2 = 29.35$, d.f. = 1, $P < 0.0001$) (Fig. 4.5). Lineage GRW06 prevalence was also significantly higher in non-native hosts (3%; $n = 148$; CI 1-7) than in native hosts (0%; $n = 288$; CI 0-1; Fisher's exact test, $P = 0.0129$). Lineage SYAT05 prevalence in non-native and native hosts showed prevalence of 1% ($n = 148$; CI 0-5) and 2% ($n = 288$; CI 1-4) respectively (no significant difference was found, Fisher's exact test, $P = 0.5654$).

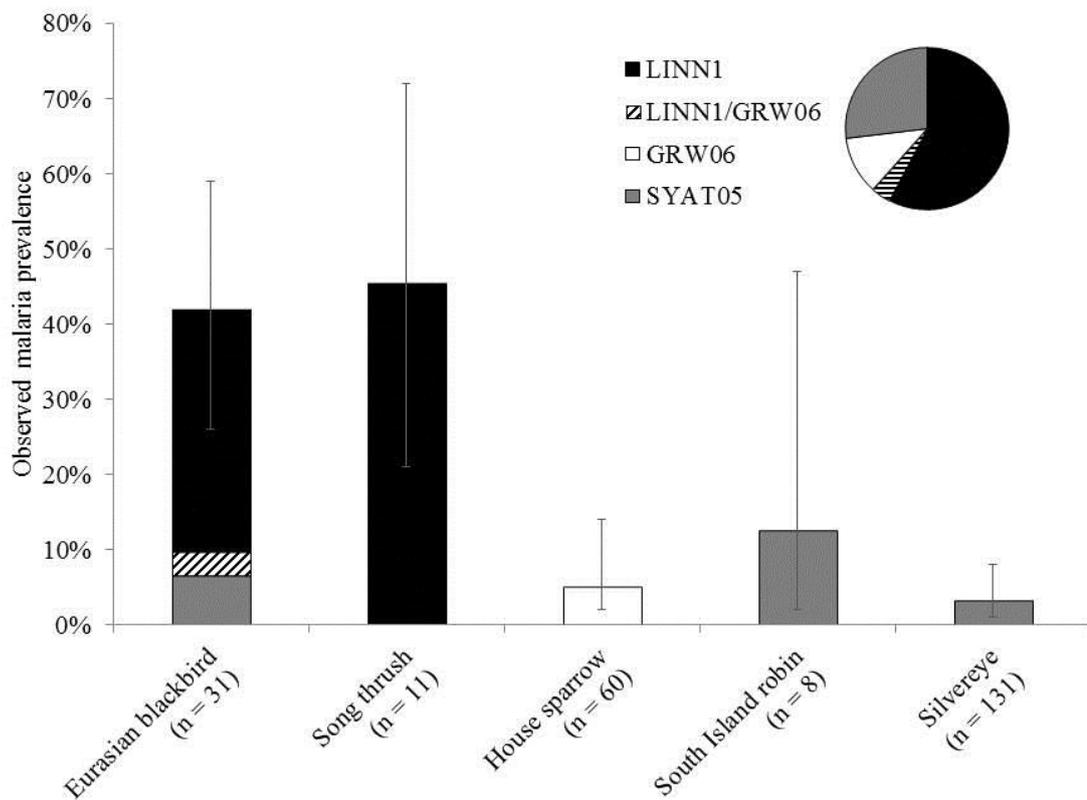


Figure 4.4. Observed prevalence of *Plasmodium* lineages in five avian species in Nelson Lakes National Park, South Island, New Zealand. Non-native host species with parasite infections are Eurasian blackbird (*Turdus merula*), song thrush (*Turdus philomelos*), and house sparrow (*Passer domesticus*), with no infections in three other species. Native species with parasite infections are South Island robin (*Petroica australis*) and silvereye (*Zosterops lateralis*), with no infections in seven other species. The error bars represent the lower and upper bounds of the 95% confidence interval. The pie chart shows relative proportion of positive infections by each lineage (n = 26).

DISCUSSION

Epizootiology

This study was the first investigation of avian malaria presence in non-native and native birds across an elevational gradient in New Zealand. The results revealed differences in the elevational distribution and species composition of the host-parasite community at the field site in Nelson Lakes National Park in the South Island. Analysis of the data also revealed prevalence varied by year and habitat, with higher *Plasmodium*

prevalence in native forest habitats. The difference between years may be correlated with a difference in mosquito emergence due to temperature or rainfall between years; however more research is needed to determine this. The difference in prevalence between habitats, with a higher prevalence observed in native forest habitat, could be due to the different number and type of host species sampled in each habitat type. Given that the observed prevalence was higher in non-native versus native birds, it is possible that the non-native versus native status could be driving differences observed by habitat as the bird species sampled are not randomly distributed across the habitat types. The difference in prevalence between habitats may also be related to vector density, with *Culex pervigilans* being the only suspected avian malaria vector reported at the study site (see Chapter 5). Although this mosquito species is known to exhibit a wide range of breeding sites, it has been reported as more common in natural breeding sites compared to artificial containers (Belkin 1968).

Although the prevalence data does not directly support the first hypothesis, as I found no significant correlation between prevalence and elevation, I do argue that an overall greater force of infection is occurring in NLNP at lower versus higher elevations for two reasons. First, a greater density of infected hosts exists at lower elevations. This is evident in non-native birds exhibiting overall higher malaria prevalence than native birds (Fig. 4.3) and existing in greater densities at lower versus higher elevations (Fig. 4.2). In other words, elevation appears to be playing an important role in the abundance of reservoir hosts, with a greater number at lower elevations. Although I acknowledge the inherent problems in comparing relative abundance of birds using mist-net capture data (Remsen *et al.* 1996), a similar pattern of density was also seen in a previous study in NLNP in blackbirds using 5-minute bird counts (Elliott *et al.* 2010). Second, a greater abundance of mosquitoes exists at lower elevations (see Chapter 5), increasing the potential for vector-host encounters. Although the higher elevation sites from the study are reported to be mosquito-free (see Chapter 5), two infected birds were sampled from these sites. One possible explanation is that transmission occurred at the same location as sampling took place, due to mosquitoes migrating from lower elevations under optimal temperature conditions (Ahumada *et al.* 2004). However it is more likely that transmission occurred at lower elevations, especially since some of these host species, such as blackbirds and song thrush, are capable of moving along an elevational gradient throughout the year in New Zealand (Dawson *et al.* 1978). Since both the

malaria parasite and reservoir host have been detected at elevations as high as the tree line, the presence of a competent mosquito vector may be the only missing component of the malaria transmission cycle at these higher elevations. This may have important implications for species or populations occurring in greater numbers at higher elevations such as the brown creeper, due to the threat of climate change and with it the potential upward elevational movement by mosquitoes (Atkinson & LaPointe 2009).

The results of this study strongly support the second hypothesis as overall higher prevalence of avian malaria parasites was observed in non-native species as compared to native species. These findings are similar to those from previous surveys conducted in New Zealand (Tompkins & Gleeson 2006; Ewen *et al.* 2012; Howe *et al.* 2012; Sijbranda *et al.* 2016), however little explanation for these patterns is provided in the literature. These differences in prevalence could be determined by numerous drivers at the vector, pathogen, or host level. Possible factors driving the prevalence observed may be rates of transmission, parasite fitness, or host susceptibility, all of which are involved in determining prevalence of infection (Atkinson & Dusek 2000; Yorinks & Atkinson 2000; Samuel *et al.* 2011). If differences in transmission rates were causing the observed prevalence patterns, I would expect native birds to be exposed to mosquitoes less often than non-native birds. However I do know that some transmission is occurring in native birds, by the evidence of malaria positive individuals. *Culex pervigilans* is a native mosquito species, and therefore existed before the introduction of the current non-native avifauna, in an ecosystem with very few native terrestrial mammals (two genera of bats only; Crump *et al.* 2001). Additionally, *Cx. pervigilans* is the most prevalent and widespread mosquito species in New Zealand (Belkin 1968), suggesting a wide range of preferences among avian hosts and providing further support against the possibility of lower transmission rates in native birds. Another possible driver of the observed patterns in prevalence may be parasite fitness. If this were the case, I would expect native bird species to be less suitable hosts for malaria parasites than non-native species found in the area, and thus affecting the survival rate of the parasite. *Plasmodium* parasites have a broad host range worldwide (Ricklefs *et al.* 2004; Beadell *et al.* 2006; Ishtiaq *et al.* 2006) and some show a high level of evolutionarily stable host-switching (Beadell *et al.* 2004). Additionally, all the host species sampled in this study are non-migratory, thus limiting the potential for parasite introductions from outside of New Zealand.

Although I am not ruling out the possibility that either transmission efficiency or parasite fitness may explain the patterns of lower prevalence in native bird species, it is more likely these patterns are related to host susceptibility (morbidity and mortality) to infection. If certain groups of birds are more impacted by infection than others, a differential impact could be resulting in more sick or dead individuals in that group. For example, in Hawaii immunologically naïve populations of birds that did not evolve in the presence of certain malaria parasites, may be highly susceptible to infection, leaving fewer birds surviving to the chronic phase of infection (Atkinson *et al.* 1995, 2001b). Therefore the impact of infection in native New Zealand birds (which would also qualify as naïve to introduced lineages and thus potentially more susceptible to infection than non-native birds), may not correlate with field generated prevalence due to lack of parasite detection. In other words, the absence of detected presence in various bird groups does not necessarily indicate a lack of successful transmission. There exists an inherent sampling bias with mist netting as sick or weak birds may not be caught due to inactivity, and only birds below a certain level of parasitemia tend to be active (Valkiunas 2005). Additionally, birds with higher parasitemia levels are at a greater risk of predation (Møller & Nielsen 2007), further biasing reported malaria prevalence in wild populations.

