Avian Malaria (*Plasmodium* spp.) in the Auckland Region: Host-Parasite Associations, Capture Technique Bias, and Landscape Disease Dynamics

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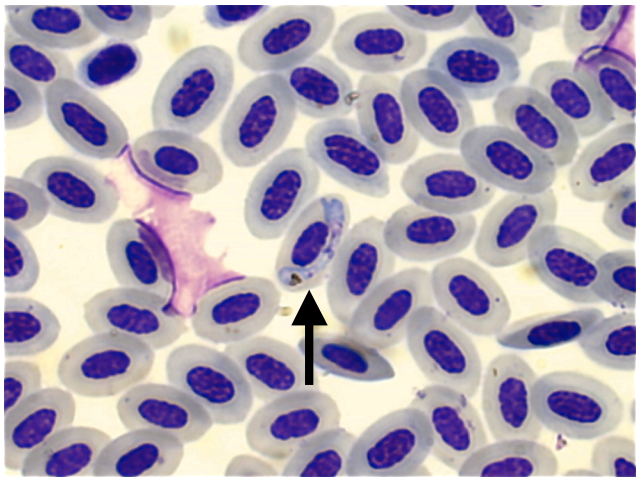
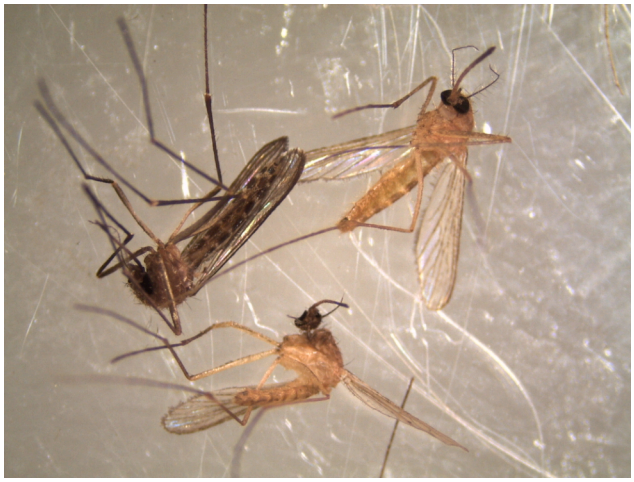
Bellbird (*Anthornis melanura*)



Plasmodium (*Novyella*) sp. found in bellbirds



Two avian malaria vectors in New Zealand
Culex pervigilans (dark) and *Culex quinquefasciatus* (light)
Photographs by D.J. Gudex-Cross

ABSTRACT

Avian malaria parasites (*Plasmodium* spp.) are distributed throughout the world and affect a vast range of bird species. However many aspects of avian malaria in New Zealand, such as the extent of parasite diversity, distribution and prevalence in host populations, are currently unknown. Thus, the first aim of this study was to examine these parameters in native and exotic bird species of the Auckland Region, North Island. A total of 21 species were sampled at two sites: Tiritiri Matangi Island and Waharau Regional Park. Of these, five native (bellbird *Anthornis melanura*, tui *Prosthemadera novaeseelandiae*, New Zealand fantail *Rhipidura fuliginosa placabilis*, North Island tomtit *Petroica macrocephala toitoi* and silvereye *Zosterops lateralis*) and three exotic (myna *Acridotheres tristis*, blackbird *Turdus merula* and song thrush *T. philomelos*) species were infected. This is the first reported incidence of avian malaria in the New Zealand fantail, and only the second record in tui. The parasite morphospecies identified in this survey were *P.* (*Haemamoeba*) *relictum*, *P.* (*Huffia*) *elongatum*, *P.* (*Novyella*) *rouxi* and two *P.* (*Novyella*) spp. not yet formally taxonomically described, one of which appears to be specific to the endemic bellbird. Parasite prevalence within the two most heavily sampled species varied strikingly: bellbird prevalence was estimated at 41.5% (*N* = 51/123) in the Tiritiri Matangi population and silvereye prevalence at 9.2% (*N* = 22/240) within the Waharau population.

The other two objectives of this study addressed: 1) the potential bias that choice of capture technique may have in avian malaria surveys and 2) the effects of forest fragmentation on avian malaria and vector distributions. The first objective was investigated by comparing prevalence and parasitaemia in adult male bellbirds on Tiritiri

Matangi Island caught *via* two live-capture techniques: mist netting and supplementary feeder trapping. In this instance it was found that the choice of capture method did influence results: mist netting of bellbirds yielded significantly higher parasitaemia than feeder trapping. The second objective was investigated through a comparison of mosquito species abundance, composition, and avian malaria prevalence in silvereyes at forest edge *versus* interior sites in Waharau Regional Park. A total of five mosquito species, three native (*Aedes antipodeus*, *Culex asteliae* and *Cx. pervigilans*) and two exotic (*Ae. notoscriptus* and *Cx. quinquefasciatus*), were trapped in this study. Significantly more exotic mosquitoes were trapped at the forest edge, with almost complete absence in the interior. Furthermore, analysis of the individual species showed a significant edge-association for *Cx. quinquefasciatus*. Although significantly more native mosquitoes were trapped in the forest interior *versus* the edge, this was due to high *Ae. antipodeus* abundance in one interior site. Consequently, no edge- or interior-associations were found for the individual native species. Finally, avian malaria prevalence in silvereyes did not significantly differ between forest edge and interior.

The outcomes of this study included: a) additional baseline prevalence data for New Zealand; b) records of two new parasite-native bird associations; c) a demonstration that different live-capture techniques can bias estimates of parasitaemia; d) confirmation that forest edge habitats are more prone to exotic mosquito invasions in New Zealand; and e) determination that avian malaria prevalence in the silvereye does not differ between birds caught at forest edge and interior and is thus not closely correlated to mosquito distribution at these sites. A discussion of the future avenues of avian malaria research in New Zealand is provided at the end of this study.

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The Real Work

It may be that when we no longer know what to do

we have come to our real work,

and that when we no longer know which way to go

we have come to our real journey.

The mind that is not baffled is not employed.

The impeded stream is the one that sings.

-Wendell Berry

(The Collected Poems of Wendell Berry ©1987, North Point Press)

PERMITTING AND BIRD NOMENCLATURE

This research was approved by the Massey university Animal Ethics Committee (AEC/13, amended 01/09) and Department of Conservation (permit AK-20666-FAU). Bird banding was carried out under Department of Conservation permit No. 2008/33. Massey University (Masterate Scholarship) and the Auckland Regional Council provided funding for this study.

The scientific names of New Zealand birds used in this study follow the nomenclature of:

Gill, B.J., B.D. Bell, G.K. Chambers, D.G. Medway, R.L. Palma, R.P. Scofield, A.J.D. Tennyson and T.H. Worthy. 2010. Checklist of the birds of New Zealand, Norfolk, Macquarie Islands, and the Ross dependency, Antarctica. Fourth Edition. Te Papa Press, Wellington, New Zealand, in association with the Ornithological Society of NZ Inc.

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CHAPTER 1.

General overview of avian malaria and research objectives

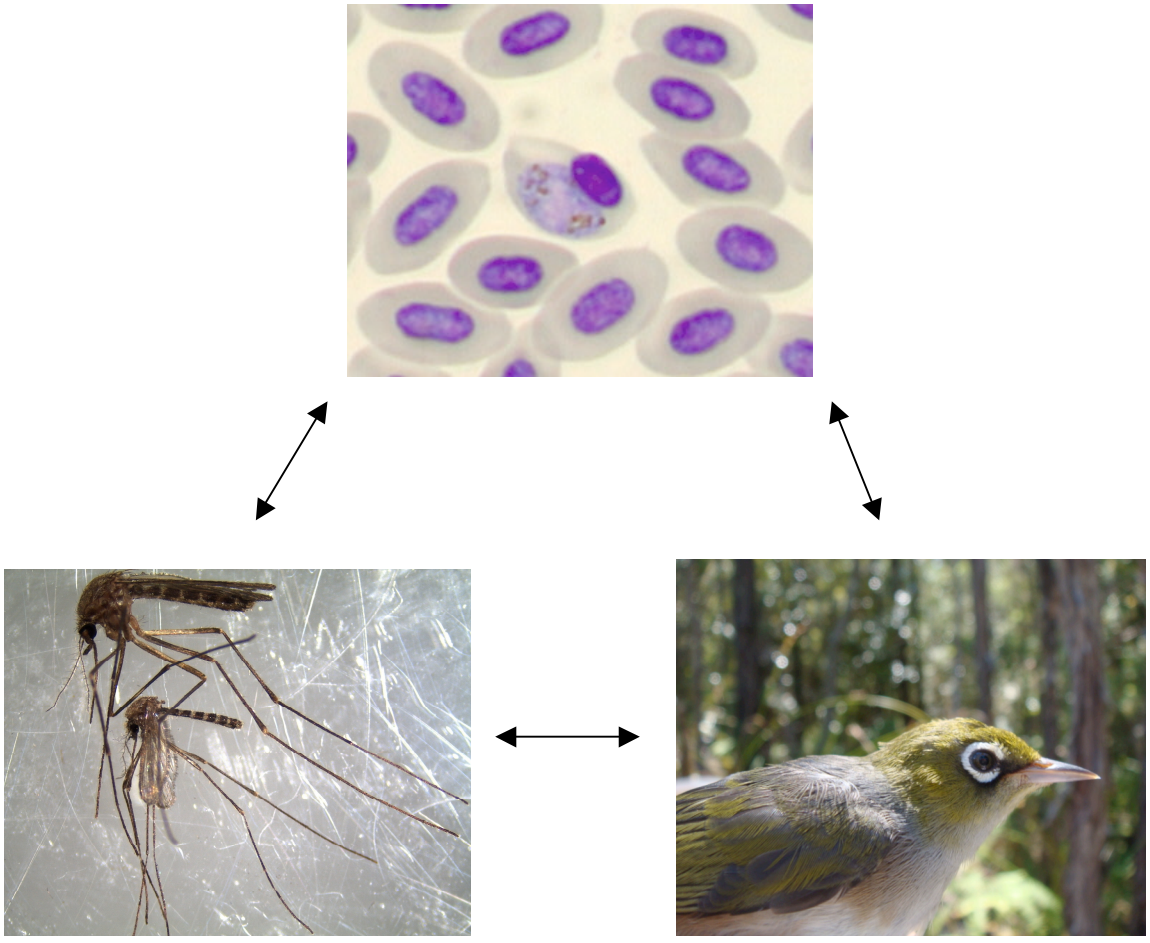


Figure 1.1. Avian malaria components. Depicted clockwise from top: parasite (*Plasmodium relictum*), host (silvereye, *Zosterops lateralis*) and vector (mosquitoes, *Culex* spp.). Photographs by D.J. Gudex-Cross.

ABSTRACT

This chapter provides a brief overview of avian malaria (*Plasmodium* spp.), including its general classification and associated nomenclature, methods of detection, known effects on host species and disease dynamics on a landscape scale. Each section synthesises historical and current research by highlighting key studies and concepts, in addition to identifying gaps in our current knowledge. This provides context to the research carried out in this project. The final section outlines the areas of avian malaria research in New Zealand targeted by this study, and my specific research objectives for each subsequent chapter.

1.1 INTRODUCTION

1.1.1 Classification and nomenclature

Protozoan parasites that utilise bloodsucking dipteran (Insecta: Diptera) vectors to infect blood and tissue cells of vertebrate hosts belong to the order Haemosporida (phylum: Apicomplexa). The three genera (*Plasmodium*, *Haemoproteus*, and *Leucoctyozoon*) that affect birds are collectively referred to as avian haemosporidians (Garnham 1966). However, traditionally, the terms "malarial parasite" and "avian malaria" have only been used in reference to *Plasmodium* parasites and clinical infections, respectively (Valkiunas et al. 2005). Whereas haemosporidiosis has been used as the term for clinical infection by *Haemoproteus* and *Leucocytozoon* (Atkinson 1999, Valkiunas 2005). The main distinctions separating *Plasmodium* are: its occurrence in squamate reptiles and mammals; asexual reproduction (schizogony or merogony) in the host's peripheral blood; the ability to increase in the blood again after the initial stage of infection (recrudescence); and the utilisation of mosquitoes (family: Culicidae) as vectors (Atkinson and van Riper III 1991, Valkiunas 2005). However, *Plasmodium* species, regardless of vertebrate host, have historically been considered more closely related to each other than to those of the other genera, *Haemoproteus* has been considered a closely related sister taxon to *Plasmodium*, and *Leucocytozoon* further removed as a much more recent coloniser of birds (Garnham 1966, Valkiunas 2005).

Recently however, Pérez-Tris et al. (2005) argued that the term avian malaria should also apply to *Haemoproteus.* They contended that recent haemosporidian phylogenies constructed on the basis of a mitochondrial gene sequence (cytochrome *b*, hereafter cyt *b*) indicated that *Plasmodium*-only terminology was taxonomically

misleading. In these phylogenies, *Plasmodium* lineages that infected birds and reptiles appeared more closely related to *Haemoproteus* than to mammalian *Plasmodium* lineages (Bensch et al. 2000, Perkins and Schaal 2002, Ricklefs et al. 2004). Thus to these authors, the use of the terms "malarial parasite" and "avian malaria" without reference to *Haemoproteus* misconstrued haemosporidian relatedness.

Yet, a caveat to these author's phylogenies (*e.g.* Bensch et al. 2000, Perkins and Schaal 2002, Ricklefs et al. 2004) was the fact that nodal support for the *Plasmodium*-*Haemoproteus* branch was weak. Furthermore, a recent groundbreaking study by Martinsen et al. (2008) clarified the apparent traditional *versus* molecular classification discrepancies (Figure 1.2). These authors constructed a haemosporidian phylogeny based on four different gene sequences of mitochondrial (e.g. cyt *b*) and nuclear origin from each parasite genus, with strong resultant nodal support. Here, the *Plasmodium* lineages were found to be more closely related to each other across their host range than to *Haemoproteus*, which remained a sister taxon to *Plasmodium*. Further, each node corresponded to the colonisation of a new group of vectors, and asexual reproduction in the host's peripheral blood arose once at the origin of *Plasmodium*. Therefore the traditional classification scheme of Garnham (1966), as amended by Valkiunas (2005), based on morphological and life history characters was best supported in this comprehensive molecular phylogeny.

In accordance with the findings of Martinsen et al. (2008), I will use the term "avian malaria" only in reference to *Plasmodium*. The issue of the more general term "malarial parasite" remained somewhat unresolved in their phylogeny, as *Plasmodium* remained paraphyletic due to the presence of *Hepatocystis* within its mammalian clade.

Thus the authors suggested including the entire clade (*Plasmodium* + *Hepatocystis* + *Haemoproteus*) under the umbrella term "malarial parasite," or otherwise making it clear which parasites are being discussed. As this study only concerns avian malaria parasites, my usage of "malarial parasites" will also refer to avian *Plasmodium*.

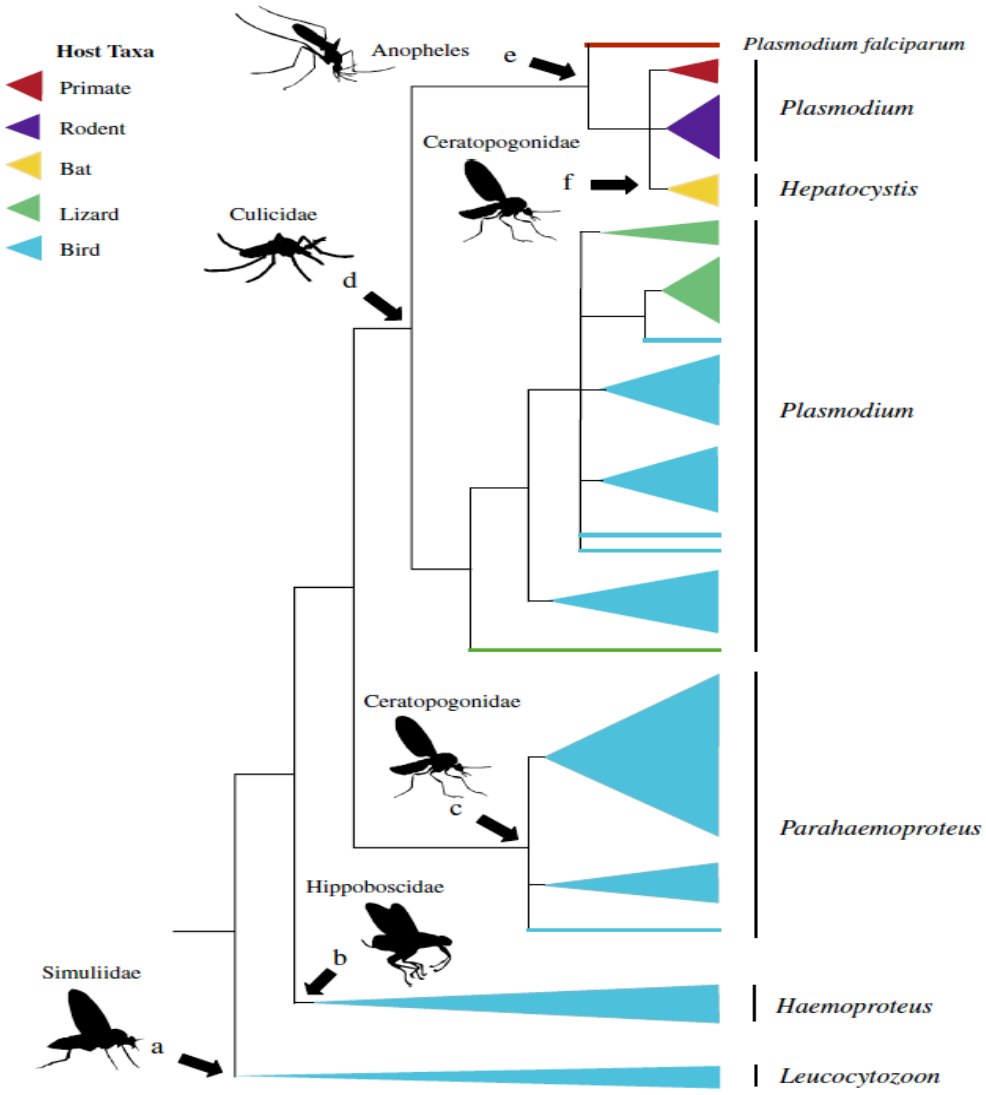


Figure 1.2. The most well supported molecular phylogeny of haemosporidian genera to date showing parasite-vector-host relationships from Martinsen et al. (2008). Each letter (a, b, c, etc.) represents the colonisation of a new group of vectors. Note: *Parahaemoproteus* is considered a subgenus of *Haemoproteus* (Valkiunas 2005), though the authors argue it should be considered a valid genus based on their results.

1.1.2 Life cycle

Avian malaria parasites undergo a complex life cycle involving reproductive stages in both host and vector (Figure 1.3). In brief (summary based on Atkinson and van Riper III 1991, Atkinson 1999, Valkiunas 2005), the cycle begins when the infective

stage (sporozites) of the parasite develops in a mosquito's salivary glands. The mosquito feeds on a bird, and sporozites invade cells of the bird's organs and tissues where they reproduce asexually, forming schizonts (schizogony). These schizonts then produce merozoites that can give rise to either asexual or sexual development stages in the host's red blood cells (erythrocytes). This round of schizogony within the host's erythrocytes is unique to *Plasmodium*.