Surveys from the last 30 years in Hawaii have shown a higher prevalence of malaria in native bird species (van Riper *et al.* 1986; Atkinson *et al.* 2005; Atkinson & Samuel 2010). These patterns, opposite of what is being reported in New Zealand, may be a result of resistance to malaria evolving in native Hawaii birds (Atkinson *et al.* 2005). Malaria parasites are thought to be introduced to Hawaii along with the introduction of Old World birds (Beadell *et al.* 2006), possibly even via New Zealand (Hellgren *et al.* 2015), but it appears now that these same non-native species may no longer be acting as the primary reservoir (Atkinson & LaPointe 2009). Species such as Hawaii 'Amakihi (*Hemignathus virens*) and 'Apapane (*Himatione sanguinea*) may be playing a role as reservoirs of malaria parasites due to an increased number of individuals surviving the acute stage of infection (Atkinson *et al.* 2001a). However not all studies in Hawaii have reported these same findings, with one study showing no significant difference in prevalence between non-native and native birds (Aruch *et al.* 2007) and another showing a higher prevalence in non-native birds (Shehata *et al.* 2001). The inconsistency in these observed patterns may again be related to host

susceptibility at the species level. For many of these studies, a large portion of the total avian malaria prevalence attributed to either non-native or native bird species are due to just one or a few species, suggesting some reservoirs may be more important than others. The same is true for the current study, as observed avian malaria prevalence for blackbird and song thrush were significantly higher than any other species (Table 4.2). With not all studies on avian malaria including samples from the same bird species, even those conducted in similar habitats or locations, it is not surprising that differences in prevalence are being reported. In studies that report high prevalence in wild native birds, lower prevalence may still be occurring in various species that have not had the same opportunity to develop resistance as others (e.g. those found only at higher elevations) and thus are highly susceptible. Given these suggestions of Hawaii species developing resistance to avian malaria, why are there no examples in New Zealand of native species showing patterns of higher malaria prevalence than non-native species? Perhaps New Zealand birds have not yet been exposed to the same type or strength of selective pressures to develop resistance at a species level; however I propose an alternative explanation. Unlike Hawaii, where only a single lineage of *Plasmodium* is present (Atkinson & LaPointe 2009), multiple lineages exist in New Zealand, some of which could be playing more of a role than others in regards to negative impacts on native birds.

Malaria lineage identification

The three malaria lineages detected in this study, *Plasmodium* sp. lineage LINN1, *P. (Novyella) vaughani* lineage SYAT05, and *P. (Huffia) elongatum* lineage GRW06 belong to separate non-native lineage clusters. Each are all globally widespread and have previously been reported in New Zealand (Ewen *et al.* 2012; Schoener *et al.* 2014). *Plasmodium* lineage LINN1 was found exclusively in blackbird and song thrush in this study, but it has been reported in bellbird, New Zealand robin (*Petroica longipes*), North Island saddleback, silvereye, and great spotted and brown kiwi in other locations in New Zealand, albeit in extremely low numbers (Castro *et al.* 2011; Ewen *et al.* 2012; Howe *et al.* 2012; Sijbranda *et al.* 2016). Additionally, the deaths of two great spotted kiwi are attributed to infection by lineage LINN1 (Alley *et al.* 2012; Howe *et al.* 2012). This could have implications at NLNP where currently exists a population of approximately 25 great spotted kiwi, with documented cases of

successful breeding (Jahn *et al.* 2013). Lineage GRW06 was found only in blackbird and house sparrows in this study, but this lineage is considered to have the widest host range in New Zealand (Schoener *et al.* 2014). Lineage GRW06 has also been reported in New Zealand bellbird, brown kiwi, New Zealand robin, South and North Island Saddleback, song thrush, silvereye, whitehead (*Mohoua albigilla*), and yellowhammer (*Emberiza citronella*) (Alley *et al.* 2010; Baillie & Brunton 2011; Castro *et al.* 2011; Ewen *et al.* 2012). Lineage SYAT05 is commonly reported in studies conducted in the North Island, however I found no references in the literature from the South Island prior to this study. Along with blackbirds, lineage SYAT05 has been previously reported in bellbird, kereru, and New Zealand tomtit (Baillie *et al.* 2012; Ewen *et al.* 2012; Howe *et al.* 2012). To the authors' knowledge, this is the first study to show the presence of lineage SYAT05 infection in silvereye and South Island robin, further highlighting the gaps in knowledge of this system in New Zealand, especially in the South Island.

Differential impacts

Interestingly, for lineage SYAT05, the same pattern of higher prevalence in non-native versus native hosts was not observed for the other two lineages identified in this study; this is especially evident when comparing with lineage LINN1 (Fig. 4.5). These contrasting patterns are likely not a result of parasite spillover (although this may be occurring), because we would expect to observe the same proportion of lineages in non-native and native hosts. This uneven distribution is also unlikely due to the local distribution of *Plasmodium* parasites at the field site in NLNP, since infected blackbird and song thrush individuals (*Turdus* spp.) were caught at five of the six mist-netting locations. So why does prevalence vary dramatically within the same host population for only two of the three lineages observed? The most likely explanation is that any negative impacts from *Plasmodium* parasites on native New Zealand birds are dependent on the specific *Plasmodium* lineage parasitising the host, with some lineages (in this case lineage LINN1) having more of an impact than others. However to further explore this hypothesis, experimental infection trials would be needed to assess host susceptibility to the various strains found throughout New Zealand, among which, lineage LINN1 would be of particular interest to the authors. Based on the prevalence data from this study, it is reasonable to assume that if malaria is affecting native birds in NLNP at a population level, then lineage LINN1 is involved.

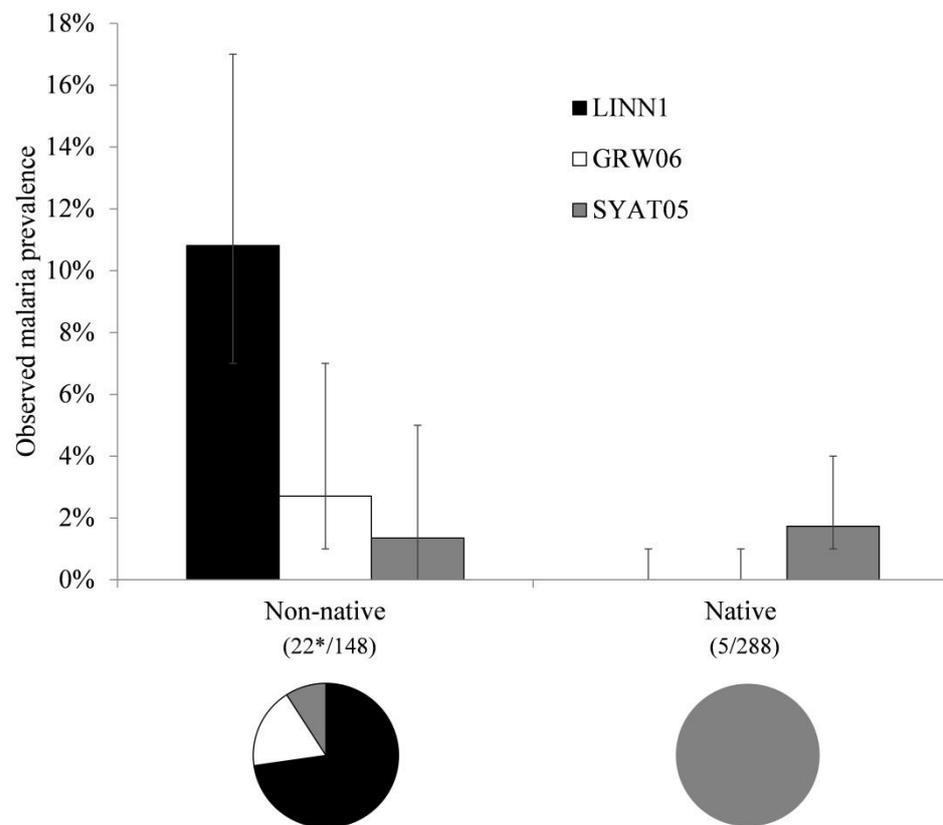


Figure 4.5. Observed prevalence of *Plasmodium* lineages in non-native and native host species in Nelson Lakes National Park, South Island, New Zealand. The error bars represent the lower and upper bounds of the 95% confidence interval. The pie charts show relative proportion of positive infections by each lineage within bird groups. The numbers in parentheses represent number of individuals infected and screened respectively for each group. *Note, one double infection (lineages LINN1 and GRW06) was found in one non-native blackbird individual and lineages are displayed here as independent observations.

Let us consider this on a broader scale. Previous research in New Zealand suggests that the bellbird is the only native species reported having populations of high malaria prevalence, up to 50% in some cases; although prevalence of 21% ($n = 33$) and 14% ($n = 36$) have been reported in silvereye and South Island saddleback, respectively (Ewen *et al.* 2012; Sijbranda *et al.* 2016). These data suggest that native New Zealand birds are capable of acting as their own disease reservoir, without exclusively relying on

non-native species, as hypothesised in the Hawaii system (Atkinson *et al.* 2001a). However the high prevalence reported appears to be dominated by only one or a few lineages, with very low prevalence of other lineages. For instance, in four studies totalling over 200 positive bellbird individuals, a suspected native *Plasmodium* lineage (“lineage ANME01”; Baillie *et al.* 2012) was present in over 80% of the samples, with only one sample positive for lineage LINN1 (Baillie & Brunton 2011; Baillie *et al.* 2012; Barraclough *et al.* 2012; Ewen *et al.* 2012). Regardless of the true native status of lineage ANME01, it is not considered to be a recent arrival to New Zealand; thus speculation has arisen that some resistance to this lineage may have developed in bellbird populations, specifically in areas of high prevalence where high transmission rates occur due to the warmer locales of higher latitudes and lower elevations (Barraclough *et al.* 2012). If native birds in New Zealand have developed resistance to only some *Plasmodium* parasites, it would support the hypothesis of a differential impact and further emphasise the need for detailed host-parasite susceptibility data. Alternatively, if these birds can develop resistance to some lineages, it is likely they can do the same for others; which raises more questions regarding simply letting nature take its course or implementing adaptive management approaches according to the level of resistance in certain species or locations.

Key reservoir hosts

Given the overall low prevalence reported in native host species, as well as the low *Plasmodium* diversity in those that do report high prevalence (e.g. identified bellbird populations), it is likely that non-native species are acting as the primary reservoirs for malaria parasites in New Zealand (Tompkins & Gleeson 2006). Based on data from the current study, I suggest that the *Turdus* spp. (especially the Eurasian blackbird) may be acting as key reservoir hosts for malaria parasites in NLNP. Both *Turdus* species have been implicated as reservoirs for the Whataroa virus (Togaviridae: Alphavirus), sourcing spillover infections to other avian species (Tompkins *et al.* 2010). The blackbird is abundant throughout both the North and South Island and is a known reservoir of *Plasmodium* parasites in other countries, specifically implicated as a key reservoir host (Hellgren *et al.* 2011). In this study, both the blackbird and song thrush showed a significantly higher prevalence than any other species (including other non-native species; Fig. 4.4). Additionally, the blackbird was the only species found to host

all three *Plasmodium* lineages identified in this study, with both *Turdus* species the only hosts infected with lineage LINN1 (Table 4.2). *Turdus* spp. individuals move frequently between habitat types during the breeding season (Greenwood & Harvey 1976) and are known to penetrate deep into the native New Zealand forests, while most other non-native bird species do not (Holdaway 1990). In the current study, *Turdus* spp. individuals were caught in all habitat types across all elevations, unlike the house sparrow which was caught only around the St. Arnaud village. Similar results and patterns have been found in other areas of New Zealand, with *Turdus* species often identified with the highest prevalence and caught in more habitat types than other non-native species (Tompkins & Gleeson 2006; Ewen *et al.* 2012; Howe *et al.* 2012; Sijbranda *et al.* 2016). By identifying potential key reservoir hosts in a disease system, we begin to better understand the factors that are driving transmission.

Conclusion

In conclusion, the results of the study support the potential for of a higher force of infection to be occurring at the lower elevations at the field site. I report an overall malaria prevalence of 6% ($n = 436$) for this study, which is slightly lower than previous records from the top half of the South Island of New Zealand and likely due to the higher elevation at which this sampling took place (Tompkins & Gleeson 2006; Baillie & Brunton 2011). I also report an overall higher prevalence of malaria in non-native versus native birds living in a shared space, suggesting the possibility of a differential impact on host species that show dissimilar reservoir competence. In other words, I suggest that despite reports of low malaria prevalence in native host species, transmission may still be occurring, however with a greater difficulty of detection due to potential impacts of infection. Of note, no parasite was detected in the five native bird species ($n = 137$) reported as declining in population in NLNP over the past 30 years, yet a high prevalence was observed in blackbirds, a species not reported as declining (Elliott *et al.* 2010).

These findings provide further support to the hypothesis that disease, in addition to predation and competition, could be playing a role in the observed population declines (Tompkins & Jakob-Hoff 2011). In Hawaii, it has been suggested that the reduction of malaria transmission or related mortality may have a larger impact on bird abundance than predator control (Samuel *et al.* 2011), however for the New Zealand situation,

more information is still needed before any management suggestions can be made. I argue that one of greatest knowledge gaps of the avian malaria system in New Zealand is the lack of detailed information on host susceptibility to infection, information that could be obtained through experimental infection studies. What we may find is that when malaria is present, a combination of factors contribute to population declines, such as infected birds having a greater risk of predation or competition of resources from disease resistant non-native birds as suggested in other parts of the world (Atkinson *et al.* 1995; Møller & Nielsen 2007). If the native avifauna is impacted by avian malaria at a population scale, and a differential impact on host species is occurring due to specific *Plasmodium* lineages, it would carry with it a whole new complexity to the conservation of native wildlife in New Zealand. Supporting evidence for such disease impacts will enable appropriate management to be developed and put in place, while a lack of evidence will enable resources to remain focused on the other issues facing New Zealand's native species (e.g. through habitat restoration, pest control, translocations and genetic management). In the case of the former, providing managers with more specific information of any potential disease impacts on native bird populations could help avoid "blanket" conservation and management efforts that can often be seen as costly and less species specific.

Chapter Five

Population dynamics of an endemic New Zealand mosquito, *Culex pervigilans*: Modeling a suspected disease vector

INTRODUCTION

Understanding the role of the environment in the transmission and distribution of disease is an important step towards identifying and managing disease threats and their impacts (Gonzalez-Quevedo *et al.* 2014), yet making these links can be difficult to demonstrate scientifically (Plowright *et al.* 2008). Climate can influence disease dynamics, impacting via a number of pathogen, host, and habitat aspects (Pascual *et al.* 2002; Freed *et al.* 2005; Altizer *et al.* 2006). For example, increased rainfall attributed to El Niño was linked to an increase in rodent reservoir populations, ultimately resulting in an emergence of hantavirus pulmonary syndrome in humans (Yates *et al.* 2002). Vector-borne diseases are especially sensitive to meteorological conditions that can influence vector distribution and abundance (Epstein 2001; Kovats *et al.* 2001; Tabachnick 2010); for example, severe winter temperatures are suspected of being capable of influencing the abundance of tick species known to vector Lyme borreliosis (Ruiz-Fons *et al.* 2012), while above-average summer temperatures are linked to the increase in West Nile virus transmitted by mosquitoes (Reisen *et al.* 2006). In addition to temperature, rainfall, and larval habitat availability can influence the density and abundance of mosquito populations (Ahumada *et al.* 2004; Barrera *et al.* 2006). Specifically, these factors play a major role in determining the transmission of malaria (LaPointe *et al.* 2012).

Malaria, transmitted by haematophagous invertebrates, has a world-wide distribution (except Antarctica; Grimaldi *et al.* 2015), and is caused by protozoan blood parasites of the genera *Plasmodium*. Avian malaria, as its name suggests, is a disease that occurs exclusively in avian hosts, belonging to a variety of species and families, but primarily affects passerines (Valkiunas 2005). These impacts have been well documented in Hawaii, where avian malaria is caused by *Plasmodium* parasites and is transmitted by the introduced *Culex quinquefasciatus* mosquito (van Riper *et al.* 1986; Atkinson & Samuel 2010). Hawaii, which has no native mosquitoes, saw the arrival of *Cx. quinquefasciatus* in the early 19th century (Fonseca *et al.* 2000; Ahumada *et al.* 2004) and a wave of native bird extinctions a century later, with avian malaria suspected of playing a key role (van Riper *et al.* 1986). The avian malaria transmission patterns in Hawaii are, in part, driven by mosquito population dynamics across an elevational gradient (LaPointe *et al.* 2012). In this system, mosquito dynamics vary

across elevation, with low elevations showing higher population abundance with little seasonal variation, and middle and high elevations consisting of lower population abundance with one or two seasonal peaks (Atkinson & Samuel 2010; LaPointe *et al.* 2012).

Avian malaria is also present in New Zealand and has been linked to mortality in multiple endemic species (Alley *et al.* 2008; Howe *et al.* 2012). However in New Zealand, there exist major gaps in the fundamental understanding of vector-borne disease dynamics, including how mosquito vectors respond to local climatic and environmental conditions (Holder *et al.* 1999; Derraik *et al.* 2005; Tompkins & Gleeson 2006). New Zealand is home to 15 of the world's 3500 mosquito species, 12 of which are endemic (Holder *et al.* 1999; Cane & Disbury 2010). Two species are suspected of being vectors of avian malaria in New Zealand, the introduced *Cx. quinquefasciatus* and the endemic *Cx. pervigilans* Bergroth (Holder *et al.* 1999; Tompkins & Gleeson 2006; Massey *et al.* 2007; Derraik *et al.* 2008). An inverse relationship between avian malaria prevalence (i.e. the proportion of infected hosts) and latitude has been reported in New Zealand, likely due to the correlation between mosquito density and temperature (Tompkins & Gleeson 2006; Baillie & Brunton 2011). The invasive *Cx. quinquefasciatus* was introduced into New Zealand in the early 19th century (LaPointe *et al.* 2012) but in recent decades has expanded its range from its introduction sites in the northern portion of the North Island to as far south as Christchurch on the South Island (Holder *et al.* 1999). Often mosquito species introduced to New Zealand are from warmer climes, with the colder local temperatures ultimately limiting their distribution (Leishnam *et al.* 2004). The native *Cx. pervigilans*, however, has a wide tolerance of environmental conditions and is the most prevalent and widespread mosquito species in New Zealand, distributed throughout the length of the country (Belkin 1968; Cane & Disbury 2010).

Although latitudinal relationships for both avian malaria prevalence and the corresponding mosquito vectors have been documented in New Zealand (Holder *et al.* 1999; Tompkins & Gleeson 2006), elevational relationships, like those seen in Hawaii (van Riper *et al.* 1986; Ahumada *et al.* 2004), have yet to be investigated. Whereas modeling has been used as an important tool in understanding the vector component within the complex avian malaria system in Hawaii (Ahumada *et al.* 2004; Samuel *et al.*

2011; Hobbelen *et al.* 2012), no such models exist to explore this system in New Zealand. Specifically, I hypothesise that temperature is playing a key role in mosquito dynamics, causing populations to vary annually, seasonally, and across an elevational gradient.

Although most studies investigating avian malaria in New Zealand ignore the vector component, it is essential to understanding the system as a whole, as highlighted by studies conducted in Hawaii (Ahumada *et al.* 2004; Aruch *et al.* 2007; Samuel *et al.* 2011). The goal was to construct a realistic model for *Culex* spp. in New Zealand to describe these conditions and make preliminary predictions of mosquito distribution. Here I take an existing model used to describe mosquito population dynamics in Hawaii, and modify it to investigate the mosquito component of the avian malaria system in New Zealand. I then use the model to investigate likely patterns of mosquito densities at previous bird mortality events linked to avian malaria. Eventually, this model can be integrated into a more comprehensive model incorporating avian host and malaria pathogen components to better explore driving factors in avian malaria transmission in New Zealand.

METHODS

Study system

The model incorporates recorded hourly temperatures and adult mosquito sampling data collected along the St. Arnaud range at Nelson Lakes National Park (NLNP), in the South Island of New Zealand. These data were collected from various elevational steps ranging from the shore of Lake Rotoiti at 620 m (the lowest elevation at this location) to the tree-line at about 1400 m. This site consists of native southern beech (*Nothofagus* spp.) rainforest home to multiple non-native and native bird species. Declines in native forest bird species over the last 30 years have been reported in NLNP (Elliott *et al.* 2010), with disease suggested as a possible cause (Tompkins & Jakob-Hoff 2011). The presence of avian malaria has been confirmed in both native and non-native bird species at this site (Chapter 4). Additional temperature data were used from nearby Abel Tasman National Park (ATNP), which consists of native temperate forests with elevations down to sea level. Abel Tasman National Park is located approximately 75 km north of the field site in NLNP.