Inside erythrocytes, merozoites mature into non-fissionable trophozoites, each of which develops into either a gametocyte (a pre-gamete sexual reproductive stage) or an erythrocytic schizont. As trophozoites the size of the parasite's nucleus increases along with the amount of cytoplasm, and the characteristic pigment of malarial parasites (hemozoin) begins to appear. The forming schizonts undergo multiple asexual divisions giving rise once again to many uninuclear merozoites. The number of merozoites in a schizont is one of the diagnostic characters for *Plasmodium* species identification (Valkiunas 2005). The size, shape and other characteristics of the gametocyte are also diagnostic for *Plasmodium* species identification (Garnham 1966; Valkiunas 2005). Finally, a mosquito ingests the gametocytes when it feeds on an infected bird. After ingestion gametocytes transform into gametes within hours, and sexual reproduction occurs in the mosquito's midgut. The zygotic products of reproduction, oocysts, become encrusted in the lining of the midgut where they eventually rupture, freeing the infective sporozites. Thus, the cycle starts again when the infective sporozites reach the salivary glands of the vector. It is interesting to note that the mosquito is actually the definitive host in the malarial cycle, as this is where the parasite undergoes sexual reproduction, and birds are the intermediate hosts.

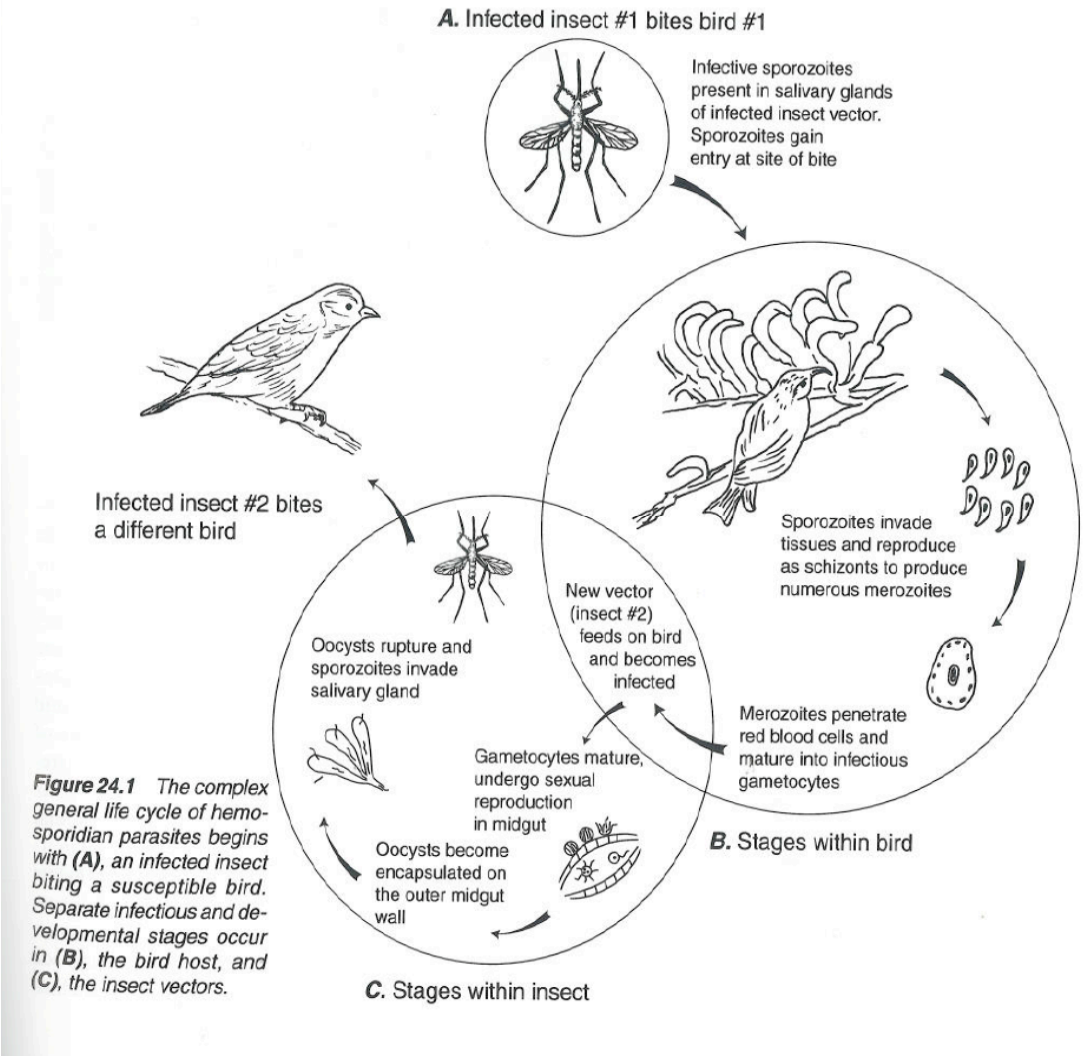


Figure 1.3. A generalised avian haemosporidian life cycle from Atkinson (1999). Note: *Plasmodium* parasites undergo an additional stage of asexual division (schizogony) inside host erythrocytes. This diagram only shows schizogony in tissues.

1.1.3 Distribution and diversity

Avian malaria parasites are found in every region of the world except Antarctica, where mosquitoes cannot breed (Bennett et al. 1993a, Atkinson 1999). Collectively they

have a wide host range, to date having been isolated in numerous bird species comprising nearly every extant order (Valkiunas 2005). Host specificity among the parasites varies, however, as some lineages are widespread in their host distribution while others are specialised to a particular family (Fallon et al. 2005, Ricklefs et al. 2005, Beadell et al. 2009). Some parasites in particular (e.g. *P. relictum*) exhibit extreme host generalist and geographic range characteristics (Valkiunas 2005, Beadell et al. 2006). The radiation of malarial parasites into many hosts throughout the world, exemplified by extreme generalists, appears to have arisen mainly through host-switching events (Bensch et al. 2000, Ricklefs et al. 2004, Fallon et al. 2005). This ability to switch hosts is likely to have been facilitated by the colonisation of new vector species, or the movement of infected birds into a new environment where suitable vectors already existed (*e.g.* through dispersal and migration), giving the parasites access to novel hosts (Ricklefs and Fallon 2002, Beadell et al. 2004, Pérez-Tris and Bensch 2005, Gager et al. 2008, Martinsen et al. 2008). However, the extent to which host-switching and global movements of parasites has led to speciation, and hence diversity, remains a subject of on-going research (Ricklefs and Fallon 2002, Bensch et al. 2004, Ricklefs et al. 2005).

Valkiunas (2005) lists five subgenera (*Bennettinia*, *Giovannolaia*, *Haemamoeba*, *Huffia* and *Novyella*) consisting of 38 valid species of avian *Plasmodium* based on morphological and life history characteristics (*i.e.* the "morphological species concept" based on observed phenotypes; *sensu* Mayden 1997, Perkins 2000). These morphospecies are classified on the basis of developmental stages observed in the host's peripheral blood. Yet, this method of defining parasite species is problematic due to several factors: the same parasite species can exhibit morphological plasticity within different hosts and

host age classes (Garnham 1966); different parasite species may have minutely different or convergent morphologies (Perkins 2000, Beadell et al. 2006); microscopy only offers a crude two-dimensional view of parasite morphology (likened to the systematic study of insect remains left on an automobile windshield by Martinsen et al. [2008]); and blood films obtained from wild birds rarely contain a wide range of developmental stages (Valkiunas et al. 2006).

Recent molecular sequencing of mitochondrial and nuclear DNA shows the number of haemosporidian species is likely to be orders of magnitude higher than previously thought (Bensch et al. 2004, Sehgal et al. 2006). This estimated increase is mainly attributed to unique DNA sequences that cannot recombine being recovered from parasites classified as single morphospecies (Perkins 2000, Bensch et al. 2004, Sehgal et al. 2006). Thereby strikingly similar parasites, which may also be sympatric, have proven to be genetically distinct and reproductively isolated (Perkins 2000, Bensch et al. 2004, Sehgal et al. 2006). For example, Beadell et al. (2006) recently demonstrated this in the ubiquitous morphospecies *P. relictum*. In a global survey, they revealed that many parasites collectively identified as *P. relictum* were actually distinct lineages, and that these lineages did not form a monophyletic clade. Unfortunately, most unique DNA sequences revealed in these studies have yet to be described at the species level (Valkiunas et al. 2007). Instead these sequences are deposited in large genetic databases such as GenBank and MalAvi (Bensch et al. 2009) at the generic level with an associated descriptor (typically the parasite genus + type host's acronym + an unique number, *e.g.* a *Plasmodium* lineage with the great reed warbler *Acrocephalus arundinaceus* as type host would read P-GRW#).

Nevertheless, recent molecular studies also corroborate some traditional morphology-based parasite designations. For instance, Martinsen et al. (2007) confirmed four of the five traditional subgenera as monophyletic, with the exception of two species of *Novyella* and the subgenera *Giovannolaia*. Moreover, the morphospecies described in Valkiunas (2005) that have been matched with a DNA sequence are unique, and thus do represent good species (Martinsen et al. 2006, Valkiunas et al. 2007). Further studies using both molecular and microscopic techniques are currently being employed to provide DNA sequences for traditional morphospecies, and morphological descriptions and species designations for new lineages (Valkiunas et al. 2007, Valkiunas et al. 2008a, Valkiunas et al. 2009).

1.2 METHODS OF DETECTION

1.2.1 Microscopy

The traditional method of detecting malarial parasites is through examination of a thin blood smear under light microscopy (Garnham 1966). Microscopic observation of malarial parasites accounts for the vast majority of our pre-21st century knowledge of their classification, life history and prevalence. Recent advances in molecular detection techniques have revolutionised the field of parasitology, yet microscopy remains essential for detecting infections involving multiple parasite species, documenting morphological characteristics of parasites, and serving as a check against molecular false positives in prevalence studies (Valkiunas et al. 2006). It also remains an accurate, low-cost alternative to molecular techniques for malarial prevalence and infection intensity

(parasitaemia) studies, but requires time, due care and expertise in thin blood smear preparation and examination (Valkiunas et al. 2008b).

This study employs a thin blood smear preparation and examination protocol similar to that described in Valkiunas (2005), which has been shown to be on par with molecular techniques in determining parasite prevalence (Valkiunas et al. 2008b). First, a drop of blood about the size of a pinhead is obtained with a capillary tube from a live bird *via* femoral (birds ≥ 300g) or brachial (birds < 300g) vein puncture. The blood is dabbed on to a microscope slide and smeared with the straight edge of a second slide tilted at a 45° angle. The angle is important to ensure the smear is thin; thick smears result in the overlapping of cell nuclei, which can mask parasite presence. The smear is air-dried, and then immediately fixed with absolute methanol. The fixed smear is stained by immersion in Giemsa stain at a 1:10 dilution with double-distilled water for up to one hour. Last, the stain is rinsed off with distilled water and allowed to air-dry. It is imperative that staining occurs within a few weeks of fixation for best results. As, after a few weeks smear quality rapidly degrades due to haemolysis, making parasite detection difficult.

Finally, stained smears are examined with a light microscope equipped with digital imagery software for a minimum of 15 minutes to a maximum of 30. For the first five to ten minutes, the smear is scanned for parasite presence under 200x or 400x magnification. This magnification allows the majority of the smear to be scanned relatively quickly, while still enabling the examiner to find small parasites. However, it is my experience that scanning at this magnification alone is insufficient in determining parasite presence. This is particularly true for wild birds, which often exhibit extremely low parasitaemia (*i.e.* the proportion of infected host cells, Zehtindjiev et al. 2008). A

telling example of the inadequacy of this technique is a study by Jarvi et al. (2003) that failed to detect parasites in 214 individual smears, but then found 59 out of 188 of the same samples to be positive by PCR. Thus, it is important that the final ten to twenty minutes of smear examination be done under oil-immersion at 1000x magnification before final determination of parasite presence. A minimum of 100 microscope fields should be examined, which is easily achievable in ten to twenty minutes. In addition, morphological observations and species descriptions (when possible) are made under this magnification.

1.2.2 Polymerase chain reaction (PCR)

In two landmark studies, Feldman et al. (1995) and Bensch et al. (2000) developed PCR diagnostic tests that revolutionised avian malaria detection methods. Feldman et al. amplified a nuclear (rRNA) and Bensch et al. a mitochondrial (cyt *b*) gene sequence isolated from the parasites to detect infections in avian blood samples. A subsequent review by Richard et al. (2002) found that amplification of the cyt *b* sequence outperformed that of rRNA in parasite detection of African rainforest birds. The authors postulated this was likely due to high nuclear sequence divergence between African birds and the Hawaiian birds on which Feldman et al.'s test was developed. Since then, however, the detection efficacy of both methods has been improved upon through the use of a nested PCR approach (rRNA, Fallon et al. 2003 and cyt *b*, Waldenström et al. 2004; reviewed by Freed and Cann 2006). In addition, they have both been shown to outperform microscopy (but see Valkiunas et al. 2008b).

There are still problematic issues associated with using PCR techniques for parasite detection. Studies utilising multiple detection methods have found PCR underestimates true prevalence (Jarvi et al. 2002, Valkiunas et al. 2006). In addition, false negatives can occur, as confirmed by failed detection in a blood sample known to be positive under microscopy (Valkiunas et al. 2008b). Freed and Cann (2006) also found variation in detection accuracy based on the type of buffer solution used for blood sample storage. These authors showed lysis buffers (Seutin et al. 1991), commonly used for its advantage of not having to be refrigerated, compromise prevalence estimates by degrading DNA quality. Last, financial costs can also be an issue for molecular diagnostics. The molecular equipment and reagents needed to conduct PCR and subsequent sequencing are expensive. Nevertheless, PCR remains a rapid way to effectively analyse large numbers of samples, and the numerous possible applications of molecular biology in avian malaria research are only beginning to be realised.

1.3 EFFECTS ON HOST SPECIES

1.3.1 General pathology and host susceptibility

Most of our knowledge regarding the pathology of avian malaria has been ascertained from captive, domesticated and experimentally infected birds (Atkinson and van Riper III 1991, Bennett et al. 1993b, Valkiunas 2005). In those instances onset of the disease can be very rapid, and diagnostic features of severe infections include anaemia, hypertrophied and dark-coloured liver and spleen, and blockage of blood capillaries to vital organs (Atkinson and van Riper III 1991, Palinauskas et al. 2008). The blockage of blood flow and other pathological changes to vital organs, namely the spleen, lungs,

heart, liver and/or brain, is associated with schizogony stages in the host's tissues (Atkinson and van Riper III 1991, Peirce et al. 2004). In heavy infections, this stage can precede schizogony in the peripheral blood and its subsequent liver and spleen effects, making malaria diagnosis difficult without tissue examination (Atkinson and van Riper III 1991). Severe anaemia is associated with red blood cell destruction brought about by erythrocytic stages of the parasite. Affected individuals often exhibit listlessness, general distress (drooping head, closed eyes), or weakness in the legs (Atkinson and van Riper III 1991, Atkinson 1999). Finally, birds that survive the initial (acute) phase of infection often harbour the parasites asymptomatically at low-levels (chronic phase) thereafter (Garnham 1966). These birds are an important part of the transmission cycle, as they serve as parasite reservoirs for further transmission to uninfected individuals (Atkinson 1999).

Host susceptibility to avian malaria is complex and influenced by many factors. These factors include, but are not limited to: age, gender, genetic resistance, immunological status, and stress (environmental, nutritional, physiological, etc) (Atkinson and van Riper III 1991, Valkiunas 2005). Young birds are most susceptible to high mortality rates, likely owing to their lack of developed immunity (Atkinson and van Riper III 1991). For example, high nestling mortality caused by avian malaria has been documented across a wide range of African wading birds (order: Ciconiiformes) (Gabaldon and Ulloa 1980). In adult birds with chronic infections, some studies have shown prevalence to be higher in males *versus* females (Atkinson et al. 1995, Wood et al. 2007). This result is not consistent across studies, however, and is likely host species-dependent (Ricklefs et al. 2005). Host susceptibility also differs among bird orders, with

some groups affected by a wide range of malarial parasites and others few to none (Valkiunas 2005). These latter groups may be evading parasitism through their habitat use, behavioural avoidance of vectors or innate immunological resistance (Deviche et al. 2001). For example, Mendes et al. (2005) and Yohannes et al. (2009) demonstrated shorebirds of coastal, saline environments effectively avoid vector transmission, whereas closely related species that inhabit warmer freshwater environments tend to be chronically infected. Birds found in extreme environments, such as penguins and resident desert species, are also less likely to harbour avian malaria due to a limited abundance of mosquito breeding habitat (Valera et al. 2003). It is these groups that can be immunologically predisposed to suffer severe infections when moved into parasite-laden novel environments such as zoos (Bennett et al. 1993b, Valkiunas 2005).

In general, the less exposure a host group, population, or individual has had to a particular parasite species the more susceptible they are to deleterious effects upon infection (Atkinson and van Riper III 1991). This is the important issue of naivety, which typically manifests in one of two ways: birds are moved into novel environments where they have not adapted to the local parasite fauna (*e.g.* zoos); or novel parasites are introduced into an area where they had not previously been found (van Riper III et al. 1986, Atkinson and van Riper III 1991, Valkiunas 2005). The naivety of birds imported into zoos to local malarial parasites is well documented (Valkiunas 2005), especially in penguins (Bennett et al. 1993b, Jones and Shellam 1999).

1.3.2 Effects on wild populations

There are few documented cases of mass mortality events caused by avian malaria in wild bird populations. Intensive sampling efforts often yield high prevalence values, yet with seemingly benign effects on host condition (Bennett et al. 1988, Bensch et al. 2007). However, Valkiunas (2005) points out that methods used to catch wild birds (namely mist-nets and set traps) may be biased towards healthier individuals. Birds have to be active enough to be caught, and those suffering from severe acute infections may be too anaemic. Moribund birds collected in the wild that have been diagnosed with avian malaria support this supposition, as these individuals often cannot fly (Stone et al. 1971). In addition, birds that succumb to malaria infection may simply go undocumented, as their carcasses would be randomly dispersed in areas infrequently used by humans and quickly scavenged (Valkiunas 2005).

Regardless, most prevalence studies reveal birds in the chronic infection stage (Jarvi et al. 2003, Bensch et al. 2007). Yet studies have only recently begun to document the survival and fitness effects of chronic infection, and little remains known with regard to behavioural effects (Atkinson and van Riper III 1991). Marzal et al. (2008) found decreased survival rates in chronically infected house martins (*Delichon urbica*). Specifically, birds with double-infections had the lowest survival rate, followed by those with single infections, when compared to uninfected individuals. Further, Möller and Nielsen (2007) correlated malaria infection to increased predation risk (and hence decreased survival probability), specifically with regard to avian predators. In terms of male fitness, Johnson and Boyce (1991) demonstrated infected male sage grouse (*Centrocercus urophasianus*) had lower and later lek attendance rates than uninfected

ones, thereby decreasing their number of successful copulations. Also, Gilman et al. (2007) found decreased song output and consistency in infected male white-crowned sparrows (*Zonotrichia leucophrys oriantha*). As for female fitness, Knowles et al. (2010) found increased hatching success, provisioning rates, and fledgling success in nesting females cleared of infection with the anti-malarial drug Malarone™ *versus* those left untreated. Taken together, these studies demonstrate chronic malarial infections can have population-level fitness effects, namely through affecting survival, reproductive success and sexual selection.