Model structure

We adapted the Samuel *et al.* (2011) epidemiological model of avian malaria in Hawaii to explore the New Zealand situation. Here I discuss only the mosquito component of the model, ignoring pathogen and bird host components, and define the parameters and values used (Table 5.1.). Although for the Hawaii model, mosquito mortality is influenced by rainfall, specifically by extreme events of drought and heavy rainfall, rainfall dynamics were ignored for the model. Since the field site has a mean annual rainfall of 1500 mm (Butler 1991) and mosquitoes in New Zealand have been reported in multiple types of water bodies held by plants including tree holes (Belkin 1968), protected from extreme rainfall events, drought and extreme rainfall were not considered to be major factors in mosquito mortality. The larval carrying capacity (K_L) for *Cx. quinquefasciatus* in Hawaii is dependent on available aquatic habitat, influenced by rainfall, which varies greatly as a function of elevation. Here I assumed *Cx. pervigilans* larval habitat to not be a limiting factor at each of the native forest elevational sites, due to the generalist nature of the species and the availability of potential breeding sites. The minimum temperature for mosquito development (*MTD*) used for *Cx. quinquefasciatus* was adjusted for the more cold tolerant *Cx. pervigilans* (see below).

*Model parameters***Table 5.1.** Symbols and definitions of abbreviations, variables, and parameters used in the model.

Abbreviation	Definition
MTD	minimum temperature for mosquito development

Variable	Description
L	density of mosquito larvae
L_i	density of mosquito larvae in maturation stage i ($i = 1, \dots, 5$)
M	density of adult mosquitoes
M_{max}	maximum adult mosquito density
T	mean daily temperature ($^{\circ}\text{C}$)

Parameter	Definition	Value	Dimension	Source
K_L	larval carrying capacity	2,710,200	numbers/km ²	This study
g_T	length gonotrophic cycle	$241T^{-1.11}$	days	Samuel <i>et al.</i> (2011)
δ_T	proportion of adults ovipositing	$\alpha e^{-\alpha g_T} / 1 - e^{-\alpha g_T}$	1/days	
b	number of female eggs per clutch	100	-	Samuel <i>et al.</i> (2011)
d_T	larval maturation rate	$T - MTD / 179$	1/days	Samuel <i>et al.</i> (2011)
MTD	minimum temperature for mosquito development	<i>Culex quinquefasciatus</i> = 10.1; <i>C. pervigilans</i> = 7.783	$^{\circ}\text{C}$	Mogi (1992); this study
α	adult mortality rate	0.073	1/days	Ahumada <i>et al.</i> (2004)
β	larval mortality rate	0.0315	1/days	Samuel <i>et al.</i> (2011)

The model consists of a series of differential equations, (see below), each incorporating parameters used to define different stages of mosquito development (Table 5.1). The values of 0.0315 and 0.073 were used for adult (α) and larval (β) mortality rates, respectively (Ahumada *et al.* 2004; Samuel *et al.* 2011). I assumed the same proportion of adults ovipositing (δ_T), length of the gonotrophic cycle (g_T), and the number of female eggs per clutch (b), for *Cx. pervigilans* as reported for *Cx. quinquefasciatus* (Samuel *et al.* 2011). The larval maturation rate (d_T), however, varies among species as it incorporates the MTD value. An estimated K_L of 2,710,000 individuals per km² was used for *Cx. pervigilans*. To obtain this value, the model output was run to equilibrium using temperature data from 2012-13 at the 680 m elevation and the MTD was set to 0. The maximum adult mosquito density (M_{max}) for this scenario was 1,000,060.

Larval mosquitoes:

$$(1) \quad dL_1/dt = b \delta_T M (1-L/K_L) - (i d_T + \beta) L_1$$

$$(2) \quad dL_i/dt = i d_T L_{(i-1)} - (i d_T + \beta) L_i$$

$$(3) \quad L = L_1 + L_2 + L_3 + L_4 + L_5$$

Adult mosquitoes:

$$(4) \quad dM/dt = 5 d_T L_5 - \alpha M$$

Adjusting the minimum temperature for mosquito development

We estimated *MTD* for *Cx. pervigilans* to be 7.783°C. *Culex pervigilans* is a more cold tolerant species than *Cx. quinquefasciatus* (Leisnham *et al.* 2004). To adjust for this difference, an *MTD* threshold for *Cx. pervigilans* was calculated by using a model fitting exercise. Since the immature stages of *Cx. quinquefasciatus* develop at a linear rate of development as a function of temperatures above the *MTD* (Ahumada *et al.* 2004, 2009), I assumed the same for *Cx. pervigilans*. This species-specific value was obtained by using data from three adult mosquito sampling events from 2013-14 to estimate the total density of adult mosquitoes (*M*) (Table 5.2). The first sampling event resulted in a total of 37 *Cx. pervigilans* individuals caught using eight traps per night for five nights (15, 27-30 November 2013) at an elevation of 650 m. The second resulted in a total of 26 *Cx. pervigilans* individuals caught using six traps per night for two nights (17-18 January 2014) at an elevation of 650 m. The third resulted in a total of four *Cx. pervigilans* individuals caught using six traps for one night (18 February 2014) at an elevation of 800 m.

Table 5.2. Mosquito trapping events used to generate MTD value (2013-2014 events) and to test model (2014-2015 event). Mosquito sampling took place in 2013-2014 and 2015 along the St. Arnaud Range, Nelson Lakes National Park, New Zealand.

Year	Dates	Model date	Total no. of traps	Elevation	<i>Culex pervigilans</i>	Calculated MTD
2013-14	15, 27-30 Nov.	22 Nov.	40	650	37	7.630
2013-14	17-18 Jan.	17 Jan.	12	650	26	7.791
2013-14	18 Feb.	18 Feb.	6	800	4	7.638
Final Calculated MTD (by minimising the sum of squares)						7.783
2014-15	17-18 Jan.	17 Jan.	12	650	14	7.945

Since the coverage distance of each CO₂ trap is estimated to be 2000 m² (Gillies & Wilkes 1969, 1972), *M* was calculated at 460, 1081, and 314 mosquitoes per km² respectively for each sampling event. While varying the MTD value and using mean daily temperature data for the corresponding dates and elevations (in this case 680 m and 780 m), the model was allowed to run to equilibrium and multiple *M* values were produced. Once the generated *M* value for the middle date of each event (22 November, 17 January, and 18 February, respectively) equalled the corresponding calculated values, the MTD values for each event were recorded. A single MTD value was then calculated by minimising the sum of squares of the three event MTD values and assigned to *Cx. pervigilans* (Table 5.2). This final MTD value of 7.783 was used in the model to simulate mosquito numbers for various elevations and years as well as to estimate maximum elevations of mosquito persistence.

Temperature data collection

Temperature data collected were used to determine the annual temperature regime along an elevational gradient as well as incorporated into the model. To obtain mean daily temperature (*T*), data were used from HOBO data loggers (Onset Corp., Pocasset, MA, USA), which were placed at eight elevational steps (680, 700, 740, 780, 1060, 1160, 1170. and 1400 m) at NLNP from 01 July 2012 to 30 June 2015 and recorded temperature every hour (Landcare Research, unpublished data). Two data loggers were used at each elevational step; the first was off the ground and shaded from the sun to collect daytime temperature and the second was on the ground exposed to the elements to collect the night-time temperature. Temperature readings between 0600 and 2200 were used to define day-time and readings between 2300 and 0500 were used for night-time. These selected 24 hourly temperature readings were then averaged to

produce T for each day. Additionally, temperature data to represent 0 m in elevation were obtained from National Institute of Water & Atmospheric Research (NIWA 2015). Hourly temperature data from Motueka (representing ATNP at 0 m) were averaged to produce T for each day. For this study low, middle, and high elevations are defined as 0, 680, and 1400 m, respectively.

Mosquito sampling

Adult mosquito sampling took place at five elevations (650, 800, 1000, 1200, and 1400 m) during 2013-2014 (November - February) and 2015 (January). EVS CO₂ Mosquito Traps (BioQuip Products, Rancho Dominguez, USA) were baited with dry ice (CO₂). Since most New Zealand mosquitoes are crepuscular and/or nocturnal (Derraik *et al.* 2005), traps were active from approximately two hours before sunset to two hours after sunrise. Female mosquitoes were quick-frozen using remaining dry ice from the traps and identified to species under a dissecting microscope (Snell 2005). A total of 83 female mosquitoes comprising two species were trapped during the study, with no mosquitoes detected above 800 m in elevation (Table 5.2.). All but two individuals were *Culex pervigilans*, with the other being identified as the winter mosquito, *Ochlerotatus antipodeus* (Edwards), also endemic to New Zealand.

Model application

Mosquito dynamics were modeled as a function of temperature using ModelMaker 4.0 (Cherwell Scientific, Oxford, UK). Simulations were run to equilibrium dynamics. I incorporated field-collected temperature data into the model to simulate the daily total density of adult mosquitoes (M). To estimate mosquito density for all elevations (not just those corresponding to the data loggers), I projected temperatures for each elevational step by plotting elevation against temperature and using the regression slopes calculated for each year. Specifically, temperatures from the 680 m data logger (middle elevation) were adjusted by adding or subtracting the corresponding slope value for each meter increase or decrease desired. To test the model, I used 2014-2015 temperature and mosquito sampling data to compare calculated and simulated densities. Specifically, 14 *Cx. pervigilans* individuals were caught using six traps per night for two nights (17-18 January 2015) at an elevation of

650 m, and the density was calculated to be 583 mosquitoes per km². The model's simulated density for the same date was 1657 mosquitoes per km².

Case study simulations (mortality events)

We used the model to investigate the possible role of mosquito-borne disease in documented mortality events in New Zealand. Four mortality events were investigated from the South Island and designated below as events 1-4. Avian malaria has been linked to events 1-3, while no similar evidence has been found for event 4. During event 1, approximately 150 yellow-eyed penguins (*Megadyptes antipodes*) deaths occurred on the Otago Peninsula, between 13 December 1989 and 13 February 1990 (Gill & Darby 1993). Avian malaria was suggested as playing a role in the declines due to a high seroprevalence of antibodies to *Plasmodium* spp., however no parasites were detected from living penguins in the area (Sturrock & Tompkins 2007). During event 2, five of eight captive Mohua (*Mohoua ochrocephala*) died from avian malaria (Derraik *et al.* 2008), with the first occurring on 9 October 2003 and the following four during March and April of 2004. The individuals had been translocated for breeding purposes from the Otago region to a more northern location near Christchurch on 10 September. Event 3, occurring in February of 2007, involved a previously translocated population of South Island saddlebacks (*Philesturnus carunculatus*) diagnosed with poor health, including two of 12 examined individuals found to be positive for avian malaria (Alley *et al.* 2010). A total of 45 individuals had been translocated from an offshore island in the Marlborough Sounds to another nearby in August of 2005. During event 4, occurring in the late austral summer of 2013, 66 yellow-eyed penguin deaths were reported from the Otago Peninsula, however of the 24 individuals necropsied, none were found to be positive for avian malaria (Alley *et al.* 2014). For each bird mortality event, I obtained hourly temperature data (NIWA 2015) for the locality involved and using the model, estimated mosquito density for each event year as well as the previous three years.

RESULTS

Temperature dynamics

Mean daily temperatures at this latitude in New Zealand showed a fluctuation of approximately 21°C throughout the year (Fig. 5.1A) while mean monthly temperatures

varied by approximately 12°C (Fig. 5.1B). The relationship between elevation and mean annual temperature shows an average -0.0048°C change per one meter increase (Fig. 5.2A). Although the temperature data obtained for ATNP were not used in defining this relationship, when incorporated, the results of the regression were similar (intercept, 12.565; slope, -0.0049; $R^2 = 0.986$). Mean annual temperatures varied by year (Fig. 5.2B), with greater variation occurring at higher elevations (Fig. 5.1C).

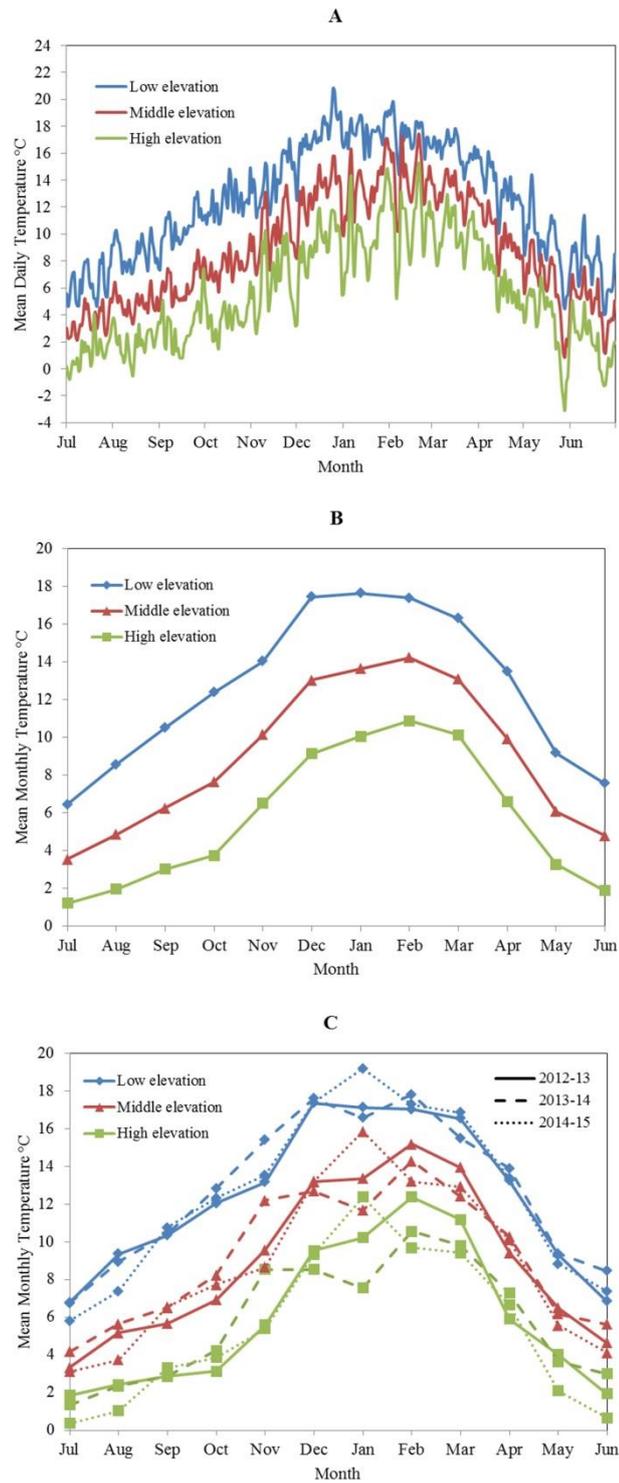


Fig. 5.1. (A) Mean daily temperatures (°C) from data collected from 01 July 2012 to 30 June 2014 at middle and high elevation sites (680 m and 1400 m, respectively) along the St. Arnaud range, Nelson Lakes National Park, New Zealand and a low elevation site (0 m) in Motueka near Abel Tasman National Park. (B) Mean monthly temperatures from the same data. (C) Mean monthly temperatures for years 2012-13 (solid lines), 2013-14 (dash lines), and 2014-15 (dotted lines) from the same data.

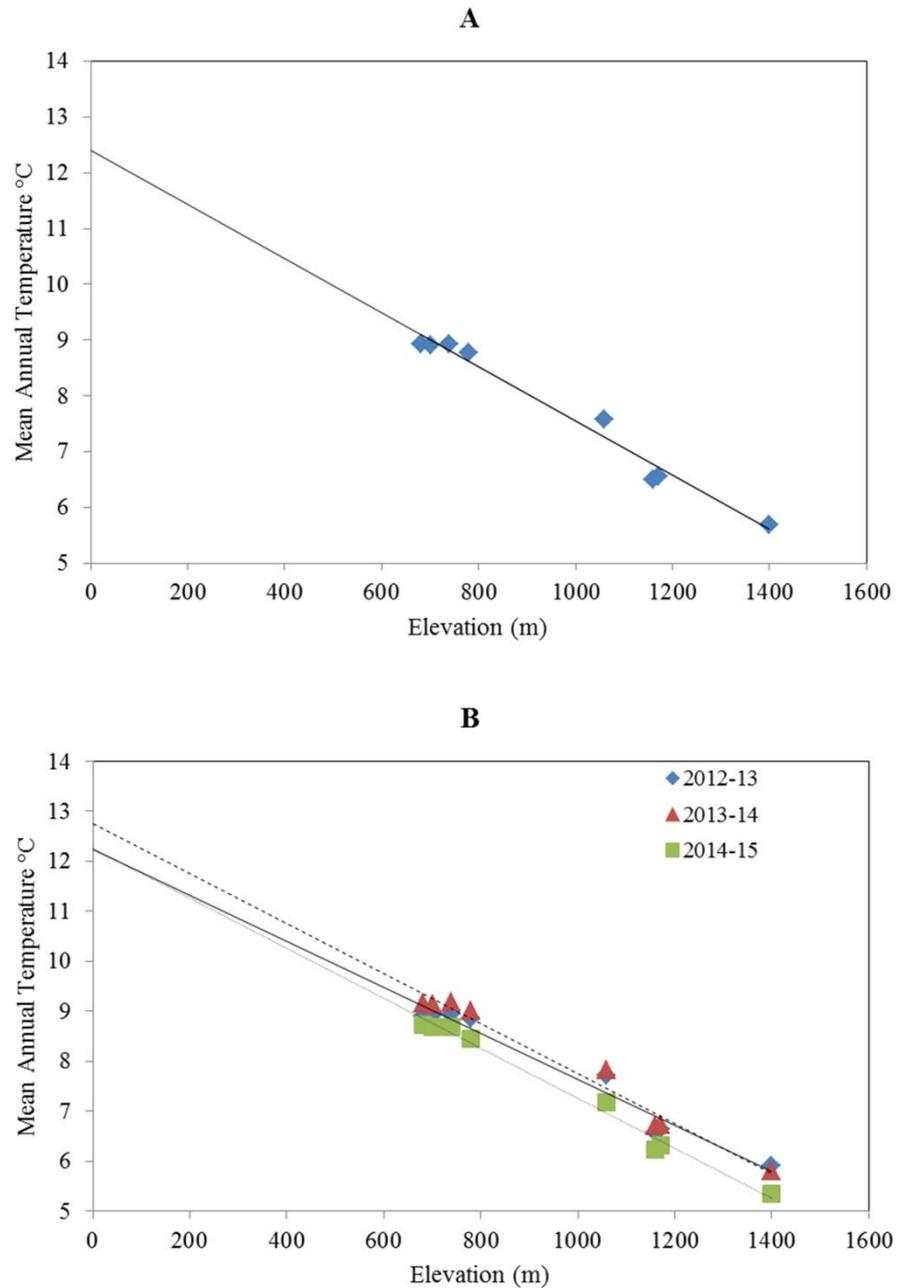


Fig. 5.2. (A) Relationship between elevation (m) and mean annual temperature (°C) from data collected between 01 July 2012 and 30 June 2015 along the St. Arnaud Range, Nelson Lakes National Park, New Zealand. Linear regression: intercept, 12.407; slope, -0.0049; $R^2 = 0.9774$. (B) Relationship between elevation and mean annual temperature for each year of the study. Linear regression for 2012-13: intercept, 12.227; slope, -0.0046; $R^2 = 0.9652$. Linear regression for 2013-14: intercept, 12.743; slope, -0.005; $R^2 = 0.9758$. Linear regression for 2014-15: intercept, 12.252; slope, -0.005; $R^2 = 0.9869$.

Mosquito simulations

The model predicted variation in mosquito dynamics seasonally, annually, and across the elevational gradient (Fig. 5.3A). The model estimated no mosquitoes at higher elevations, showing mosquito density approaching zero within the 700-800 m elevational range (Fig. 5.3B). The mean monthly temperatures crossed the *MTD* threshold approximately three months earlier at low elevations than at the middle elevations in each year, and one month earlier at the middle elevations than at high elevations (Fig. 5.4). Similarly, high mosquito densities were sustained for a longer period of time at low elevations compared to middle elevations, correlating with the longer duration that mean monthly temperatures remained above the *MTD* threshold.