1.4 DYNAMICS ON A LANDSCAPE SCALE

1.4.1 The case of Hawaii

The most well known case of avian malaria causing severe declines in naïve wild populations involves the epizootic experienced by native honeycreepers (family: Drepanidinae) on the islands of Hawaii. The pre- and post-epizootic research carried out on Hawaii also provides the most comprehensive linking of avian malaria host, parasite and vector dynamics to date.

Van Riper III et al. (1986) wrote the first extensive monograph on Hawaii's avian malaria epizootic. They correlated honeycreeper declines and subsequent range restrictions with their exposure to an introduced subspecies of *P. relictum* (*P. r. capistranoae*). From historical introduction records, they deduced the parasite likely originated in Asian birds brought to Hawaii for game or husbandry purposes. However, it was not until a sufficient number of these reservoir hosts established in native habitats that the parasite was then able to spill over into endemic populations. In addition, they

demonstrated through experimental infections that most endemic honeyeaters had very little to no innate resistance to malaria. Yet, the mechanistic link that facilitated the epizootic was the introduction of a mosquito (*Culex quinquefasciatus*) capable of readily transmitting the parasite (Hawaii lacked any mosquitoes until human settlement, Warner 1968). The proliferation of *Cx. quinquefasciatus* in low- to mid-elevation habitats likely enabled the rapid dissemination of malaria from exotic to endemic host populations in these areas (Warner 1968). Thus, Hawaii's epizootic was the product of a naïve host population, abundant reservoir hosts, and the proliferation of a competent vector species.

Van Riper III et al. (1986) also found that the populations most severely affected were those with seasonal movements from upper to lower elevations. Sedentary species that remained at low elevations year-round exhibited some resistance upon experimental infection. The authors postulated this discrepancy was due to low elevation species evolving some innate resistance in the face of prolonged exposure to the "malarial zone." Conversely, they reasoned endemic populations that bred at higher elevations lacking mosquitoes experienced less selective pressure to evolve resistance.

Encouragingly, Woodworth et al. (2005) recently demonstrated that one low elevation species, the common amakihi (*Hemiphagus virens*), has evolved malarial resistance genes. Yet mid to high elevation species such as the apapane (*Himatione sanguinea*) continue to experience high intensity infections and mortalities, especially in the younger demographic (Atkinson and Samuel 2010). Further, *Cx. quinquefasciatus* and malaria prevalence are both increasing at upper elevations, and it is believed endemics such as the common amakihi that have evolved resistance are now serving as reservoir hosts for the parasites (Freed et al. 2005).

1.4.2 Effects of landscape variation on disease dynamics

There is evidence that environmental changes, such as increases in climatic temperature and forest fragmentation, are promoting the emergence of parasitic diseases, largely through their effect on vector communities (Holmes 1996). For example in human malaria, small increases in climate have been shown to sharply increase malarial transmission (Patz et al. 2000). In addition, deforestation and subsequent land use changes can alter vector communities and their distributions in a way that promotes increased transmission (Patz et al. 2000). As shown in the case of Hawaii (van Riper III et al. 1986), avian malaria is also largely mediated by vector community abundance, distribution and competency (efficacy of transmitting a particular parasite). Further, Freed et al. (2005) demonstrated warmer summer climates have led to increased vector invasion into upper elevations in Hawaii. Yet the effects of local or large-scale landscape variation and change, particularly forest fragmentation, on avian malaria disease processes are only beginning to be explored (Sehgal 2010).

Some landscape-level patterns revealed by recent research include both localised and regional differences in parasite demography. For example, Wood et al. (2007) showed marked spatial heterogeneity in avian malaria prevalence and lineage distribution in blue tits (*Cyanistes caeruleus*) within a single forest patch in the United Kingdom. In this case, overall malaria prevalence (pooled lineages) decreased moving away from the Thames River. In Brazil, Ribeiro et al. (2005) found higher avian malaria prevalence in larger *versus* smaller forest fragment size when pooled across species. However, when the authors accounted for potential host variation in prevalence by focusing on one species, this relationship was not significant. Bonneaud et al. (2009) found a similar

result in Cameroon when pooling prevalence values across species. However, in a follow-up study to Bonneaud et al. (2009) conducted by Chasar et al. (2009) with paired-site sampling of two widespread African birds, these authors found a much more complex malarial infection pattern between deforested and pristine areas. First, they identified 20 lineages and grouped them into four morphospecies clades. Of these four morphospecies, they found a landscape interaction in two of them (*P. megaglobularis* and *P. lucens*). The prevalence of one morphospecies group (*P. megaglobularis*) was higher in undisturbed *versus* disturbed areas, and *vice versa* for the other group (*P. lucens*).

Sehgal (2010) notes that these spatial differences, especially the lineage-specific ones, may be driven by vector specificity for a particular parasite lineage. Thus, changes in the vector's habitat would ultimately drive changes in host prevalence patterns. Alternatively, environmental stress at different sites may be affecting an individual's susceptibility to infection by a particular lineage. However, more studies examining the effects of forest structure on avian malaria dynamics are needed.

1.5 RESEARCH OBJECTIVES

This research seeks to address three main areas of deficiency in our knowledge of avian malaria in New Zealand: 1) the prevalence and diversity of malarial parasites across a wide range of native and exotic birds; 2) the potential capture technique bias inherent in most studies of avian malaria in the wild; and 3) the relationship between vector ecology and malaria prevalence on a landscape scale. I will address the first area through a malarial survey of two Auckland Region sites, Tiritiri Matangi Island and Waharau Regional Park. For the second issue, I will examine malaria prevalence and

parasitaemia in Tiritiri Matangi bellbirds (*Anthornis melanura*) caught *via* two different capture techniques: mist nets and supplementary feeder traps. Finally for the third area, I will investigate the relationship between the relative abundance of potential native and introduced mosquito vectors, as well as the prevalence of malaria in avian hosts, at forest edge and forest interior sites in Waharau Regional Park. Thus, my specific research objectives are as follows:

1) Survey the presence or absence of avian malaria across a range of exotic and native bird species in the Auckland Region (Chapter 2)
2) Determine prevalence rates for affected bird species (Chapter 2)
3) Identify the types of malarial parasites found in the survey to subgenus, and species level where possible (Chapter 2)
4) Examine whether parasite prevalence and parasitaemia differ in bellbirds caught using two different capture techniques: mist nets and supplementary feeder traps (Chapter 3)
5) Determine the species composition and relative abundance of potential mosquito vectors at two forest edge and two forest interior sites in Waharau Regional Park (Chapter 4)
6) Explore whether mosquito abundance and species composition correlates to malaria prevalence at Waharau forest edge and interior habitat sites (Chapter 4)
7) Discuss research findings and present general conclusions (Chapter 5)

CHAPTER 2.

A survey of avian malaria at two sites in the Auckland Region, North Island, New Zealand: Tiritiri Matangi Island and Waharau Regional Park

Figure 2.1. A field station used for avian malaria sampling at Waharau Regional Park. Pictured clockwise from left: D.J. Gudex-Cross and volunteers Brielle Christine, Emily Gardiner, Erikka Lewis and Sam Mass. Photograph by Emily Gardiner.

ABSTRACT

The full extent of avian malaria (*Plasmodium* spp.) prevalence, host range, and parasite diversity in New Zealand remains unknown. To increase knowledge of avian malaria in the Auckland Region, I conducted a survey at two sites: Tiritiri Matangi Island in the Hauraki Gulf and Waharau Regional Park near the Firth of Thames. Parasite presence and identification were determined from thin blood smears using light microscopy. A total of 21 bird species were sampled in the survey. Of these, five native (bellbird *Anthornis melanura*, tui *Prosthemadera novaeseelandiae*, New Zealand fantail *Rhipidura fuliginosa placabilis*, North Island tomtit *Petroica macrocephala toitoi* and silvereye *Zosterops lateralis*) and three exotic (myna *Acridotheres tristis*, blackbird *Turdus merula merula* and song thrush *T. philomelos*) species were infected. The parasites found in this survey were consistent with the morphospecies *Plasmodium* (*Haemamoeba*) *relictum*, *P.* (*Huffia*) *elongatum*, *P.* (*Novyella*) *rouxi* and two *P.* (*Novyella*) spp. not yet formally taxonomically described, one of which appears specific to an endemic honeyeater, the bellbird. Most affected birds harboured single infections, but double infections of *P. relictum* and *P. rouxi* were found in all three blackbirds sampled at Waharau. Of the two most heavily sampled species, bellbird prevalence was estimated at 41.5% (N = 51/123) for the Tiritiri Matangi population and silvereye prevalence at 9.2% (N = 22/240) for the Waharau population.

2.1 INTRODUCTION

There is limited information regarding the historical distribution, diversity and prevalence of avian malaria parasites (*Plasmodium* spp.) in New Zealand. The first documented evidence of their presence in the country dates back to the early to mid 1900's (Doré 1920, Fantham and Porter 1944). These initial parasites, identified as *Plasmodium* (*Haemamoeba*) *relictum*, were isolated from four exotic passerine and two native penguin species (Laird 1950). Yet a large-scale blood parasite survey conducted by Fallis et al. (1976) failed to detect avian malaria in 43 bird species, though many of their sample sizes were very small (ca. 1-10 birds). These survey results, along with the death of four endemic parrots (kea *Nestor notabilis*) in a Malaysian zoo attributed to avian malaria, led some authors to conclude malarial parasites were largely absent from New Zealand (*e.g.* Bennett et al. 1993 and Valkiunas 2005). Consequently, the detection of avian malaria parasites in a number of native and exotic birds around the turn of the century (McKenna 1998) raised concerns about the potential for an epizootic similar to that of Hawaii (see Section 1.4.1) being reproduced here (Tompkins and Poulin 2006, Massey et al. 2007, Hale 2008).

The findings of recent avian malaria studies in New Zealand initially suggest several parallels to Hawaii. First, generalist parasites (*e.g. P. relictum* and *P. elongatum*) have been found in both exotic and endemic birds throughout much of the country (Tompkins and Gleeson 2006, McKenna 2008, Alley et al. 2010, Howe et al. *under review*, Castro et al. *accepted*, R.K. Barraclough, Massey University, unpub. data). This sharing of parasites, as in Hawaii, provides a transmission pathway that can potentially

lead to severe consequences in threatened native populations lacking malaria resistance genes (van Riper et al. 1986, de Castro and Bolker 2005). Second, two threatened endemic species have recently suffered avian malaria-induced mortality events. In the first instance, five yellowheads (*Mohoua ochrocephala*) brought into Orana Wildlife Park in Canterbury, South Island for breeding died soon thereafter with pathologies consistent with avian malaria infection (Alley et al. 2008). The parasites are believed to have originated in local blackbird (*Turdus merula merula*) populations (Derraik et al. 2008). In addition, two separate South Island saddleback (*Philesturnus carunculatus*) populations on offshore islands have suffered mortalities attributed to *P. elongatum*, though affected birds also suffered from concurrent avipox virus infections (Hale 2008, Alley et al. 2010). Third, the mosquito (*Culex quinquefasciatus*) responsible for facilitating the Hawaiian epizootic was first introduced on the North Island around the mid 1800's (Laird 1995). Since then, it has been repeatedly reintroduced from different parts of the world *via* commercial ships and aircraft (Derraik 2004a), thereby providing a persistent risk of exotic parasite introduction. Further, *Cx. quinquefasciatus* has now become one of the most widespread mosquito species in the country (Weinstein et al. 1997). It appears to already play a major role in avian malaria transmission here, as Tompkins and Gleeson (2006) found a positive correlation between its distribution and that of *P. relictum* in exotic birds. Finally, reports of avian malaria incidence in native bird species have increased substantially over the past ten years (McKenna 2010). However, this increased incidence may simply reflect an increase in sampling effort since detailed historical data is lacking.

While these factors appear to parallel those that led to Hawaii's epizootic, recent malarial research in New Zealand has also revealed important key differences between the two systems. For example, New Zealand has at least one native *Plasmodium* species: Barraclough et al. (*in press*) recently uncovered a highly prevalent parasite of the subgenus *Novyella* in the Auckland Region that is a Meliphagidae specialist, specific to bellbirds (*Anthornis melanura*). Molecular sequencing of this parasite (cyt *b*) has shown that it is distinct from malarial lineages found thus far in other New Zealand native and introduced bird species, and is most similar to sequences isolated by Beadell et al. (2004) from Australian and New Guinea honeyeaters (Barraclough unpub. data). In addition, the *P. elongatum* parasite involved in the South Island saddleback mortalities has been identified *via* both microscopy and molecular sequencing (cyt *b*) in other endemic birds, including North Island robin (*Petroica longipes*), North Island brown kiwi (*Apteryx australis mantelli*), North Island saddleback (*Philesturnus rufusater*), and silvereye (*Zosterops lateralis*); and this parasite may yet prove to be native (Alley et al. 2010, Howe et al. *under review*, Castro et al. *accepted,* Barraclough, unpub. data). Furthermore, *P.* (*Novyella*) *rouxi* has also been found in a New Zealand pigeon (*Hemiphaga novaeseelandiae*) (Barraclough, unpub. data, Howe et al. *under review*). Taken together, the presence of a host specialist parasite in bellbirds and several different morphospecies in other asymptomatic endemic birds demonstrates that avian malaria is native in New Zealand. Finally another crucial way that New Zealand differs from Hawaii is that it has a native mosquito fauna, and the most widespread native mosquito (*Culex pervigilans*) is suggested to be a competent malaria vector (Holder et al. 1999, Massey et al. 2007).

Despite this recent increase in avian malaria research, we still lack fundamental information regarding host-parasite associations, as well as the distribution and impact of these parasites. In sum, the uncertainty surrounding avian malaria in New Zealand indicates the need for more baseline data and further systematic malarial surveys (Tompkins and Poulin 2006). This information is vital in serving as a reference point for future monitoring (Thompson et al. 2010). Subsequent surveillance will then be able to distinguish changes in infection rates and identify any potential novel parasite introductions in New Zealand bird populations (Plowright et al. 2008). This information is particularly vital for threatened populations, especially those on islands, which may have lost disease resistance genes through genetic bottlenecking or prolonged isolation from the mainland (Tompkins and Poulin 2006). Thus to contribute to baseline avian malaria knowledge, the purposes of this study were to: 1) survey the presence or absence of avian malaria across native and exotic host species in the Auckland region; 2) determine prevalence rates for affected host species; and 3) where possible, to identify the parasite species found within the survey *via* light microscopy.

2.2 METHODS

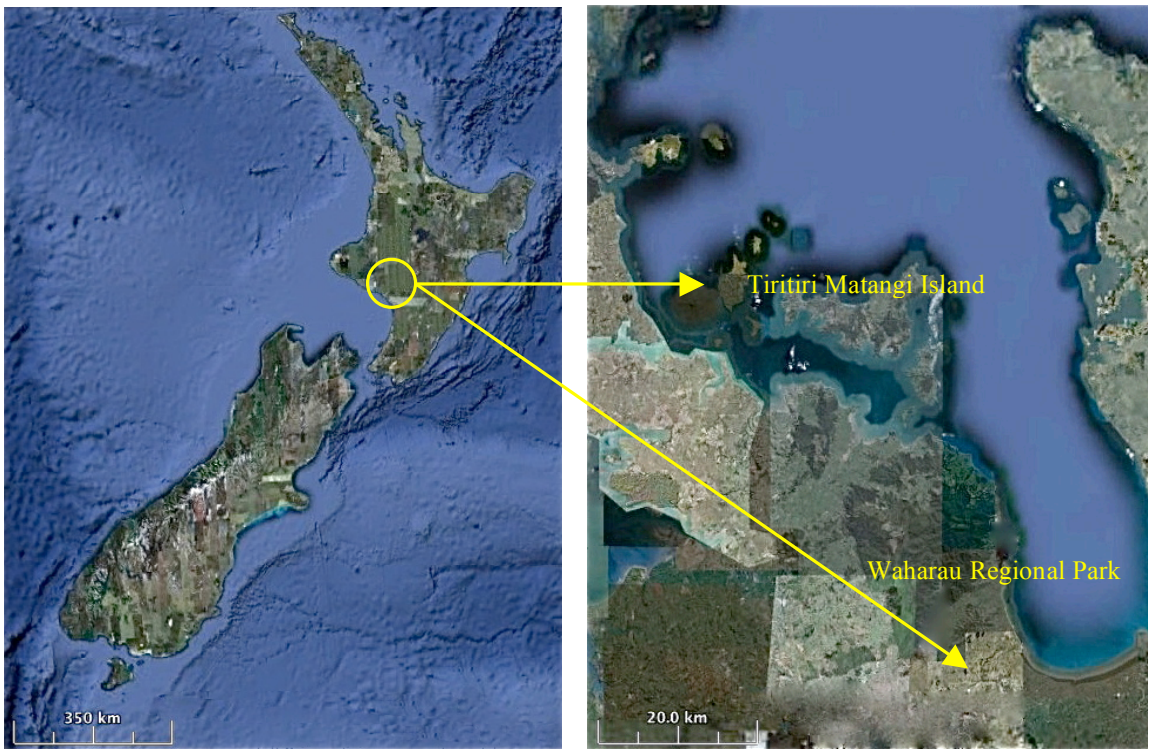
2.2.1 Survey locations

The survey was carried out at two locations on the North Island: Tiritiri Matangi Island (36°36′07″S, 174°17′98″E) in the Hauraki Gulf and Waharau Regional Park (37°02′95″S, 175°17′98″E) near the Firth of Thames on the mainland (Fig. 2.2). Tiritiri Matangi Island is a 220 ha predator-free scientific reserve lying just off the Whangaparoa Peninsula, approximately 25 km north of Auckland City. It was mostly farmland with

small remnant patches of mature pohutakawa (*Metrosideros excelsa*) and puriri (*Vitex lucens*) forest until around thirty years ago, when a major programme was initiated to re-vegetate the island. Now native forest cover is around 60%, and a number of native and exotic birds have naturally re-colonised the island (Graham and Veitch 2002). In addition, Tiritiri Matangi has been host to a number of endemic bird translocations, with many being of species that are still rare to absent on the mainland (Galbraith and Hayson 1995). The more numerous of these translocated species include hihi (*Notiomystis cincta*), kakariki (*Cyanoramphus novaezelandiae*), North Island saddleback and whitehead (*Mohoua albicilla*). Thus given its isolation from the mainland, recent colonisation events, and unique assemblage of potential exotic and native host species, Tiritiri Matangi was a prime location for the purposes of this survey.

Waharau Regional Park extends from the eastern foothills of the Hunua Ranges to the shore of the Firth of Thames. The forest types change along an elevational gradient from the higher foothills to the lower base at the firth, and deep valleys run through the middle of the park. The upper slopes contain mature mixed beech (*Nothofagus* spp.), kauri (*Agathis australis*), and puriri forest. The valley and mid-elevation forests are dominated by tawa (*Beilschmiedia tawa*), rimu (*Dacrydium cupressinum*), totara (*Podocarpus totara*) and regenerating kauri. The lower slopes have a long history of sheep grazing, which still exists in the park to date. Here most of the forest is a mix of early successional kanuka (*Kunzea ericoides*) and manuka (*Leptospermum scoparium*), with scattered patches of second-growth celery pine (*Phyllocladus glaucus*). Many of the native bird populations have been decimated or extirpated due to the presence of mammalian predators, but a few species are particularly numerous throughout the park:

silvereye, grey warbler (*Gerygone ignata*), and New Zealand fantail (*Rhipidura fuliginosa placabilis*). Some uncommon native birds are also found in the valleys and upper slopes abutting the Hunua Ranges where there is mammalian pest control, namely the bellbird and North Island tomtit (*Petroica macrocephala toitoi*). Finally, there are a number of exotic species in the park around the sheep grazing paddocks at the base, but they tend to be infrequent in the forests. This native and exotic bird species assemblage is typical of North Island forests (pers. obs.), yet differs markedly from that of Tiritiri Matangi. Thus these two sites were chosen together to cover as many common and rare native species as possible present in the Auckland region, while also allowing for sampling of common exotics found throughout New Zealand.