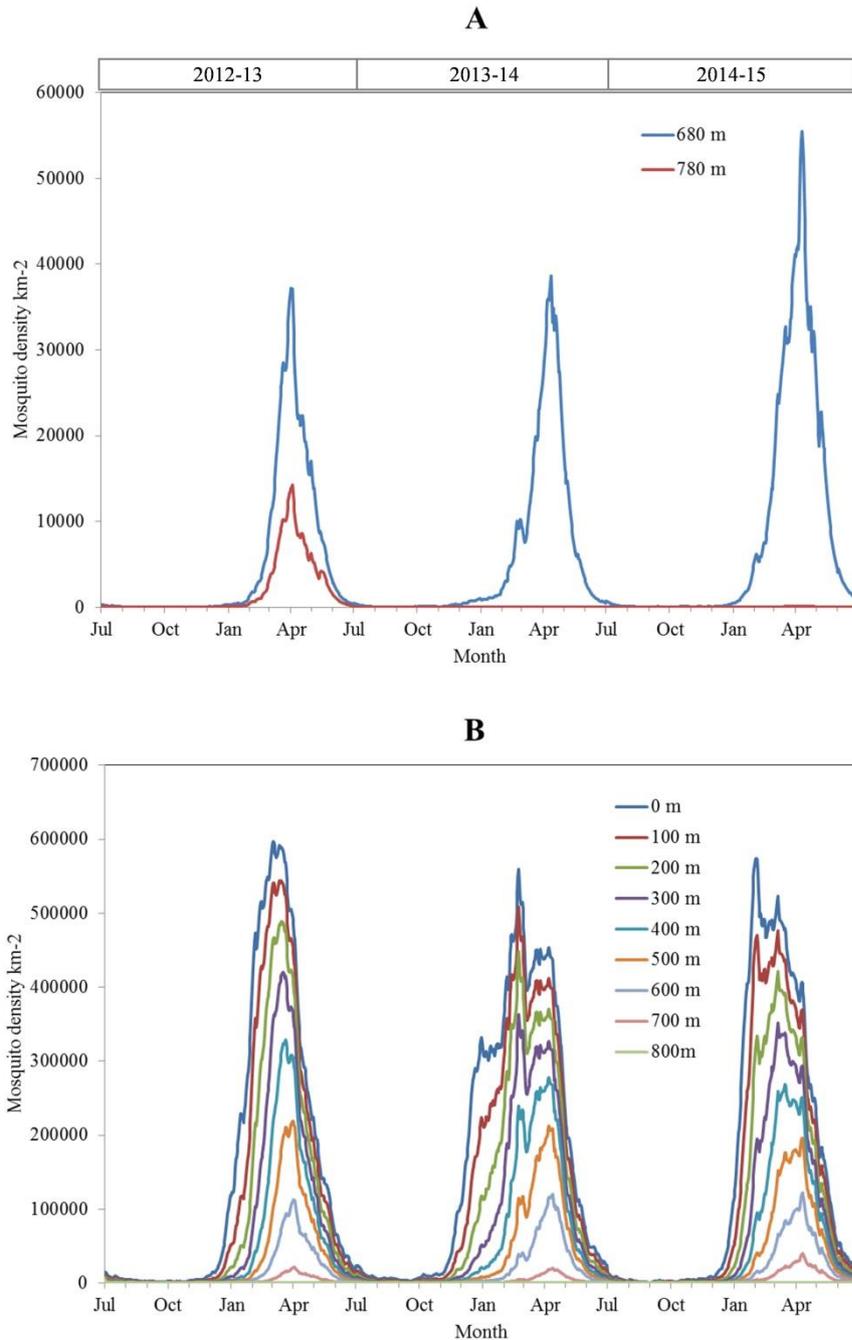


Fig. 5.3. (A) Model predictions of adult mosquito (*Culex pervigilans*) density per elevation (m) using actual temperature data collected for three years (2012-13 to 2014-15) along the St. Arnaud Range, Nelson Lakes National Park, New Zealand. Data from elevations not shown resulted in no mosquito presence in the model. (B) Model predictions of adult mosquito densities for 100-meter elevational steps using projected temperatures based on actual temperatures from a single elevation (in this case 680 m.). Note the scale differences between figures.

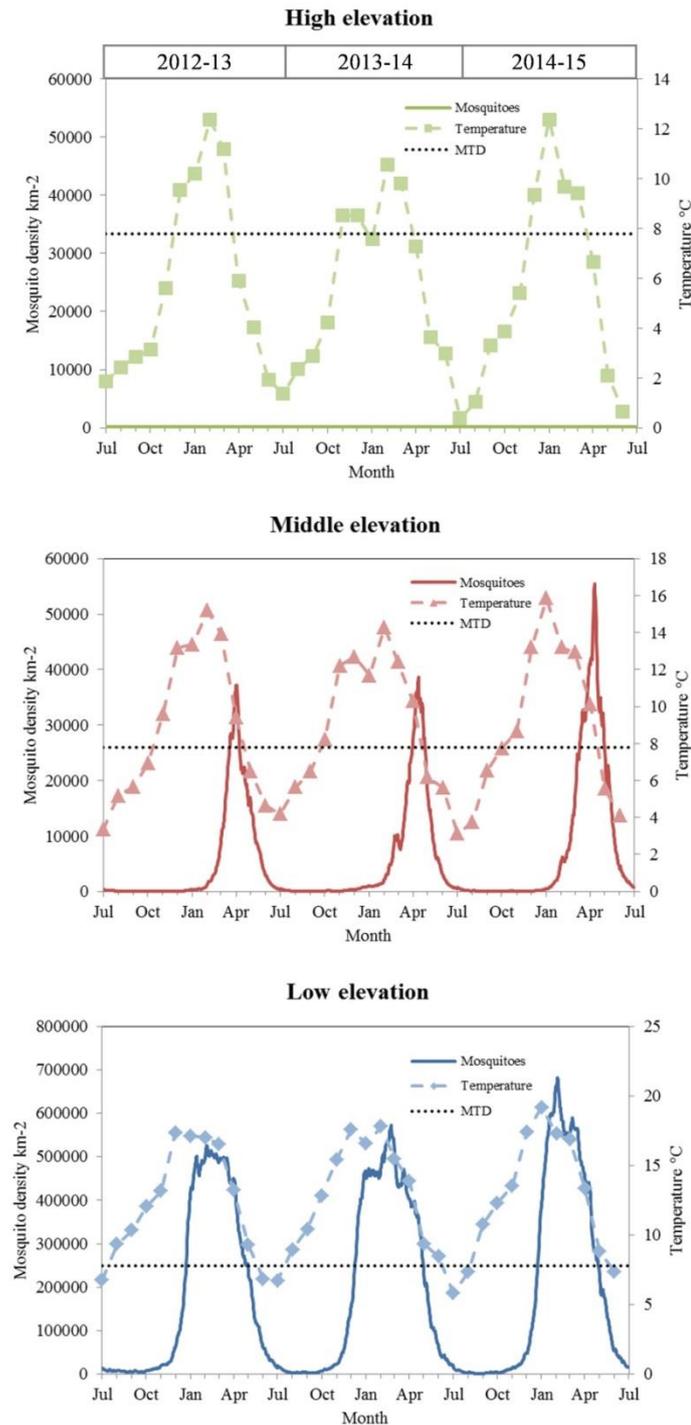


Fig. 5.4. Model predictions of adult mosquito (*Culex pervigilans*) density per elevation (m) using mean monthly temperature from data collected for three years (2012-13 to 2014-15). The high and middle elevation sites (1400 and 680 m) are along the St. Arnaud Range, Nelson Lakes National Park and the low elevation site (0 m) is Motueka, near Abel Tasman National Park, New Zealand. Note the scale differences for low elevation.

Case study simulations (mortality events)

The patterns of simulated mosquito abundance at the time and place of each bird mortality event differed for each scenario (Fig. 5.5). For event 1, mosquito numbers both increased and peaked over a month later in the event year as compared to the previous year. In addition, approximately four times less mosquitoes were estimated in the season leading up to the last recorded mortality as compared to the previous year. For event 2, the model estimated a slight earlier increase and peak in mosquito density in the event year as compared to the previous year, although total mosquitoes produced prior to the final mortality were similar. For event 3, mosquito density both increased and peaked later in the event year as compared to the previous year, with similar overall numbers produced for the entire year. For event 4, the peak mosquito numbers were nearly twice that of the previous year as well as almost twice as many mosquitoes estimated for the entire season.