2.2 **Figure 2.2.** Map of the two North Island locations in the Auckland Region surveyed for avian malaria. Images modified from ©2010 Google Earth.

The Tiritiri Matangi survey was carried out over six short trips during the 2009 non-breeding season to minimise potential negative effects on the island's breeding bird populations. Sampling occurred over eight days in autumn (March 27th-29th; May 16th-20th), nine days in winter (July 15th-18th; August 5th-9th) and eleven days in spring (November 17th-19th and 23rd-30th). Since the final sampling period occurred at the onset of the breeding season, females were inspected for brood patches during this time (this was done for all birds in species without marked gender differences). If a brood patch was present, the bird was quickly banded in case of recapture and released.

Sampling sites were distributed across the island to cover as wide a range of individuals as possible and to minimise recaptures (Fig. 2.3). In addition, sampling was alternated among sites on either a daily or every other day basis. Most birds were captured from early morning until dusk using mist-nets. Additionally, bellbirds were caught in supplementary sugar-water feeders designed for hihi use on the island. Bellbirds tend to dominate the feeders, often excluding hihi, especially during the non-breeding season (pers. obs.). Thus, ample opportunity exists to target them when sampling at these locations. The feeders have three small openings on two of their opposing sides, of which a trapdoor is latched above. A net is placed so it covers all the holes on one side, and the other sides' trapdoor is propped open with an object (*e.g.* a piece of wood) attached to a string. When a bird enters the feeder, the string is pulled, and the latch door closes. The bird must then escape through the open side, where is becomes caught in the net.

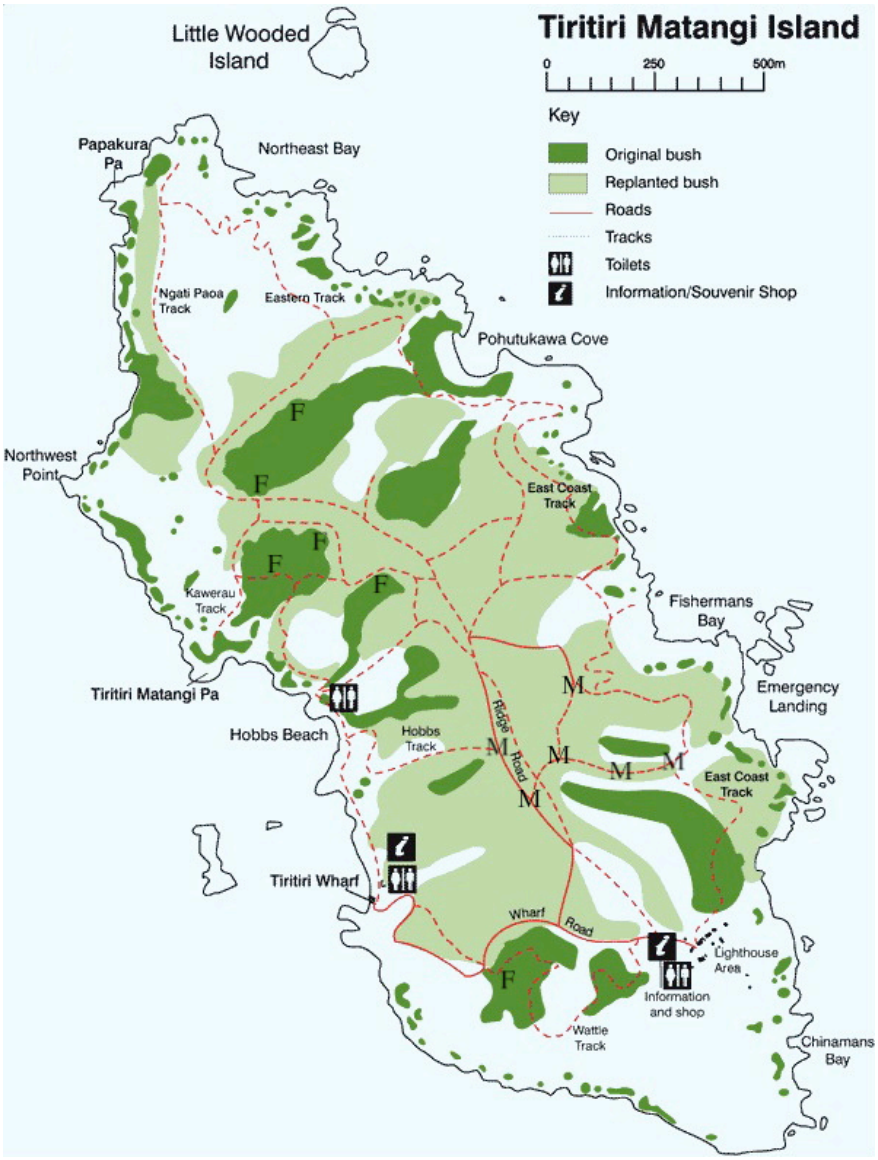


Figure 2.3. Map of the Tiritiri Matangi Island sites surveyed for avian malaria. M = mist-netting sites and F = feeder sites partially used for bellbird sampling.

Waharau Regional Park was surveyed in autumn (Mar. 1st-Apr. 26th) of 2010. Sampling occurred at four sites; two sites at low-mid elevation at the forest edge and two at higher elevation in the forest interior (Fig. 2.4). Each site was sampled for approximately two weeks at a time. All birds were captured from early morning to dusk using mist-nets. Playback recordings of songs and contact calls were used as lures to maximise capture rates.

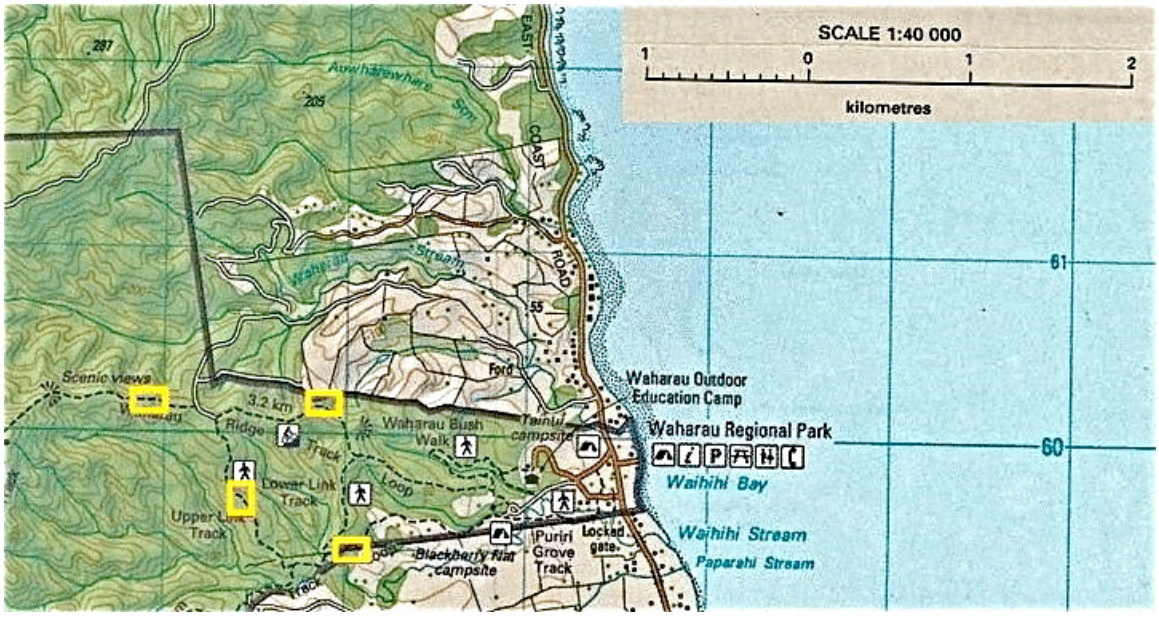


Figure 2.4. Map of the Waharau Regional Park sites (outlined in yellow) surveyed for avian malaria.

Upon capture, each bird was placed in a cloth bag, weighed, and then banded to prevent re-sampling. Morphometric measurements including tarsus, wing, tail and head/beak lengths were taken to the nearest 0.1 mm. A small blood sample (a maximum of 20 uL) was extracted from the brachial vein, and a thin blood smear prepared (see Section 1.2.1, second paragraph).

2.2.3 Data analysis

Thin blood smears were examined using the light microscopy procedure described in Section 1.2.1 (third paragraph). Digital photographs were taken of anything resembling a possible parasite. Questionable parasite photos were then re-examined by an expert microscopist (Barraclough, Massey University) for positive or negative confirmation. This was done to minimise the number of potential false positives, which can be substantial for novice microscopists (Ohrt et al. 2002). The presence of erythrocytic

schizonts, in conjunction with gametocyte structure, was used to confirm infection with avian malaria (following Valkiunas 2005). Parasites were identified according to descriptions given by Garnham (1966) and Valkiunas (2005), as well as with the aid of Barraclough.

Prevalence values were calculated in percent as the total individuals positive over total individuals sampled. Bellbird prevalence was pooled across seasons and capture techniques (*i.e.* mist-net and feeder) on Tiritiri Matangi, as prevalence did not significantly differ in either case (Log-linear model: season $P = 0.77$, technique $P = 0.57$; see Section 3.4.1). Silvereye prevalence was pooled across sampling sites at Waharau, as prevalence did not significantly differ among them (Log-linear model: site $P = 0.80$; see Section 4.4.2). Sample sizes for other species caught in this survey were too small to examine potential differences across seasons for Tiritiri Matangi or sites for Waharau.

2.3 RESULTS

2.3.1 Tiritiri Matangi Island survey

A total of 15 bird species were sampled in the Tiritiri Matangi survey, of which four tested positive for avian malaria (Table 2.1). Positive hosts were comprised of two endemic (bellbird and tui *Prosthemadera novaeseelandiae*) and two exotic species (blackbird and myna *Acridotheres tristis*). Only the bellbird, North Island saddleback and tui were caught in numbers greater than ten individuals (N = 123, 20 and 12, respectively). None of the North Island saddlebacks were detectably positive *via* microscopy. Across all species, 56 of 191 (29.3%) individuals were infected, the vast majority of which were bellbirds ($N = 51$).

All positive bellbirds had single infections of the meliphagid-specific *P.* (*Novyella*) sp. not yet formally taxonomically described. This parasite was highly prevalent (41.5%) in the 123 birds sampled, of which 119 were adult males. The other four birds were adult females and two were parasitised. Three of 12 tuis (25%) sampled were also infected with a *P.* (*Novyella*) sp. The *P.* (*Novyella*) sp. in these tuis had some similarity to the bellbird parasite, but also unique characteristics. The blackbird and myna were positive for a parasite consistent with the morphospecies *P.* (*Novyella*) *rouxi*. This parasite was found in one of two mynas (sex unknown), and the single adult male blackbird sampled.

Table 2.1. Results of avian malaria surveys conducted in the Auckland Region: positive individuals per total sampled (+ve/*n*) and prevalence (prev %).

	Tiritiri		**Waharau**		**Total**	
	+ve/*n*	prev (%)	+ve/*n*	prev (%)	+ve/*n*	prev (%)
Galliformes						
Phasianidae	0/4	0.0	-	-	0/4	0.0

Coturnix ypsilophora (Brown Quail)						
Charadriiformes						
Laridae						
Chroicocephalus scopulinus (Red-billed Gull)*	0/2	0.0	-	-	0/2	0.0
Psittaciformes						
Psittacidae						
Cyanoramphus novaezelandiae (Kakariki)*	0/5	0.0	-	-	0/5	0.0
Coraciiformes						
Halcyonidae						
Todiramphus sanctus vagans (New Zealand Kingfisher)**	0/4	0.0	-	-	0/4	0.0
Passeriformes						
Meliphagidae						
Anthornis melanura (Bellbird)*	51/123	41.5	0/9	0.0	51/132	38.6
Prosthemadera novaeseelandiae (Tui)*	3/12	25.0	0/3	0.0	3/15	20.0
Acanthizidae						
Gerygone igata (Grey Warbler)*	-	-	0/35	0.0	0/35	0.0
Callaeaidae						
Philesturnus rufusater (North Island Saddleback)*	0/20	0.0	-	-	0/20	0.0
Rhipiduridae						
Rhipidura fuliginosa placabilis (New Zealand Fantail)*	-	-	1/19	5.3	1/19	5.3
Petroicidae						
Petroica longipes (North Island Robin)*	0/2	0.0	-	-	0/2	0.0
P. macrocephala toitoi (North Island Tomtit)*	-	-	1/8	12.5	1/8	12.5
Hirundinidae						
Hirundo neoxena (Welcome Swallow)**	0/1	0.0	-	-	0/1	0.0
Zosteropidae						
Zosterops lateralis (Silvereye)**	-	-	22/240	9.2	22/240	9.2
Sturnidae						
Acridotheres tristis (Common Myna)	1/2	50.0	-	-	1/2	50.0
Turdidae						
Turdus merula (Blackbird)	1/1	100.0	3/3	100.0	4/4	100.0
T. philomelos (Song Thrush)	0/3	0.0	1/1	100.0	1/4	25.0
Passeridae						
Passer domesticus (House Sparrow)	0/2	0.0	-	-	0/2	0.0
Prunellidae						
Prunella modularis (Dunnock)	-	-	0/1	0.0	0/1	0.0
Fringillidae						
Carduelis chloris (Greenfinch)	-	-	0/1	0.0	0/1	0.0
Fringilla coelebs (Chaffinch)	0/3	0.0	0/5	0.0	0/8	0.0
Subfamily: Mohouinae						
Mohoua albicilla (Whitehead)*	0/7	0.0	-	-	0/7	0.0
Total	56/191	29.3	28/325	8.6	84/516	16.3

* = Endemic species; ** = Native species

2.3.2 Waharau Regional Park survey

A total of 11 bird species were sampled in the Waharau survey, of which five tested positive for avian malaria (Table 2.1). Positive hosts were comprised of one native

(silvereye), two endemic (New Zealand fantail and North Island tomtit) and two exotic species (blackbird and song thrush *Turdus philomelos*). Of species with sample sizes greater than ten individuals, the silvereye was most numerous, followed by grey warbler and fantail (n = 240, 35 and 19, respectively). None of the grey warblers were found to be positive. In addition, no parasites were found in any of the nine bellbirds caught at Waharau. Across all species, 28 of 325 (8.6%) individuals were infected, the majority of which were silvereyes (n = 22).

A total of 22 of 240 (9.2%) silvereyes had single infections of avian malaria. Twenty of those (8.3%) were infected with a parasite consistent with the morphospecies *P.* (*Huffia*) *elongatum*. The other two (0.8%) were infected with *P.* (*Haemamoeba*) *relictum*. All positive silvereyes were adult birds, but sex cannot be visually ascertained in this species. One fantail (sex unknown) of 19 sampled (5.3%), one tomtit (adult male) of eight sampled (12.5%), and the single song thrush (sex unknown) also had infections of *P. elongatum*. All three blackbirds, which were female, harboured double-infections of *P. relictum* and *P. rouxi*. In addition, this survey revealed a new lankesterellid in four of eight tomtits sampled.

2.3.3 Overall Auckland Region survey

Between Tiritiri Matangi and Waharau, a combined total of 21 species were sampled in the Auckland Region. Of these, eight tested positive for avian malaria (Table 2.1). Across species, parasites were found in 84 of 516 (16.3%) individuals. Aside from bellbirds, most positive species harboured single infections consistent with one of the following morphospecies: *P.* (*Haemamoeba*) *relictum*, *P.* (*Huffia*) *elongatum* or *P.*

(*Novyella*) *rouxi* (Table 2.2). The *P.* (*Novyella*) sp. believed to be specific to the bellbird was highly prevalent at Tiritiri Matangi, but absent in nine birds sampled at Waharau. The tui proved to carry what appeared to be a new variety of *P. (Novyella)* sp.: characterised by the presence of bow-tie schizonts and large merozoites. Further malarial survey work on the tui, and sequencing of these parasites, is needed to determine their relationship with the bellbird parasite and other New Zealand *P.* (*Novyella*) varieties. *P. elongatum* and *P. relictum* were only found in the Waharau survey, and at low prevalence outside the song thrush (though only four of these were sampled) (Table 2.2). All blackbirds sampled were infected with *P. rouxi*, and hence this parasite was present at both sites. The three Waharau blackbirds were also co-infected with *P. relictum* (Table 2.2).

Table 2.2. Avian malaria species found per host in the Auckland Region survey: positive individuals per total sampled (+ve/*n*) and prevalence (prev %). T = Tiritiri Matangi Island and W = Waharau Regional Park.

Parasite Species	Host Species	Site	+ve/n	Prev (%)
P. (*Huffia*) *elongatum*	New Zealand fantail	W	1/19	5.3
	Silvereye	W	20/240	8.3
	Song thrush	W	1/4	25.0
	North Island tomtit	W	1/8	12.5

Total	4	W	23/271	8.5
P. (*Haemamoeba*) *relictum*	Blackbird	W	3/4	75.0
	Silvereye	W	2/240	0.8
Total	2	W	5/244	2.1
P. (*Novyella*) *rouxi*	Blackbird	T/W	4/4	100.0
	Myna	T	1/2	50.0
Total	2	T/W	5/6	83.3
P. relictum/P. rouxi (Double)	Blackbird	W	3/4	75.0
P. (*Novyella*) sp.	Bellbird	T	51/132	38.6
P. (*Novyella*) sp.	Tui	T	3/15	20.0

2.3.4 Parasite descriptions

The *P. elongatum* (Fig. 2.5)*, P. relictum* (Fig. 2.6) and *P. rouxi* (Fig. 2.7) parasites were consistent with morphospecies descriptions given by Garnham (1966) and Valkiunas (2005). The key diagnostic features outlined by these two authors are provided in the context of the parasites observed in this survey. The *P.* (*Novyella*) spp. of the bellbird (Fig. 2.8) and tui (Figure 2.9) were identified and described with the aid of Barraclough (Massey University).

2.3.4.1 *P.* (*Huffia*) *elongatum*

Asexual stages (erythrocytic schizonts) were found chiefly in young or polychromatic erythrocytes (Fig. 2.5 a, j, k). These schizonts had elongate merozoites, and nuclei of mature schizonts were arranged in fan-like form (Fig. 2.5 j-l). Gametocytes were generally found in mature erythrocytes (Fig. 2.5 b-i, m-o) and predominated in

blood smears, whereas asexual stages were rarely observed (only four schizonts from three birds were observed out of 23 total infected birds). Gametocytes did not or only slightly displaced the nucleus of the host cell (Fig. 2.5 b-i, m-o) and were generally emaciated in appearance with an irregular or wavy internal margin (Fig. 2.5 b-i, m-o). Microgametocytes (Fig. 2.5 f, g, n, o) were seen to often have a drawn out, thread-like nucleus. No trophozoites were observed.