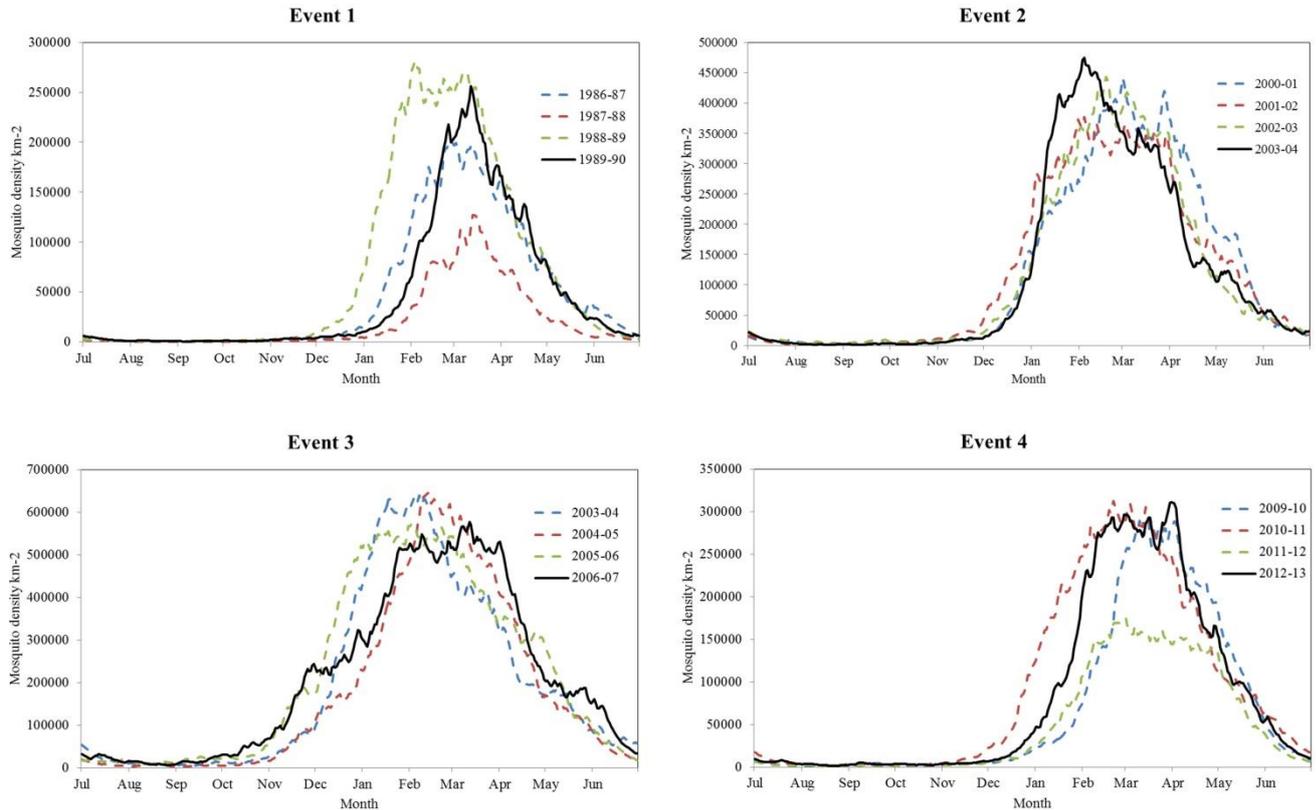


Fig. 5.5. Patterns of simulated mosquito abundance during four bird mortality events, three of which have been associated with avian malaria (Events 1-3). Event 1: yellow-eyed penguins (*Megadyptes antipodes*) on Otago Peninsula. Event 2: Translocated Mohua (*Mohoua ochrocephala*) from Otago to near Christchurch. Event 3: Translocated South Island saddlebacks (*Philesturnus carunculatus*) within the Marlborough Sounds. Event 4: yellow-eyed penguins on Otago Peninsula. Black line represents year that deaths occurred.

DISCUSSION

Temperature dependence

The model shows mosquito population dynamics to be influenced greatly by temperature, with annual, seasonal, and elevational differences in mean densities and maximums as seen in Hawaii (Ahumada *et al.* 2004). Although the concept of a temperature dependent mosquito population may not be surprising given the nature of the *MTD* parameter and its role in the model, beginning to identify the patterns occurring is a crucial first step in understanding mosquito dynamics in New Zealand. Using accurate temperature data, I was able to estimate densities of *Cx. pervigilans* as well as the timing of seasonal population growths and peaks at the latitude of the field

site at NLNP. As expected, mosquito abundance was greater at lower elevations (Fig. 5.3). Although the seasonal timing of temperature increase was relatively consistent across elevation, the timing of mosquito emergence was not, with population growth and peaks in density occurring later at higher elevations (Fig. 5.4). The magnitude and structure of the *Cx. pervigilans* annual cycle also differed among elevations. At low elevations, mosquito densities increased dramatically in late spring with multiple peaks throughout the summer. At middle elevations, mosquito densities increased dramatically in mid-summer with a single peak in early autumn. Temperature conditions at high elevations did not allow for mosquito populations to persist. Due to the annual differences in temperature patterns, both mosquito abundance and the timing of emergence varied by year. A positive correlation was seen between overall mosquito densities and temperature. Both the higher the temperatures, and the longer these temperatures were sustained, influenced mosquito abundance. The findings also emphasise that the timing of mosquito population growth correlates with the rate of temperature increase as well as how early in the season the mean monthly temperature reaches the *MTD* threshold. By beginning to describe how temperature influences mosquito dynamics, we may be able to better understand and predict patterns in vector abundance and potential pathogen transmission.

Application of model to mortality events

By using the model to estimate mosquito abundance at the time and place of a known bird mortality event, I was able to speculate on potential mosquito-borne disease impacts based on the patterns observed. For example, during event 1, the model shows both an earlier and greater mosquito emergence in the year previous to penguin deaths than the event year itself (as well as the other two simulated years) (Fig. 5.5.). Additionally, the peak mosquito numbers for the year before the event were sustained for approximately two months, as compared to the brief, sharp peak in numbers of the event year. One possible explanation linking the cause of penguin mortality to disease is an increase in avian malaria presence in other bird species for the year prior, due to greater mosquito exposure as a result of a particularly significant mosquito season. This increased malaria transmission would essentially be priming the surrounding bird populations to then act as reservoirs for disease spillback during the following mosquito season. In this scenario, although overall exposure to mosquitoes may be lower than the

previous year, the probability of successful transmission may be greater due to an increase in background prevalence of malaria. A similar pattern of high mosquito abundance and/or early emergence in the year prior to the mortality event was not found for event 4, which involved the same species and location. In fact the opposite was seen for, with almost twice as many mosquitoes estimated for the event year as compared to the previous year. Therefore, based on the model, this scenario of bird mortality linked to high malaria transmission from the previous year's mosquito presence for event 4 is unlikely, which is consistent with reports of no evidence of malaria presence (Alley *et al.* 2014).

When using the model to compare mosquito emergence and abundance between event years and the previous years, opposite patterns can be seen between event 2 and event 3 (Fig. 5.5.), both of which involve translocations. However for both mortality events, when comparing the event year and the previous year, the year with the earlier mosquito emergence is the same as that of the translocation (the event year for event 2 and the previous year for event 3). Translocated birds can undergo considerable stress due to capture, transport, and quarantine (Fix *et al.* 1988; Dickens *et al.* 2010), which can suppress immune responses (Dhabhar & McEwen 1997) leading to increased disease. In addition to the examples above, other cases exist where naïve birds, susceptible to new local malaria parasites, have experienced mortality following a translocation (Fix *et al.* 1988; Bennett *et al.* 1993). Since it may be difficult to determine if the greater impact from exposure to malaria parasites during a translocation is due to the mosquito dynamics of a particular season or rather the translocation itself, both factors should be taken into account before implementing such a strategy. The application of the model can be used to aid in minimising the potential threat of mosquito-borne diseases by identifying higher risk years and/or sites for an intended translocation. Although the model can be used to identify sites as higher risk by comparing temperatures from previous years, predictions of mosquito dynamics for the current season may be limited to detecting possible early mosquito emergence as opposed to overall seasonal abundance. However, information on the timing of mosquito emergence could be of particular importance when taking into account the unique biology of a given bird species, such as the timing of hatching or fledging.

The implications of these data for avian malaria having population scale impacts on native bird populations are unclear. The exclusion of both host and malaria pathogen components is likely reducing the predictive power of the model to predict the involvement of avian malaria in the mortality events discussed previously; therefore the lack of congruence in the predictions above cannot be used to rule out malaria, a better model is needed for that. The impacts of avian malaria may in fact be interacting with other factors to cause these population scale effects, making it difficult to determine the role of disease.

Future direction

Although drought and rainfall were not considered to be limiting factors at the field site due to estimated annual rainfall and the abundance of protected breeding sites, respectively, it is possible that the inclusion of rainfall data in the model could have an effect on the model output. The addition of rainfall may be particularly important when comparing results between sites with different rainfall patterns, such as multiple sites involved in a bird translocation. Additional data describing *Cx. pervigilans* densities and life cycle would help to refine parameters used, many of which were estimated for *Cx. quinquefasciatus*. Although more sampling is recommended to better describe the mosquito community at NLNP, the presence of *Cx. pervigilans* but not *Cx. quinquefasciatus* at a site known to have malaria-positive birds provides further support for *Cx. pervigilans* as a vector for avian malaria in New Zealand. Also, as mentioned above, further model development is needed to assess malaria transmission dynamics in New Zealand, by integrating both avian host and malaria pathogen components.

Conclusion

The model can also be used to explore the effects of potential climate change on mosquito dynamics. For example, I can increase the daily temperature data in the model by 1°C, simulating a 1°C warming event, and compare the results of mosquito densities. This could allow us to predict possible increased mosquito-borne disease threats and identify risk sites or seasons, similar to Hawaii (Benning *et al.* 2002). Additionally, the model, which is currently designed to describe the population dynamics of *Cx. pervigilans*, could be used as a basis for developing similar models of other mosquito species, either already in New Zealand or threatening to make their way into the

country. These species-specific models could in turn be beneficial to the understanding of other mosquito-borne disease threats such as Ross River virus, West Nile virus, and human malaria.

Chapter Six

General Discussion

Emerging infectious diseases have impacted the health of both individuals and populations of wild animals, posing a serious threat to the conservation of global diversity (Harvell *et al.* 1999; Daszak *et al.* 2000). Often it is native wildlife species that are threatened by non-native parasites they have not evolved with or adapted to (Tompkins & Poulin 2006; Tompkins *et al.* 2015). Avian malaria may be of concern to the New Zealand avifauna, considered to be the most extinction-prone in the world (Sekercioğlu *et al.* 2004; Tompkins & Poulin 2006). Although avian malaria has impacted both captive populations and wild individuals in New Zealand, whether or not it is a cause of concern to native bird populations in the wild is unknown (Alley *et al.* 2008; Banda *et al.* 2013).

This research provides insight into the general ecology and epidemiology of avian malaria, highlighting differences in patterns of prevalence as well as offering recommendations to improve detection via molecular techniques. Also, this study was an investigation into avian malaria in New Zealand and its effects on the local avifauna. Various factors potentially influencing transmission dynamics were explored, such as elevation, vector abundance, and native or non-native status. This research incorporates the first investigation of avian malaria presence across an elevational gradient in New Zealand. Avian malaria prevalence was assessed at a site experiencing native bird declines in which malaria has been suggested as playing a role (Elliott *et al.* 2010; Tompkins & Jakob-Hoff 2011). Associations between pathogens and hosts were identified and a model was created to make preliminary predictions of mosquito abundance based on temperature.