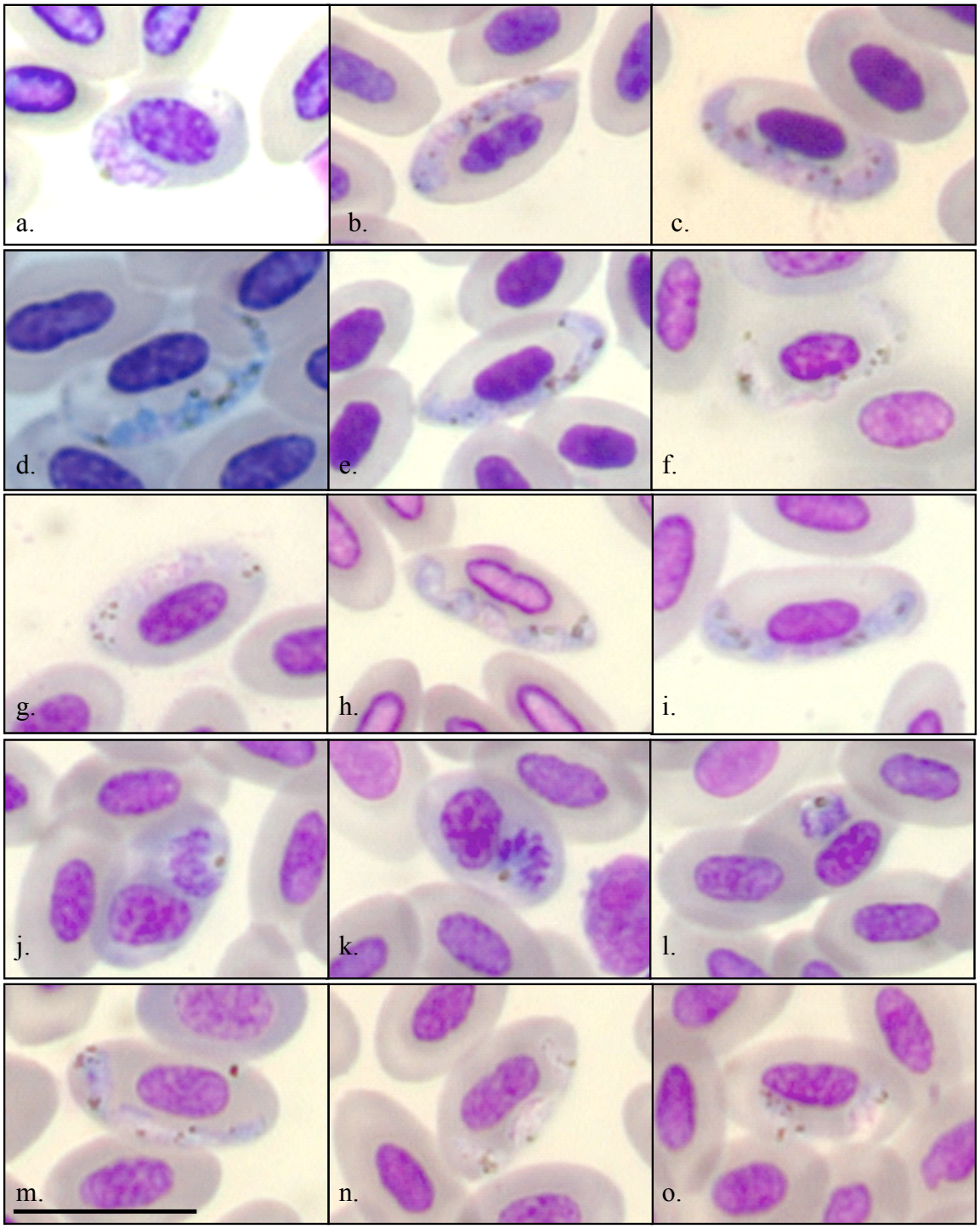


Figure 2.5. *Plasmodium elongatum* from silvereye (a-g), New Zealand fantail (h, i) and song thrush (j-o). Silvereye: a. developing erythrocytic schizonts; b-e. macrogametocytes; f-g. microgametocytes. Fantail: h,i. macrogametocytes. Song thrush: j-l. erythrocytic schizonts; m. macrogametocyte; n,o. microgametocytes. Scale bar c.10µm.

2.3.4.2 *P.* (*Haemamoeba*) *relictum*

Trophozoites were roundish or amoeboid (Fig. 2.6 j, k) in shape, displaced the host cell nucleus, and often contained pigment granule close to the edge of the parasite. Clear vacuoles were also found in some of the trophozoites (Fig. 2.6 k). Schizonts greatly displaced or entirely expelled the host cell nucleus (Fig. 2.6 a-h, l), and mature (or near mature) schizonts and gametocytes often occupied more than half, but not all, of the host cell's cytoplasm (Fig. 2.6 a-i, l-o). Schizonts contained pigment granules clumped into one or two foci (Fig. 2.6 a-h, l), and contained between 10-16 merozoites (mean = 12, *N* = 11), although most of the schizonts observed were not yet fully differentiated. Mature gametocytes were round or oval in shape and greatly displaced or entirely expelled the host nucleus (Fig. 2.6 i, m-o), and their length did not exceed 10μm. Pigment granules of the gametocytes were round or oval in shape and usually scattered in the parasite's cytoplasm (Fig. 2.6 m-o).

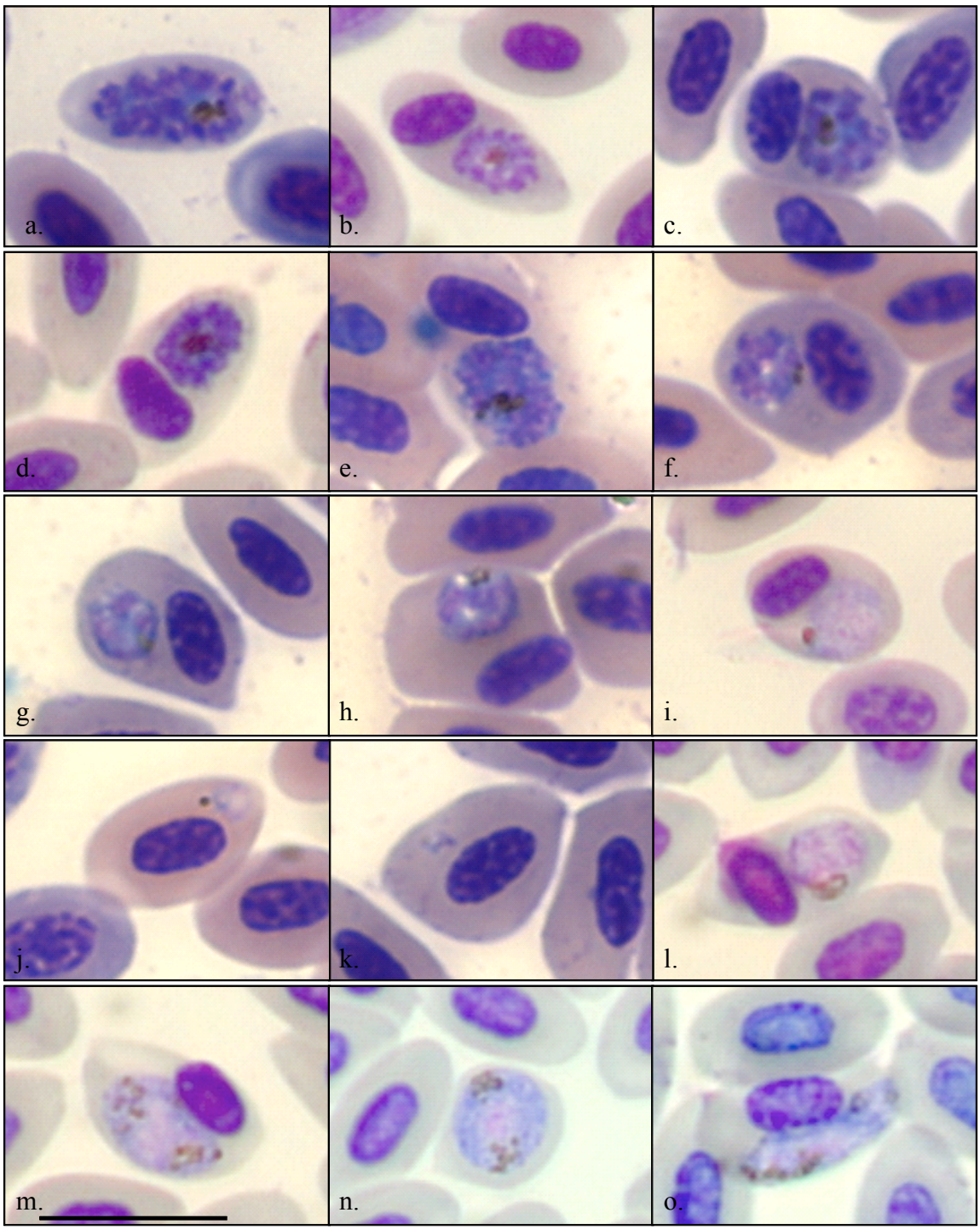


Figure 2.6. *Plasmodium relictum* from blackbird (a-k) and silvereye (l-o). Blackbird: a-h. erythrocytic schizonts; i. macrogametocyte; j. developing parasite; k. trophozoite. Silvereye: l. erythrocytic schizont; m-o. macrogametocytes. Scale bar c.10µm.

2.3.4.3 *P.* (*Novyella*) *rouxi*

Trophozoites had an amoeboid shape with one pigment granule and some contained a "wisp" of cytoplasm (Fig. 2.7 i, o). Mature schizonts were often quadrangular or in a "bow-tie" configuration with a double wing emanating from a central pigment granule (Fig. 2.7 a, c, d, j). Schizonts were located in a polar or subpolar position in the host cell, and contained four merozoites (Fig. 2.7 a-d, j-n). A single clear, round vacuole was present in most trophozoites and schizonts (Fig. 2.7 a-d, i-o). Gametocytes were oblong or irregularly shaped and elongated with part of their outer margin abutting the outer edge of the host cell (Fig. 2.7 e-h). Mature gametocytes did not, or only slightly, wrap around the host nucleus (Fig. 2.7 f-h). Macrogametocytes stained pale blue and usually did not fill the host cell to the poles (Fig. 2.7 e-g). The gametocyte characteristics, bow-tie configuration, and relatively small size distinguished these parasites from *P. vaughani*, which is a more robust parasite whose mature gametocytes wrap around the nucleus (Garnham 1966).

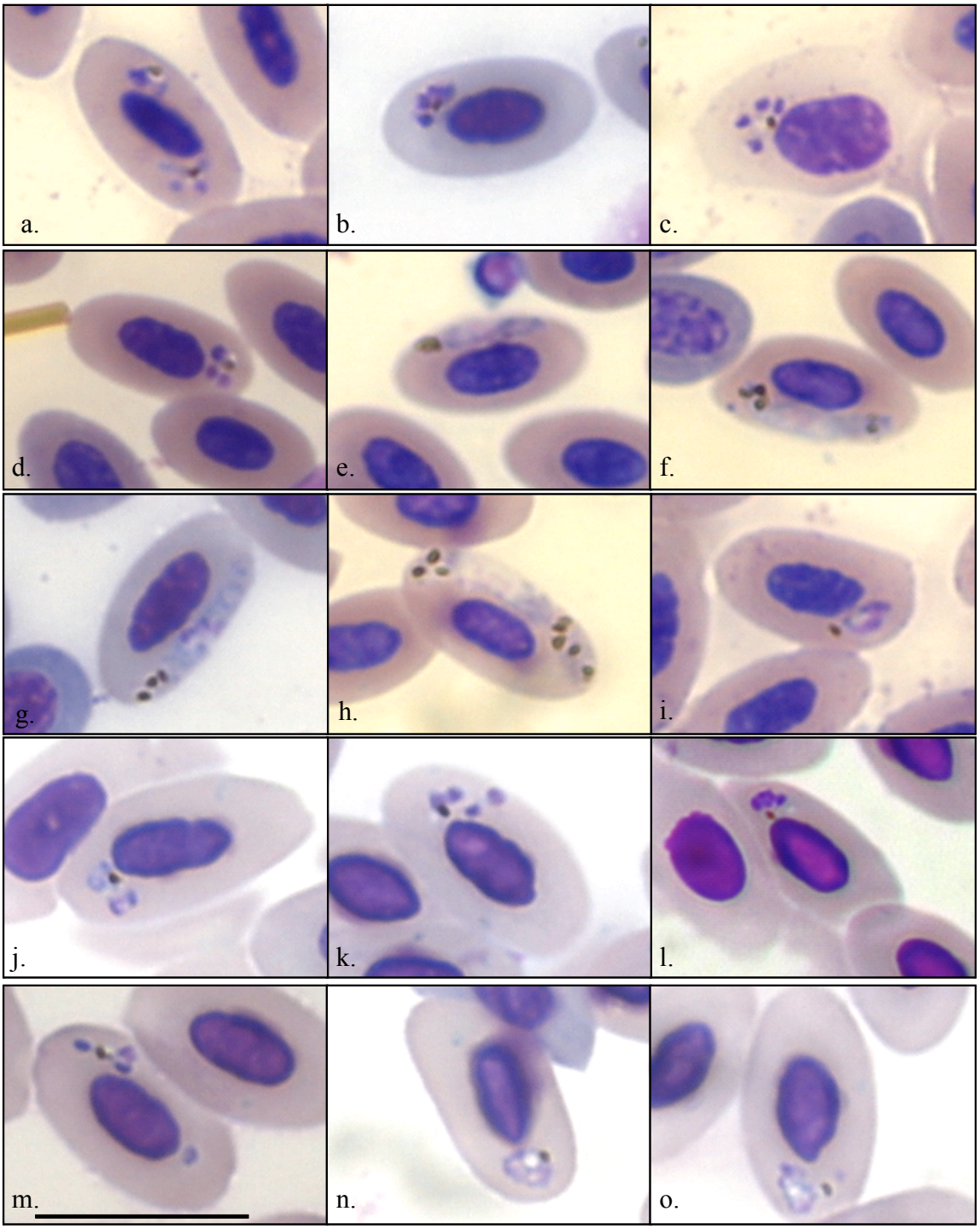


Figure 2.7. *Plasmodium rouxi* from blackbird (a-i) and myna (j-o). Blackbird: a-d. erythrocytic schizonts; e-g. macrogametocytes; h. microgametocyte; i. trophozoite. Myna: j-o. erythrocytic schizonts. Scale bar c.10µm.

2.3.4.4 Bellbird *Plasmodium* (*Novyella*) sp.

The parasite stages found in the Tiritiri Matangi bellbirds were consistent with those of a *Plasmodium (Novyella*) sp. previously found in bellbirds on this island and a nearby mainland site, Tawharanui Regional Park (Barraclough et al. *in press*). As outlined in the introduction, this parasite has been characterised through sequencing of mitochondrial cyt *b* (Barraclough unpub. data). Of those sequences available on GenBank, it was found to be most similar to *Plasmodium* sequences isolated from Australian and New Guinea honeyeaters (Beadell et al. 2004). Erythrocytic schizonts sometimes slightly touch the host cell nucleus but usually do not (Fig. 2.8 f-g). The number of merozoites varies, including within a single infected bellbird, but usually range from four to eight (Fig. 2.8 f-g). Schizonts may be positioned in any part of the infected cell, and sometimes displace the host cell nucleus laterally, but this displacement is generally not extreme (Fig. 2.8 f-h). A single clump of pigment cells is seen in schizonts (Fig. 2.8 f-j). Cytoplasm in developing schizonts is most obvious in those with numerous merozoites (Fig. 2.8 i). Gametocytes are elongated, typically do not touch the host cell nucleus or lie against the outer margin of the infected cell, and often slightly displace the host cell nucleus (Fig. 2.8 a-d). Small vacuoles may be seen in mature gametocytes (Fig. 2.8 b, c). Pigment granules in both macrogametocytes (Fig. 2.8 a-c) and microgametocytes (Fig. 2.8 d) may be clumped together or separated. These often number three or four, but can range up to six. Trophozoites are often seen in a subpolar position in infected cells (Fig. 2.8 e). Double erythrocytic infections were also seen (Fig. 2.8 j-l), and these can be associated with greater host cell nucleus displacement (Fig. 2.8 j).

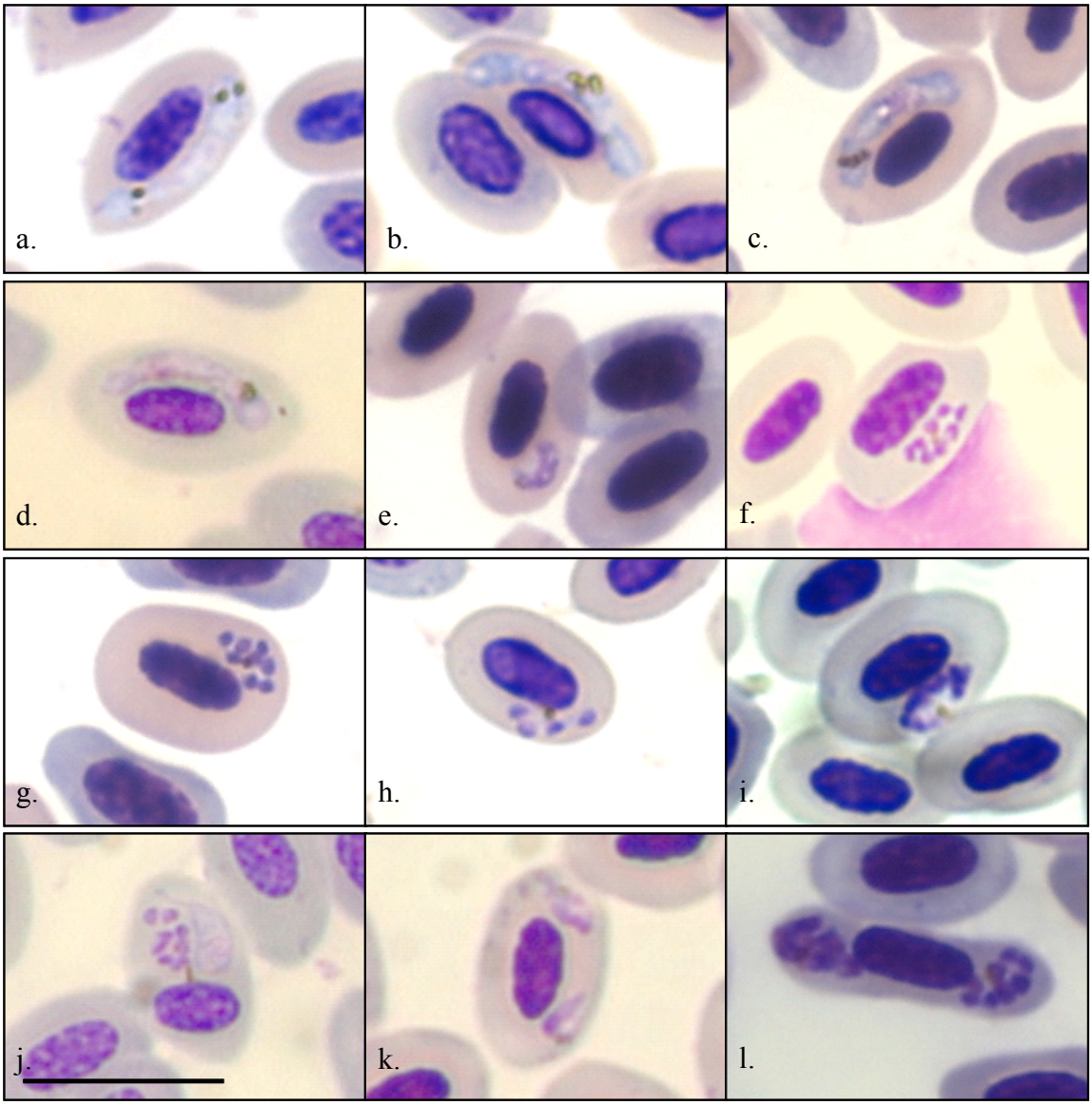


Figure 2.8. *Plasmodium* (*Novyella*) sp. from Tiritiri Matangi bellbirds: a-c. macrogametocytes; d. microgametocyte; e. trophozoite; f-l. erythrocytic schizonts. Scale bar c.10μm.

2.3.4.5 Tui *Plasmodium* (*Novyella*) sp.

This parasite has yet to be characterised by molecular sequencing, which is recommended to aid in determining its relationship with the *Plasmodium* sp. found in the bellbird. Also, further thin blood smear collections are recommended to provide more examples of its erythrocytic stages. Within the scope of the available examples, this parasite bore some resemblances to the bellbird parasite but differed primarily in the appearance of its schizonts.

There were a variable number of merozoites in the erythrocytic schizonts (Fig. 2.9 a-e). The majority of schizonts seen in this study contained four merozoites, however these numbered over eight in some cases (Fig. 2.9 a-e). A distinct stalk-like attachment connected to merozoites was usually seen within erythrocytic schizonts (Fig. 2.9 a-d). The three schizont examples carrying four merozoites were located at a subpolar or polar position and did not displace the host nucleus. Larger schizonts extended down the lateral side of the host cell and displaced the host nucleus laterally (Fig. 2.9 d, e). A double pigment granule was generally seen in erythrocytic schizonts (Fig. 2.9 a-e). One example of a trophozoite was located in a subpolar position (Fig. 2.9 f). Gametocytes were elongated and sometimes featured a thread-like nucleus (Fig. 2.9 h,i). They also slightly displaced the host cell nucleus (Fig. 2.9 g-i). Pigment granules in gametocytes usually numbered two or three and were found together towards one end of the gametocyte (Fig. 2.9 g-i). However, these granules were sometimes separated, and two gametocytes were seen carrying five and six granules respectively, each with a scattered distribution. The size of the merozoites in these erythrocytic schizonts are larger those of the bellbird and are notable for their stalk-like appendages (Fig. 2.9 a-e).

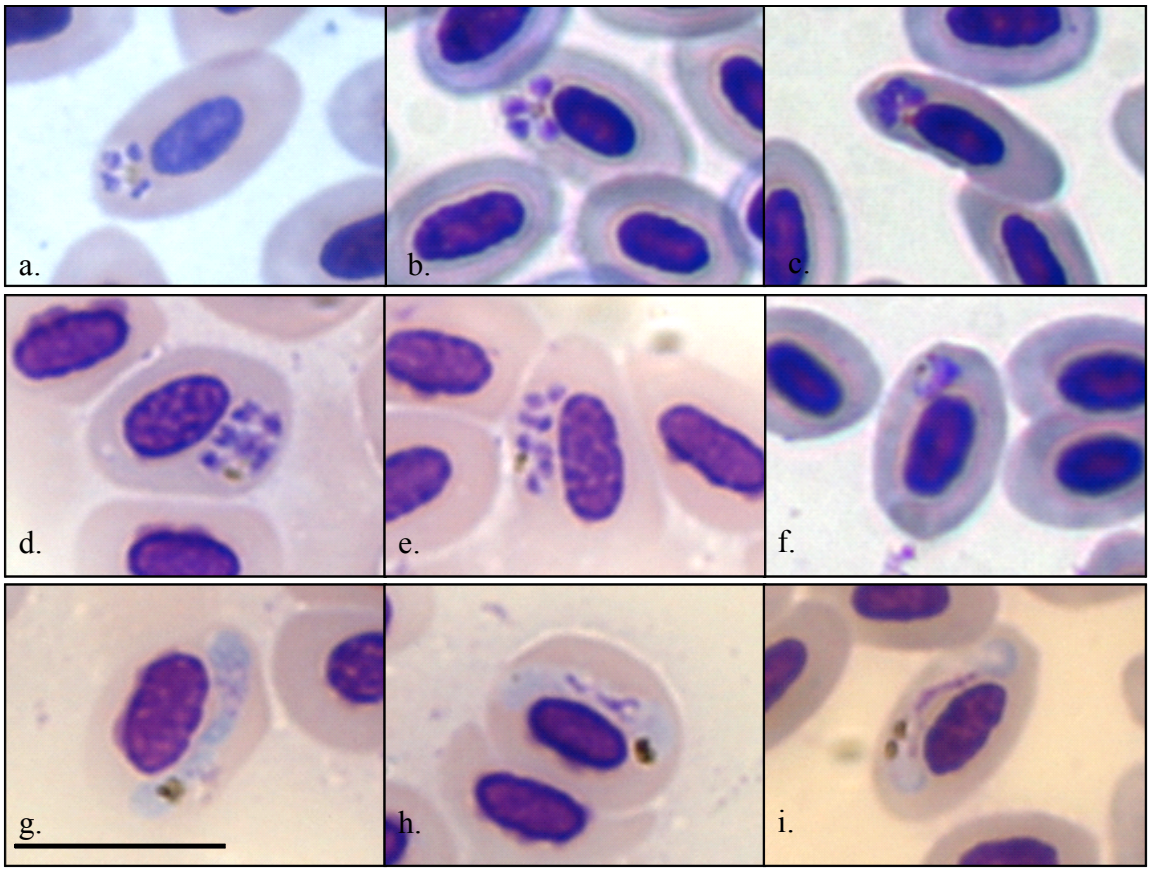


Figure 2.9. *Plasmodium* (*Novyella*) sp. from Tiritiri Matangi tui: a-e. erythrocytic schizonts; f. trophozoite; g-i. gametocytes. Scale bar c.10µm.

2.4 DISCUSSION

2.4.1 Infected host species

This survey revealed avian malaria presence in both native and exotic birds in the Auckland Region. Of the exotic host species, every blackbird sampled was infected with at least one species of avian malaria, and three of the four birds sampled harboured doubled infections (*P. relictum* and *P. rouxi*). Infected myna and song thrush each harboured single infections, the former at Tiritiri Matangi and latter at Waharau. Blackbirds and song thrushes are well-documented carriers of avian malaria in New

Zealand (Tompkins and Gleeson 2006, Howe et al. *in review*) and have been implicated as parasite reservoirs in malaria outbreaks involving endemic species (Derraik et al. 2008, Hale 2008). Currently, the myna is less widely acknowledged as a potential reservoir species in New Zealand. Yet Ishtiaq et al. (2006) found a *P. relictum* lineage closely related to the one involved in Hawaii's epizootic in New Zealand myna populations, along with six other unique lineages of avian malaria. These results, along with the finding of an infected myna in this survey, support this possibility.

Of the native birds, four of the five infected species (bellbird, tui, New Zealand fantail and North Island tomtit) in this survey are endemic to New Zealand, and the other (silvereye) a self-introduced native from Australia (Kikkawa 1961). Each of these except the tui is listed as a confirmed host of avian malaria on the most recent checklist of New Zealand blood parasites (McKenna 2010). Despite the fact that avian malaria has been found at least once in tui prior to this survey (Barraclough, pers. comm.), it has not been reported in the literature thus far. The parasites found per host at each Auckland Region site are discussed in detail below.

2.4.2 Host-parasite associations

On Tiritiri Matangi, *P. rouxi* was found in a single individual of the blackbird and myna (Table 2.2). This is in accordance with its previous detection on Tiritiri Matangi in a blackbird (Howe et al. *under review*). It was not found in any native species sampled at Waharau, but was present in all three blackbirds sampled at this site as a co-infection with *P. relictum*. The single other recorded report for *P. rouxi* in New Zealand was from a New Zealand pigeon sampled at an Auckland bird rescue centre, which tested positive

for this parasite via PCR and cyt *b* sequencing (Howe et al. *under review,* Barraclough unpub. data). Nonetheless, this is likely the first incidence of it in myna populations in New Zealand, and the first time it has been recorded in a wild mainland population. *P. rouxi* is a host generalist with a cosmopolitan distribution known to cause mortality in experimentally infected canaries (*Serinus canaria*) (Valkiunas 2005). Thus its presence in the Auckland Region, particularly on Tiritiri Matangi, which holds many rare and threatened bird populations, is of note and warrants further investigation. Aside from *P. rouxi*, the *P.* (*Novyella*) sp. found in the bellbird was the only other parasite found on Tiritiri Matangi in this survey, although the first known finding of a tui infected with the parasite depicted in Figure 2.9, was also from this island (Barraclough, unpub. data).

The *P.* (*Novyella*) sp. of the bellbird has previously been documented as highly prevalent in the Auckland region (Barraclough et al. *in press*, S. Baillie, Massey University, unpub. data). The prevalence estimated in this survey for the Tiritiri Matangi population (41.5%) corroborates these findings. This value is very high when compared to prevalence estimates for meliphagids of Australia (10%, Beadell et al. 2004). However, Jarvi et al. (2003) found high prevalence (73%) in an American Samoa meliphagid, the wattled honeyeater (*Foulehaio carunculata*). They postulated that the combination of native vectors, stable host populations and chronic infection rates likely meant the malarial strains were native in these birds. This also seems likely to be the case for the bellbird parasite found in the Auckland region, as it appears to be a highly prevalent host specialist with low pathogenicity (Barraclough et al. *in press*). In addition, unpublished surveys and anecdotal reports demonstrate bellbird populations are stable and expanding in the Auckland region (K. Parker, Massey University, pers. comm.).

Finally, it is of note that the bellbird parasite was not found in any of the nine individuals sampled at Waharau. Unfortunately the small sample size limits conclusions, but the absence of this parasite from these birds raises the possibility that it exists at a lower prevalence in this area of the Auckland region. Thus, further research into its mainland distribution is recommended.

P. elongatum was the most prevalent parasite at Waharau, being found in the New Zealand fantail, silvereye, North Island tomtit and song thrush. This survey provides a baseline estimate of *P. elongatum* prevalence in the Waharau silvereye population at 8.3%. However, only single individuals of the other three species were infected, with relatively low prevalence in the New Zealand fantail (5.3%) and North Island tomtit (12.5%). The only song thrush sampled at this site was infected. *P. elongatum* has previously been found in South Island saddleback (Hale 2008, Alley et al. 2010), North Island saddleback (Castro et al. *accepted*), North Island robin (Barraclough unpub. data) and North Island brown kiwi (Howe et al. *under review*). In addition, it has previously been identified *via* both light microscopy and cyt *b* sequence in an Auckland silvereye (Howe et al. under review, Barraclough unpub. data). In contrast, the incidence of *P. elongatum* in the New Zealand fantail and North Island tomtit in this survey is the first on record for both of these endemic host species, as well as for the exotic song thrush in New Zealand. Thus, further research into the distribution and prevalence of this parasite in endemic host species is recommended.

The wide host range of *P. elongatum* found in this study is consistent with its life history as a host generalist with one of the broadest geographical and host distributions in the world (Bennett et al. 1993a). Wild passerines are the most commonly infected

species, and infection is typically non-lethal in these birds (Valkiunas 2005). Consistent with this trend, this study found low prevalence of *P. elongatum* in the Waharau silvereye population. Relatively low prevalence and parasitaemia have also been documented in other native species, such as the North Island saddleback (Castro et al. *accepted*). However since this parasite has been associated with the death of South Island saddleback (Alley et al. 2010), further pathological studies of infected passerines would be useful to determine the degree to which this parasite impacts native hosts. Furthermore, both introduced and native passerines may also serve as reservoir hosts for more susceptible species such as penguins (Valkiunas 2005). Considering New Zealand has six species of penguin, further research on the transmission of this parasite is warranted where the ranges of these species overlap.

P. relictum is a host generalist known to be present in New Zealand *via* microscopy (Doré 1920, Fantham and Porter 1944, Laird 1950). In addition, *Plasmodium* lineages closely related to *P. relictum* sequences in GenBank have been isolated from North Island saddleback and a dead hihi (Howe et al. *under review*). Yet, this parasite was rarely recorded in this survey and only at Waharau. All three blackbirds sampled harboured this parasite (with a co-infection of *P. rouxi*), consistent with this species being a well-known carrier of *P. relictum* in New Zealand (Tompkins and Gleeson 2006). However, this is the first recorded occurrence of this parasite in a New Zealand silvereye. In addition, the mosquito survey conducted at Waharau (see Chapter 4) revealed the presence of a known *P. relictum* vector, *Culex quinquefasciatus,* at low relative abundances. The study by Tompkins and Gleeson (2006) found a positive correlation between the distribution of this parasite and vector, which is corroborated in this study by

the fact that *P. relictum*-infected blackbirds were sampled at Waharau sites containing *C. quinquefasciatus*. The low prevalence of *P. relictum* outside of blackbirds at Waharau may also be a reflection of its potentially high pathogenicity in New Zealand. Many mortality events and severe outbreaks described from wild populations in other parts of the world have involved a strain of *P. relictum* (van Riper et al. 1986, Valkiunas 2005). Thus, the possibility exists that *P. relictum*-infected birds were less likely to be captured due to more severe pathologies. However, the susceptibility of native New Zealand birds to *P. relictum* is currently unknown and requires further research.

2.4.3 Prevalence interpretation

This study presents a cross-section of avian malaria prevalence at two sites in the Auckland Region. However, it should be noted that the use of mist-nets at both sites, and playback recordings at Waharau, potentially influenced the results towards healthier individuals (Valkiunas 2005). An infected bird has to be healthy enough to fly into the net, which may be an issue for infected birds since anaemia and lethargy are characteristic symptoms of heavy infections (Atkinson and van Riper 1991). However, since mist netting is the standard capture technique for these species in New Zealand, the relative prevalence values reported here should be comparable to future studies.

The use of recorded song as a lure may also have selected for birds healthy enough to defend a territory or investigate a novel song. However this seems unlikely in the case of silvereyes, the only species for which population-level prevalence was estimated using recorded song in this study, as this species is highly social and travels together in small flocks (Kikkawa 1961). As observed in the field, one or two individuals

in a flock responded to the playback recording, and then once trapped in the net would emit an alarm call that resulted in most of the flock being captured.

Another important consideration in interpreting prevalence estimates is that the proportion of individuals sampled to that of the total population is unknown (Jennelle et al. 2007). Thereby if a particularly infected or uninfected portion of the total population has been sampled, this would skew the prevalence estimate either positively or negatively. Thus, future avian malaria prevalence studies in New Zealand should consider the use of mark-recapture to assess detection probabilities of uninfected *versus* infected hosts (Jennelle et al. 2007). Similarly, microscopy generally underestimates true prevalence (Valkiunas et al. 2008b). This is because birds suffering from a chronic infection can have parasitaemia (proportion of infected cells to total cells examined) as low as one parasite per one million cells (Zehtindjiev et al. 2008). Hence, the prevalence values found in this survey should be interpreted as low-end estimates.

2.5 CONCLUSION

This study furthered our knowledge on the host distribution, prevalence and diversity of avian malaria parasites in the Auckland Region. In addition, new host-parasite associations were reported for both this region and New Zealand. Baseline prevalence rates, particularly for the silvereye, have been provided as a means for future comparison and surveillance.

The high prevalence of a native avian malaria species in Tiritiri Matangi bellbirds, as well as other parasites in additional hosts, warrants a vector survey on the island. No such survey has been conducted to date, and the widespread native *Cx. pervigilans* is a

likely candidate for malaria transmission at this site. Identifying the vectors present on Tiritiri Matangi would contribute greatly to our understanding of native malaria transmission dynamics. It would also help inform vector management options if a malaria outbreak were to occur in any of the rare or threatened bird populations on the island.

Chapter 3.

Does avian malaria prevalence and parasitaemia in the bellbird (*Anthornis melanura*) differ between two capture techniques: mist nets and supplementary feeders?

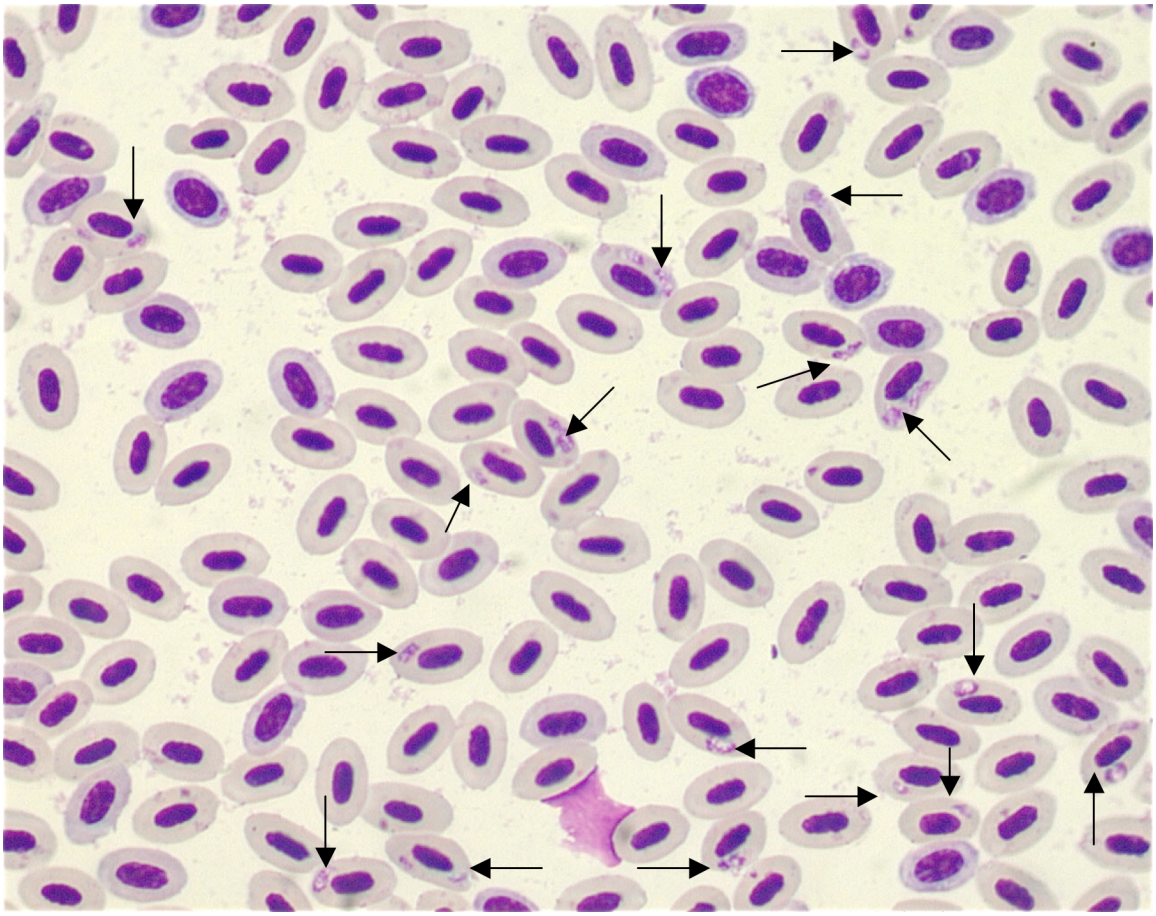


Figure 3.1. An example of high parasitaemia in a bellbird infected with avian malaria. Each arrow points to an infected cell**.** Photograph by D.J. Gudex-Cross.