Drivers of malaria prevalence in the Australasia-Pacific region

The ability of parasites to infect host populations depends on a number of factors leading to differences in prevalence of infection between and within animal populations. Many of these differences in prevalence have been associated with the exposure of the host to vectors and susceptibility of the host to infection (Atkinson & Dusek 2000; Hellgren *et al.* 2008; Samuel *et al.* 2011). Results from the comparative analysis (Chapter 2) help to identify likely drivers of avian malaria prevalence in the Australasia-Pacific region. Characteristics such as adult body mass of host species, elevation, and latitude may be influencing avian malaria prevalence. These patterns underpin the hypothesis that avian malaria has had a negative impact on population

dynamics of native bird species, influencing their elevational abundance and distribution (van Riper *et al.* 1986; LaPointe *et al.* 2010; Samuel *et al.* 2015). Differences in patterns of infection were also seen across locations, with an interesting inverse trend seen for prevalence in non-native and native hosts between New Zealand and Hawaii, leading to questions related to resistance to infection in different regions. By beginning to identify likely drivers of malaria prevalence, we are able to better understand large-scale patterns of variation across multiple avian populations.

Further research is needed to investigate other factors that may be driving parasite prevalence, such as nest type (i.e. within tree cavity or out in the open), height above ground of nest, coastal versus inland populations, and colonial versus solitary species. Additionally, the expansion of the data set to other regions of the world beyond the Australasia-Pacific region would allow for the identification of global patterns and the possibility of identifying differences between regions. An important next step in New Zealand will be to determine whether malaria affects native and non-native birds differently on a population level. Although difficult, studying this under field conditions could provide key information on host susceptibility that is currently lacking. This could be achieved using long-term mark-recapture studies of wild birds, allowing comparisons between the survival of malaria-infected and uninfected individuals of different species. Based on the number of recaptures in this study, an increased trapping effort would be needed to achieve enough recapture data for statistical analysis.

Improving detection of avian malaria from host bird blood

Molecular diagnostic techniques, such as PCR, have played an important role in the advancement and understanding of avian malaria taxonomy and ecology. However problems arise when parasites in the blood cannot be amplified, resulting in false negatives (Ricklefs & Fallon 2002; Hellgren *et al.* 2004). Results from this study (Chapter 3) show that, when attempting to detect *Plasmodium* spp. from bird blood via PCR, modifying the elution step of a silica-membrane-based extraction protocol can be an effective approach to decreasing the occurrence of false negatives. Any increase in sensitivity of detection of avian malaria parasites could prove extremely beneficial in the diagnosis or necropsy of high priority individuals as well as during large-scale disease surveys, when numerous samples must be screened quickly and on a limited budget. Additional research into the efficacy of molecular techniques for identifying

parasites within a host is recommended, since it is unlikely that false negatives can be eliminated completely when working with avian malaria (Freed & Cann 2006). Further research into optimisation strategies such as sample storage and DNA extraction and amplification is also recommended, to help minimise the potential for false negatives. Since the birds screened for this study were wild caught, I was not able to assess false negatives. Further investigation involving known positive individuals may be necessary to estimate the rate of false negatives occurring in various species and at various levels of parasitemia.

Modeling Culex pervigilans, a suspected disease vector

In this study, a model for mosquito abundance (Chapter 5) was used to estimate densities of the native *Culex pervigilans* mosquito, as well as the timing of seasonal population growths and peaks. The model shows that temperature influences on mosquito population dynamics in New Zealand likely drives annual, seasonal and elevational differences in mean densities and maximums, such as are seen in Hawaii (Ahumada *et al.* 2004). The model was used to estimate mosquito abundance surrounding known bird mortality events, allowing for insight into the possible role of vector-borne diseases in these events. Model applications may include use for potentially minimising the threat of mosquito-borne diseases by identifying higher risk years and/or sites for an intended translocation. The information on the timing of mosquito emergence could be of particular importance when taking into account the unique biology of a given bird species, such as the timing of hatching or fledging. Other model applications include exploring the effects of potential climate change on mosquito dynamics, as well as the adaptation into similar models of other mosquito species, either already in New Zealand or threatening to make their way into the country.

However, further model development is needed to assess malaria transmission dynamics in New Zealand. The addition of rainfall may be particularly important when comparing results between sites with different rainfall patterns, such as multiple sites involved in a bird translocation. The recommended next step is to integrate both avian host and malaria pathogen components into the model as has been carried out for avian malaria in Hawaii (Samuel *et al.* 2011).

Avian malaria prevalence along an elevational gradient

While studies exist that have investigated latitudinal relationships of avian malaria in New Zealand (Tompkins & Gleeson 2006; Baillie & Brunton 2011), no such studies have explored elevational patterns. The results (Chapter 4) support the potential for of a higher force of infection to be occurring at the lower elevations at the field site. This may have important implications for avian species or populations occurring in greater numbers at higher elevations, due to the threat of climate change and with it the potential upward elevational movement by mosquitoes (Atkinson & LaPointe 2009). The concordance of evidence between these field results and the results from the comparative analysis (Chapter 2) strongly argue for universal elevation patterns of avian malaria prevalence. These observations, along with the strong elevational patterns of mosquito abundance observed from the mosquito model simulations discussed earlier (Chapter 5), further emphasise the importance of the vector component in the avian malaria system. This in turns highlights the need for further mosquito research in New Zealand, where most studies investigating avian malaria ignore the vector component. Such efforts could include more detailed surveys of mosquito distribution, especially at higher elevations, and studies that focus on the native *Cx. pervigilans*, as a species for which limited knowledge exists with respect to its ecology and vector potential (Derraik *et al.* 2005).

Results from this study also show an overall higher prevalence of malaria in non-native versus native birds living in a shared space, suggesting the possibility of a differential impact on host species that show dissimilar reservoir competence. In other words, I suggest that despite reports of low malaria prevalence in native host species, transmission may still be occurring, however with a greater difficulty of detection due to potential impacts of infection. These results are similar to the general patterns for New Zealand, reported from the comparative analysis presented earlier (Chapter 2). Additionally, no avian malaria parasite was detected in five native bird species reported as declining in population in Nelson Lakes National Park, yet a high prevalence was observed in blackbirds, a species not reported as declining (Elliott *et al.* 2010). Based on data from the current study, I suggest that the *Turdus* spp. (blackbirds and song thrushes) may be acting as key reservoir hosts for malaria parasites at this site. These findings provide further support to the hypothesis that disease, in addition to predation

and competition, could be playing a role in the observed population declines (Tompkins & Jakob-Hoff 2011).

One of greatest knowledge gaps of the avian malaria system in New Zealand is the lack of detailed information on host susceptibility to infection, information that could be obtained through experimental infection studies (Atkinson *et al.* 1995). Such studies could provide valuable information on differences in susceptibility between New Zealand species as well as the timing of potential relapses. These data would complement those from the previously mentioned proposed mark-recaptures studies conducted under field conditions. If avian malaria is impacting the native avifauna at a population scale, and a differential impact on host species is occurring due to specific *Plasmodium* lineages, it would carry with it a whole new complexity to the conservation of native wildlife in New Zealand. Supporting evidence for such disease impacts will enable appropriate management to be developed and put in place, while a lack of evidence will enable resources to remain focused on the other issues facing New Zealand's native species (e.g. through habitat restoration, pest control, translocations and genetic management). In the case of the former, providing managers with more specific information of any potential disease impacts on native bird populations could help avoid "blanket" conservation and management efforts that can often be seen as costly and less species specific.

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Appendix One

**Studies included in the data set used to obtain
malaria prevalence in Chapter 2**

APPENDIX ONE: Studies included in the data set used to obtain malaria prevalence in Chapter 2

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Appendix Two

Poster summarising research from my PhD

Study and presented at the 64th Annual

International Conference of the Wildlife Disease

Association in Queensland, Australia, July 2015

Appendix Two: Poster summarising research from my PhD Study and presented at the 64th Annual International Conference of the Wildlife Disease Association in Queensland, Australia, July 2015

Investigating Avian Malaria Transmission Dynamics in New Zealand



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² Landcare Research, Dunedin, New Zealand



Introduction:

Avian malaria, caused by protozoan blood parasites of the genus Plasmodium, is a concern for native New Zealand birds; this mosquito-borne disease has impacted both captive populations and wild individuals in the country. However, whether or not it is a cause of concern to native, wild populations is still unclear. In Hawaii avian malaria has been a major factor in the population declines of native forest bird species and limits the elevational distribution of many remaining species.

Objective:

Identify factors predicted to influence the transmission dynamics of avian malaria in New Zealand.

Study Site:

Nelson Lakes National Park, South Island, New Zealand



Methods:

Bird Surveys



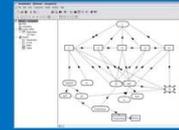
Mosquito Surveys



Molecular Diagnostics



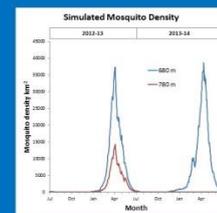
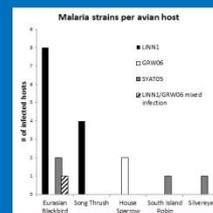
Mathematical Modeling



Results:

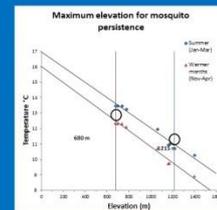
Elevation (m)	Malaria Prevalence		
	Non-natives (n=126)	Natives (n=134)	Total
High (1200-1400)	22.2% (2)	0%	3.3% (2)
Mid-alt. (650-800)	12.8% (15)	2.4% (2)	6.9% (17)
Total	18.5% (17)	1.5% (2)	6.3% (19)

Elevation (m)	Mosquito Presence	
	Culex pervigilans	Culex quinquefasciatus
800	4	0
650	73	0
Total	77	0



Conclusions:

- Confirmed avian malaria presence at Nelson Lakes NP, with higher prevalence in non-native vs. native bird hosts
- Confirmed presence of the native *Culex pervigilans* mosquito but not the introduced *Culex quinquefasciatus*
- Begun to identify host-strain associations
- Estimated the maximum elevation for mosquito persistence during mosquito breeding season (680 m) and summer months (1215 m)



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