ABSTRACT

In studies of avian malaria (*Plasmodium* spp.), it has been suggested that the capture technique of mist netting may select for healthier birds, leading to an underestimate of true prevalence and parasitaemia. Mist netting relies on birds being active, yet listlessness induced by anaemia is a common outcome of avian malaria infection. However, few studies have empirically tested for differences between prevalence and/or parasitaemia yielded from mist netting and an alternative technique. On Tiritiri Matangi Island in New Zealand, the high prevalence of a host specific *Plasmodium* (*Novyella*) sp. in adult male bellbirds (*Anthornis melanura*), and their use of supplementary feeders, presented a unique opportunity to compare prevalence and parasitaemia between mist-net- and feeder-caught individuals. Birds were captured during three seasons: autumn, spring and winter. Using light microscopy, it was determined that there was no difference in prevalence across seasons ($P = 0.7714$) or per capture technique ($P = 0.5696$). Most birds exhibited low parasitaemia, regardless of season or capture technique. However, parasitaemia was significantly higher in birds captured in mist-nets *versus* feeders ($P = 0.002$). In addition, there was a weak seasonal effect ($P = 0.07$) as autumn-caught birds had much higher parasitaemia than those caught in spring and winter. Since Tiritiri Matangi bellbirds are used in reintroduction programmes these results have important implications for conservation, as higher parasitaemia may affect reintroduction survival rates. Finally the potential relationship between feeder access and parasitaemia is discussed, as well as important considerations and recommendations for future avian malaria surveys.

3.1 INTRODUCTION

One of the underlying assumptions in disease prevalence and infection intensity studies is that each disease state in question (*e.g*. uninfected or infected, chronic or acute infection) has an equal probability of being detected within a given sampling period (Courchamp et al. 2000, Jennelle et al. 2007). Yet in such studies of avian malaria (*Plasmodium* spp.), Valkiunas (2005) pointed out the most common capture technique employed (mist netting) is likely to be biased towards sampling healthier individuals in a population. The reason for this potential bias is that acute infections of avian malaria are often associated with severe anaemia (Atkinson and van Riper III 1991). Thus, individuals suffering from acute infections would be underrepresented due to decreased activity or morbidity (Valkiunas 2005). This supposition is inferentially supported by numerous studies where the majority of infected birds caught in mist-nets harboured chronic infections of low intensity (measured in parasitaemia – the percentage of blood cells infected with parasites) (Booth and Elliott 2003, Valkiunas 2005, Bensch et al. 2007, Ricklefs and Sheldon 2007). Nonetheless, the literature is almost devoid of empirical studies that have tested for differences in avian malaria prevalence and/or parasitaemia between mist netting and alternative capture techniques.

In the only study of its kind I could find in the literature, Valkiunas (2005) compared the parasitaemia of young chaffinches (*Fringilla coelebs*) infected with *Haemoproteus fringillae* between individuals caught by standard methods used for this species (mist-nets and stationary traps), and individuals sampled by shooting. *Haemoproteus* is a genus closely related to *Plasmodium* (see Section 1.1.1 for further discussion) that also causes anaemia in hosts with acute infections (Atkinson and van

Riper 1991) and would thereby also likely affect bird activity levels. In this instance, young chaffinches sampled by shooting had significantly higher parasitaemia than those captured by mist-nets and stationary traps, lending support to the supposition that mist nets are likely to capture healthier individuals. No similar studies have compared avian malaria prevalence and/or parasitaemia results from two live-capture techniques. Yet, this fundamental information is crucial to understanding how different capture techniques may be biasing survey results.

The high prevalence of a *Plasmodium* (*Novyella*) sp. in bellbirds (*Anthornis melanura*) on Tiritiri Matangi Island in New Zealand (see Ch. 2), and their use of supplementary feeders, presented the unique opportunity to compare prevalence and parasitaemia between mist net- and feeder-caught individuals. Bellbirds are nectar-feeding honeyeaters (Family: Meliphagidae) abundant on Tiritiri Matangi. They form strict linear dominance hierarchies around high-quality food resources, with males maintaining nearly exclusive access over females and juveniles (Craig and Douglas 1986). The supplementary feeders on Tiritiri Matangi contain sugar water meant for hihi (*Notiomystis cincta*) use. However, bellbirds exploit this food resource and males dominate access to them (pers. obs.). Therefore, this presented an opportunity to compare the two capture techniques using male bellbirds. In addition, detection of recaptures between mist-nets and feeders could be determined by banding birds.

Dominant social position and/or constant access to high-quality food may affect parasitaemia levels, or *vice versa*, as a function of host condition (Beldomenico and Begon 2009). Thus, this study cannot establish a definitive causal link to parasitaemia levels observed between the two capture techniques. Prevalence, on the other hand,

should be less affected by these factors as each bird presumably has an equal chance of being bitten by a mosquito. Most mosquitoes in New Zealand are nocturnal or crepuscular feeders (Derraik et al. 2005), and thus malaria transmission would largely occur while the birds are roosting. However, most crucially, this study will determine if the two different capture methods yield differing representations of avian malaria prevalence and parasitaemia in a wild endemic passerine population.

3.2 NULL HYPOTHESES

I tested two null hypotheses regarding avian malaria in male bellbirds and capture technique employed on Tiritiri Matangi Island:

1) There is no difference in prevalence between mist-net- and feeder-caught individuals.

2) There is no difference in parasitaemia between infected mist-net- and feeder-caught individuals.

3.3 METHODS

3.3.1 Study site and sampling techniques

This study was carried out on Tiritiri Matangi Island in the Hauraki Gulf of the Auckland Region (see Fig. 2.2 and Sec. 2.2.1 for a description of the site). Sampling occurred over approximately the same duration for three seasons (8 days in autumn, 9 days in winter and 11 days in spring of 2009) and employed the same processing protocol as that outlined in Chapter 2 (Sec. 2.2.2). Birds were captured using two techniques: mist-netting and supplementary feeder traps. In addition to metal banding, unbanded birds captured during this study were fitted with unique combinations of plastic colour bands.

The colour banding of individuals allowed me to target unbanded or previously unsampled birds at the feeders, thereby minimising the number of times a given bird had to undergo the stress of capture. It also allowed for the quick identification of any potential recaptures at a given sampling site, or between the two capture techniques. Mist-netting sites were placed at least 200m from the nearest feeder to avoid the potential bias of capturing birds that predominately use the feeders (see Fig. 2.3). Finally, only adult male bellbirds were sampled in this study to control for potential juvenile/adult and gender differences in avian malaria prevalence and parasitaemia (see Section 1.3.1, 2nd paragraph).

3.3.2 Prevalence and parasitaemia determination

Thin blood smears were initially examined for parasite presence or absence using the light microscopy procedure described in Section 1.2.1 (3rd paragraph) and Section 2.2.3 (1st paragraph). The amount of time spent examining a slide was standardised to a minimum of 15 minutes and maximum of 30 per bird, and a minimum of 100 frames were examined. Prevalence was calculated as the proportion of infected individuals to the total number sampled and expressed as a percentage.

Following Godfrey et al. (1987), parasitaemia was calculated as the number of parasites counted in 100 fields divided by an estimate of the total erythrocytes examined and expressed as a percentage. Godfrey et al. (1987) found that accurate parasitaemia estimation requires the complete enumeration of erythrocytes in a given field of view, and that at least 2,000 erythrocytes must be examined. In this study, the number of parasites per 100 fields was counted using the full x1000 oil immersion fields visible

through the eyepiece. However, photographs of every 10th field were used to calculate the total erythrocytes examined. The number of erythrocytes was accurately counted from each of the ten photographs. Then, since the photographed frames were smaller than the field of view through the microscope eyepieces, these counts were multiplied by a correction factor of 2.04 (Barraclough, Massey University, unpub. data) to account for the estimated number of erythrocytes outside the photographic frame. Each frame total was added and multiplied by ten to estimate the total erythrocytes examined per 100 fields. Thus, parasitaemia was calculated by dividing the total number of parasites counted in 100 fields of view by this estimate of total erythrocytes viewed. Table 3.1 provides an example of my parasitaemia estimation technique taken from the actual data. Finally, every total erythrocyte count estimated in this study was more than the 10,000-20,000-erythrocyte benchmark used in or recommended by other studies (N = 49, mean = 27,658 ± 3,912SD) (Godfrey et al. 1987, Booth and Elliott 2003, Valkiunas 2005, Ricklefs and Sheldon 2007).

Table 3.1. An example of the parasitaemia estimation method used in this study.

Photographic Frame	**Cells**
10th	142
20th	159
30th	109
40th	171
50th	184
60th	69
70th	182
80th	168
90th	95
100th	146
Sum of Cell Counts	1425
Correction Factor for Cell Counts	2.04
Total Erythrocytes Examined (Cell Counts*Correction Factor*10)	29,070
Parasites Counted/100 microscope frames	4
Estimated Parasitaemia % (Parasites Counted/Total Erythrocytes*100)	0.014

3.3.3 Statistical analyses

3.3.3.1 Prevalence analysis

Prevalence data involves nominal variables (parasitised and unparasitised) associated with values 0 and 1, thereby violating the assumptions of linear modelling techniques (O'Brien et al. 2009). I ran a log-linear model using a maximum likelihood estimator (PROC CATMOD, Statistical Analysis Systems, version 9.1, SAS Institute Inc., Cary, North Carolina, USA 27511) to test for differences in the prevalence of malaria in bellbirds (response variable 0/1) associated with two main effects: 1) *season* (spring/summer/winter) and 2) *capture technique* (mist netting/feeders). Avian malaria prevalence is generally known to vary by season in high latitudes (Valkiunas 2005), often with peaks in autumn and spring (Atkinson and van Riper III 1991). This is also true for bellbirds, as autumn and spring peaks in prevalence have been found in Hauraki Gulf populations (S. Baillie, Massey University, unpub. data). Finally, a α-value of ≤ 0.05 was used as the standard for statistical significance.

3.3.3.2 Parasitaemia analysis

The frequency distribution of parasitaemia values is usually strongly skewed towards very small values (Booth and Elliott 2003, O'Brien et al 2009). This was true of the bellbird parasitaemia data analysed in this study (Fig. 3.1). Such data violate the assumption of normality used in parametric statistics, and thus require a log-transformation, the use of generalized linear models based on the negative binomial or Poisson distribution, or nonparametric analyses for hypothesis testing (McDonald 2009). I applied log-transformations (natural and base_{10}) to normalise my parasitaemia data

(following: van Riper et al. 1986, Atkinson et al. 2000, Zehtindjiev et al. 2008) Normality was tested using a Shapiro-Wilk test (available at http://epitools.ausvet.com.au. Sergeant 2009) ($P = 0.377$) (Fig. 3.2).

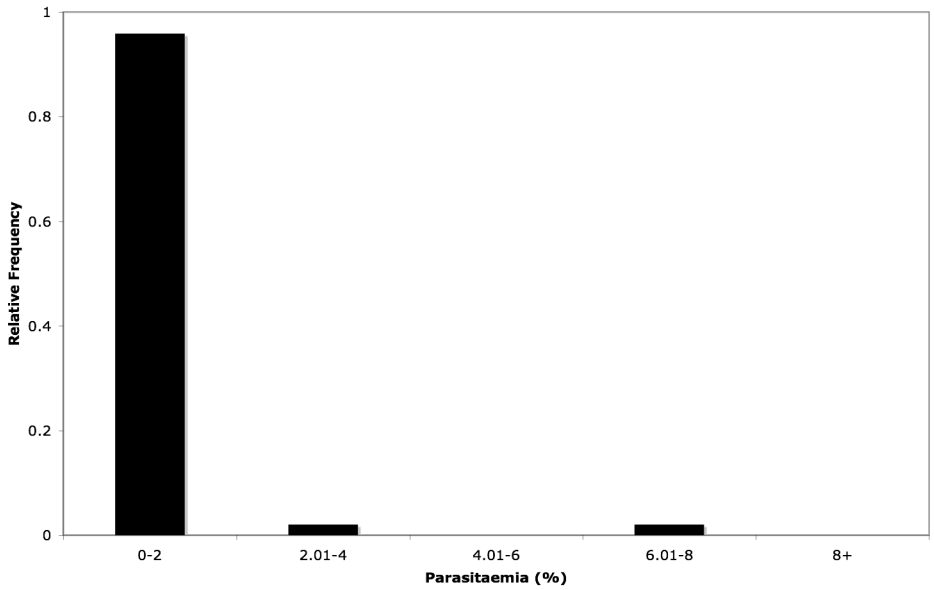


Figure 3.2. The strongly skewed frequency distribution of the bellbird parasitaemia data ($N = 49$).

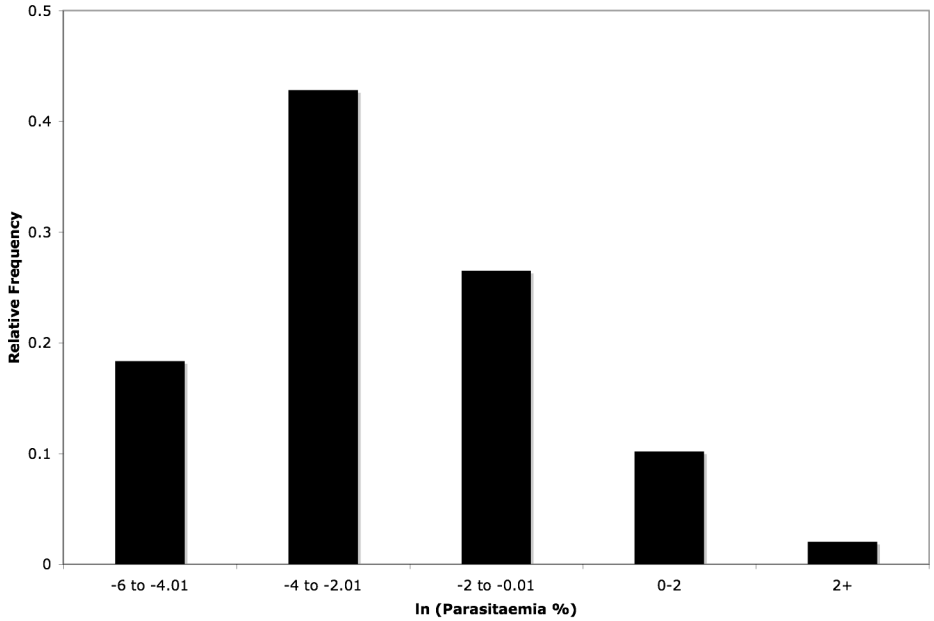


Figure 3.3. The normalised frequency distribution of the bellbird parasitaemia data following natural log (ln) transformation.

I ran a general linear model (GLM) for unbalanced ANOVA (PROC GLM, Statistical Analysis Systems, version 9.1, SAS Institute Inc., Cary, North Carolina, USA 27511) to test for differences in the parasitaemia (transformed) associated with two main effects: 1) *season* (spring/summer/winter) and 2) *capture technique* (mist netting/feeders). As sample sizes were small and the design unbalanced, I interpreted the results based on the Type III sum of squares as recommended in the SAS/STAT User's Guide for unbalanced sample sizes (available at http://www.technion.ac.il/docs/sas/stat/index.htm). The model included a planned comparison (orthogonal contrast) of the grouped spring and autumn means *versus* winter. Spring and summer were predicted to be the seasons associated with peaks in prevalence, and parasitaemia to be lower in winter because of the absence of vector activity. A α-value of ≤ 0.05 was used as the standard for statistical significance for each test.

3.3.4 General parasitaemia trends

Box-plots of the pooled season and capture technique data, and of the capture technique data within each season, were constructed to examine general trends in the untransformed parasitaemia data. I compared median values instead of means, since the standard deviation exceeded the mean across all variables except birds mist-netted in winter (see Section 3.4.2, Table 3.3), a reflection of the strong skew towards lower parasitaemia shown in Figure 3.2. This skew also resulted in the box-plots having a flattened appearance, making some of the data points difficult to visualise, so numeric values of the important parameters were provided (see Section 3.4.2, Table 3.3).

3.4 RESULTS

3.4.1 Prevalence

Table 3.2 provides a summary of avian malaria prevalence in male bellbirds per capture technique, season, and capture technique within each season. A total of 49 of the 119 (41.2%) adult male bellbirds sampled in this study were infected with a *Plasmodium* (*Novyella*) sp. (see Section 2.3.4.4 for parasite description). Prevalence was highest in autumn (44.2%), followed by spring (41.5%) and winter (34.8%). Per capture technique, prevalence was higher in mist-nets (44.2%) *versus* feeders (38.8%). None of the birds sampled at the feeders were recaptured in mist-nets or *vice versa*. Within each season, prevalence was consistently higher at mist-nets *versus* feeders.

Table 3.2. Tiritiri Matangi adult male bellbird prevalence per season and capture technique: positive individuals per total sampled (+ve/*n*) and prevalence (prev %). MN = mist-nets and F = feeders.

Season	Technique	+ve/n	Prev (%)
Autumn	MN	8/17	47.1
Autumn	F	11/26	42.3
Spring	MN	12/27	44.4
Spring	F	10/26	38.5
Winter	MN	3/8	37.5
Winter	F	5/15	33.3
Autumn	All	19/43	44.2
Spring	All	22/53	41.5
Winter	All	8/23	34.8
All	MN	23/52	44.2
All	F	26/67	38.8
All	All	49/119	41.2

However, neither the seasonal ($\chi^2 = 0.52$, $P = 0.77$) or capture technique ($\chi^2 = 0.32$ $P = 0.57$) prevalence differences were significant (see Appendix 3.1 for statistical outputs). Thus, my null hypothesis of no difference in prevalence between capture techniques

could not be rejected. This was due to the large standard error overlap in each of the expected probabilities generated by the log-linear model's maximum likelihood analysis (Fig. 3.4), which may be a product of the relatively small sample sizes.

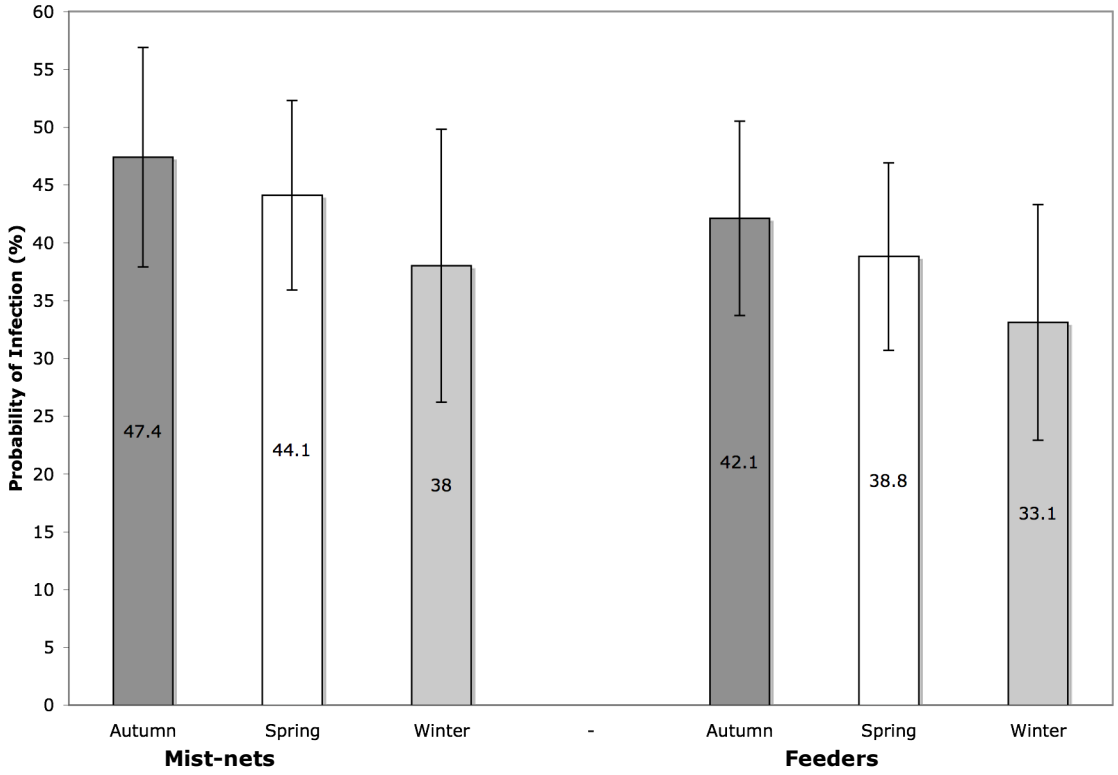


Figure 3.4. The expected probabilities of malaria infection (±SE) per capture technique within each season for Tiritiri Matangi adult male bellbirds. The data reflect the downward trend in prevalence from autumn to winter in both capture techniques, and from mist-nets to feeders within each season, but with large standard error overlap.

3.4.2 Parasitaemia

Box plots of the pooled season and capture technique data (Fig. 3.5), and of the capture technique data within each season (Fig. 3.6), indicate several interesting trends in bellbird parasitaemia. The overall median parasitaemia was low (0.074%), but exhibited a wide range from extremely low (0.003%) to relatively high (7.876%) (Fig. 3.5). Across

seasons, the medians were similarly low, with autumn having the highest (0.074%), followed by spring (0.065%) and winter (0.051%). However, the range of parasitaemia was much greater in autumn (0.009-7.876%) than spring (0.004-1.244%) or winter (0.003-0.298%), which is reflected in the dispersion around each respective median (Fig. 3.5). Between capture techniques, the median parasitaemia was much higher in mist-nets (0.182%) than feeders (0.042%) (Fig. 3.5).

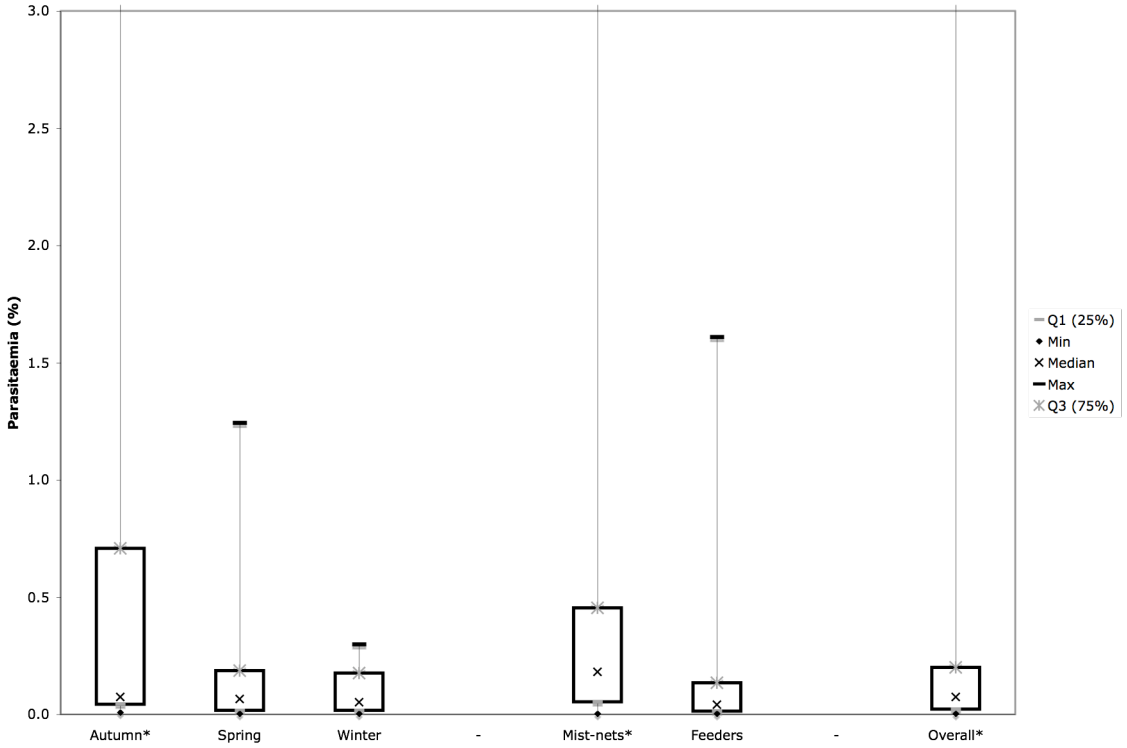


Figure 3.5. Box-plot of the untransformed bellbird parasitaemia data pooled per season, capture technique and overall. Q1 = 25th percentile and Q3 = 75th percentile.

* The max parasitaemia for autumn, mist-nets, and overall was 7.876%, not shown because of its effect on the diagram's scale.

Bellbirds captured in mist-nets during autumn had by far the highest median parasitaemia (0.633%) observed in this study, which was also much higher than autumn

birds caught in feeders (0.053%) (Fig. 3.6). In addition, the most heavily parasitised individual (7.876%) was caught in a mist-net during autumn. Birds mist-netted in spring and winter also carried higher median parasitaemia (0.185% and 0.079%, respectively) than those caught in feeders during the same period (0.018% and 0.023%, respectively). All feeder-caught birds had relatively similar parasitaemia, but with autumn again the highest, followed by winter, and then spring (Fig. 3.6). Thus, the lowest median parasitaemia observed in this study was in birds caught at the feeders during spring.

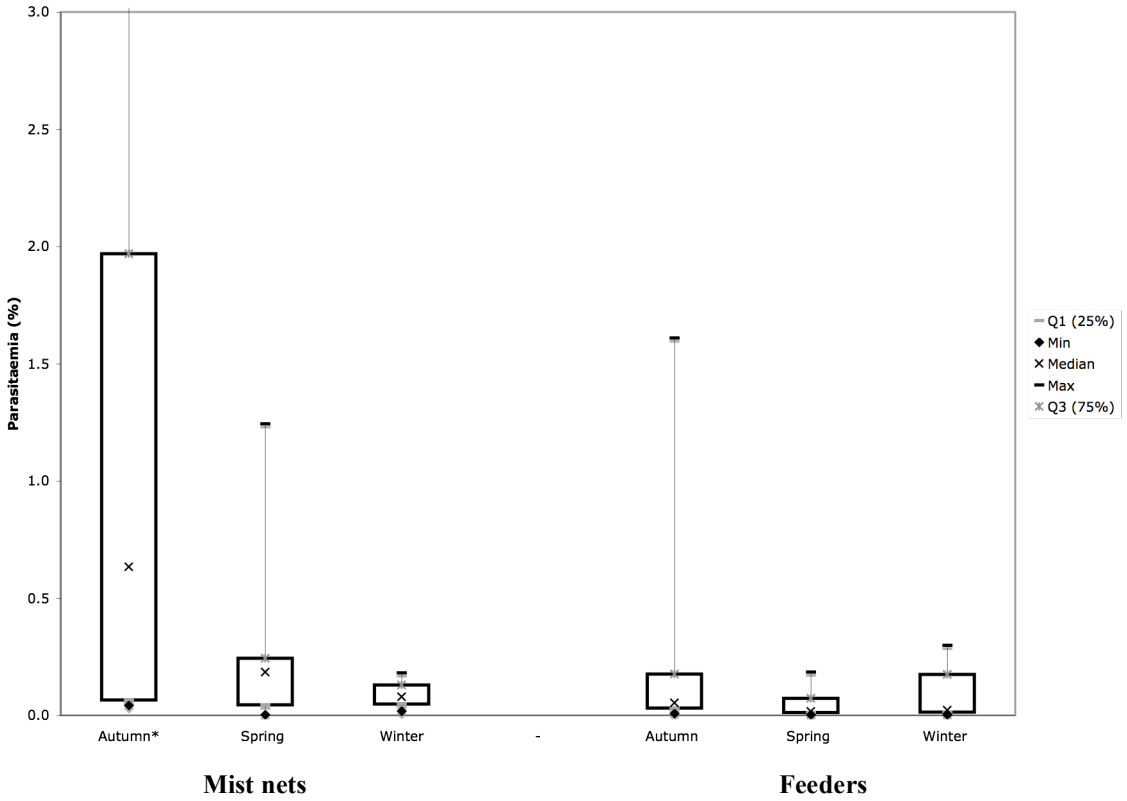


Figure 3.6. Box-plot of the untransformed bellbird parasitaemia data per capture technique within each season. Q1 = 25th percentile and Q3 = 75th percentile.

* The max parasitaemia for birds mist-netted in autumn was 7.876%, not shown because of its effect on the diagram's scale.

Table 3.3. Descriptive statistics of the parasitaemia levels found in Tiritiri Matangi bellbirds per season, capture technique, and capture technique within each season. All parasitaemia values are percentages. *N* = sample size, MN = mist-nets and F = feeders.

Season	Technique	*N*	Range	Median	Mean (SD)
Autumn	All	19	0.009-7.876	0.074	0.836 (1.85)
Spring	All	22	0.004-1.244	0.065	0.167 (0.281)
Winter	All	8	0.003-0.298	0.051	0.099 (0.108)
All	MN	23	0.004-7.876	0.182	0.736 (1.677)
All	F	26	0.003-1.61	0.042	0.132 (0.312)
Autumn	MN	8	0.043-7.876	0.633	1.68 (2.659)
Autumn	F	11	0.009-1.61	0.053	0.223 (0.466)
Spring	MN	12	0.004-1.244	0.185	0.267 (0.352)
Spring	F	10	0.004-0.185	0.018	0.047 (0.057)
Winter	MN	3	0.019-0.182	0.079	0.093 (0.083)
Winter	F	5	0.003-0.298	0.023	0.103 (0.13)
All	All	49	0.003-7.876	0.074	0.415 (1.197)

The GLM constructed from the transformed parasitaemia data, modelling parasitaemia against season and capture technique, was highly significant ($F = 5.19$, $P = 0.0037$) (see Appendix 3.2 for statistical outputs). The interaction between these main effects was not significant ($P = 0.7121$) and thus removed from the final model. Parasitaemia was weakly related to season in the model ($F = 2.82$, $P = 0.07$), with autumn higher than spring and winter. The planned comparison of winter *versus* autumn and spring was not significant (CONTRAST, $P = 0.3479$). However, the relationship between parasitaemia and capture technique was highly significant ($F = 10.83$, $P = 0.002$). Bellbirds caught in mist nets had higher parasitaemia than those caught in feeders. Thus, my null hypothesis of no difference in parasitaemia between infected mist-net- and feeder-caught individuals is rejected.

3.5 DISCUSSION

3.5.1 Prevalence and parasitaemia

The two survey methods successfully detected similar seasonal trends in avian malaria prevalence and parasitaemia in adult male bellbirds on Tiritiri Matangi Island. For example, both measures were highest in autumn, followed by spring, and then winter. Prevalences detected by both methods were also not significantly different. However, the parasitaemia levels detected by the two capture techniques did significantly differ: bellbirds captured in mist nets were found to carry higher parasitaemia than those captured in feeders. Since parasitaemia is widely considered an indication of virulence in avian malaria (e.g. Garnham 1966, Fallon and Ricklefs 2008), this result supports the suggestion by Valkiunas (2005) that healthier birds may be selected by capture techniques targeting active individuals. This is because the method targeting competing birds, feeder trapping, produced the lowest levels of parasitaemia.

O'Brien et al. (2009) demonstrated that measures of parasite abundance (*i.e.* parasitaemia) always outperform prevalence in statistical power at sample sizes below 1,000 individuals. Therefore the fact that significant differences in parasitaemia but not prevalence were detected, despite higher prevalence being recorded from mist net-caught birds across and within seasons, is likely a function of sample size (49/119 positive birds). Future blood parasite studies concerned with infection status comparisons should consider the use of parasitaemia rather than, or in addition to, prevalence.

Naturally, this methods comparison cannot determine if mist netting underestimates true parasite prevalence and parasitaemia in the bellbird population. It simply demonstrates that the feeder-capture method underestimates these measures

relative to mist netting. However, these results do confirm that the capture method employed can significantly bias disease survey results. In this case, different outcomes were yielded from two techniques that are frequently used in New Zealand. Thereby underscoring the importance of considering how methodology may bias the representation of avian malaria yielded from field surveys.

Interestingly, the effect of season on parasitaemia approached significance (P = 0.0696). Considering the relatively low number of birds sampled, it seems likely that an increase in sample sizes across seasons, particularly winter, would reveal a significant seasonal parasitaemia trend. Thus, further research on seasonal patterns in avian malaria parasitaemia in bellbirds and other confirmed New Zealand host species is recommended.

3.5.2 Feeder access and parasitaemia

Feeder trapping sampled a significantly healthier portion of the male bellbird population than mist netting. Yet, a causal link as to why parasitaemia differed between the two capture techniques cannot be made within the scope of this study. However, there are two likely explanations: firstly, individuals caught in mist-nets may have higher parasitaemia because of their lower social status and consequent exclusion from the feeders. If so, dominant males may be gaining some immunological benefit from having constant feeder access, or subordinate birds excluded from the feeders may be immunologically compromised by increased nutritional stress, or both. Thus, higher parasitaemia would be a result of feeder exclusion. For dominant males, constant access to high-quality food likely keeps them in good physiological condition. These individuals would be better prepared to limit parasitaemia, as parasites would face a stronger immune

response (Beldomenico and Begon 2009). Similarly, studies of human malaria have shown that good nutrition can preclude high parasitaemia through stronger haematological response (*e.g.* synthesis of more erythrocytes to moderate anaemia) (Shankar 2000, Nussenblatt and Semba 2002). Equally, subordinate birds excluded from the feeders may face food limitations due to competition with conspecifics and other nectar-feeding species present on the island, such as hihi and tui (*Prosthemadera novaeseelandiae*). Nutritional stress can lead to decreased immune function (Apanius 1998) and has been implicated in relapse or recrudescence events in avian malaria (Atkinson and van Riper III 1991), both of which could result in higher parasitaemia.

Conversely, the development of high parasitaemia in a bird may result in feeder exclusion. In which case, the feeders are not providing an immunological benefit *per se*, but rather an individual's ability to cope with infection dictates their social status and feeder access. Thus, increased parasitaemia may be a cause of feeder exclusion. For example, Lindström et al. (2005) showed physiological condition is a major determinant of social status in house sparrows (*Passer domesticus*). As, when in large groups, individual sparrows maintained a social position that minimised their energetic (as incurred by dominance interactions) and immunological costs. Hence, male bellbirds that develop high parasitaemia may forego costly dominance interactions at the feeders in favour of allocating more energetic resources to immune response. Ultimately, it may be an interaction between these two explanations that define the relationship between higher parasitaemia and feeder access.

3.6 CONCLUSION

The parasitaemia difference detected between these capture techniques has important implications for the application of conservation management tools, such as species translocations. For example, Tiritiri Matangi bellbirds have recently been used in translocations aimed at re-establishing populations in areas of New Zealand where they were once found, but have since been extirpated (K. Parker, Massey University, pers. comm.). Birds intended for translocation in these operations were caught in mist nets. However, this study illustrates that this technique is likely to capture adult male bellbirds with relatively higher-intensity avian malaria infections than feeder trapping, particularly in autumn. As translocation is a stressful process for the animal (Teixeira et al. 2007), and stress reduces immune function (Apanius 1998), mist net-caught birds may have a lower survival rate either during or post-translocation. Thus, the use of feeder trapping in future translocations from Tiritiri Matangi should be considered.

Finally, at a time when research in to avian malaria in New Zealand is growing, the results of this study have repercussions for future studies. This is because, although mist-netting is the primary method for capturing passerines, when available, the use of feeders for capturing birds offers a convenient way to sample a large number of birds for blood parasite surveys. However, comparisons of results from these two methods will be flawed. Thus such potential biases must be taken into account when designing baseline data collection or long term studies, and it is important that capture techniques be standardised across sites and through time.

Appendix 3.1. Statistical outputs for the prevalence data

The CATMOD Procedure

Data Summary

Response	RESP	Response Levels	2
Weight Variable	FREQ	Populations	6
Data Set	TIRIPREV	Total Frequency	119
Frequency Missing	0	Observations	12

Maximum Likelihood Analysis

Maximum likelihood computations converged.

Maximum Likelihood Analysis of Variance

Source	DF	Chi-Square	Pr > ChiSq
Intercept	1	3.59	0.0581
Technique	1	0.32	0.5696
Season	2	0.52	0.7714
Likelihood Ratio	2	0.01	0.9971

Analysis of Maximum Likelihood Estimates

Parameter		Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept		0.3850	0.2032	3.59	0.0581
Technique	F	0.1079	0.1897	0.32	0.5696
Season	AU	-0.1733	0.2686	0.42	0.5187
	SP	-0.0390	0.2602	0.02	0.8808

Maximum Likelihood Predicted Values for Response Functions

			------Observed------ Function	Standard	------Predicted----- Function	Standard	
LOC	SEASON	Number		Error		Error	Residual
F	AU	1	0.310155	0.396958	0.319523	0.343055	-0.00937
F	SP	1	0.470004	0.403113	0.453853	0.341559	0.016151
F	WI	1	0.693147	0.547723	0.705221	0.459361	-0.01207
M	AU	1	0.117783	0.485913	0.103762	0.382431	0.014021
M	SP	1	0.223144	0.387298	0.238092	0.333602	-0.01495
M	WI	1	0.510826	0.730297	0.48946	0.500709	0.021365

Maximum Likelihood Predicted Values for Probabilities

			--------Observed------- Probability	Standard Error	-------Predicted------- Probability	Standard Error	Residual
LOC	SEA	RESP					
F	AU	0	0.5769	0.0969	0.5792	0.0836	-0.002
		1	0.4231	0.0969	0.4208	0.0836	0.0023
F	SP	0	0.6154	0.0954	0.6116	0.0811	0.0038
		1	0.3846	0.0954	0.3884	0.0811	-0.004
F	WI	0	0.6667	0.1217	0.6693	0.1017	-0.003
		1	0.3333	0.1217	0.3307	0.1017	0.0027
M	AU	0	0.5294	0.1211	0.5259	0.0954	0.0035
		1	0.4706	0.1211	0.4741	0.0954	-0.003
M	SP	0	0.5556	0.0956	0.5592	0.0822	-0.004
		1	0.4444	0.0956	0.4408	0.0822	0.0037
M	WI	0	0.625	0.1712	0.62	0.118	0.005
		1	0.375	0.1712	0.38	0.118	-0.005

Appendix 3.2. Statistical outputs for the parasitaemia data

The GLM Procedure

Class Level Information

Class	Levels	Values
Season	3	AU SP WI
Technique	2	F MN

Number of Observations Read	49
Number of Observations Used	49

Dependent Variable: ln(parasitaemia)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	37.5504540	12.5168180	5.17	0.0037
Error	45	108.9276276	2.4206139		
Corrected Total	48	146.4780816			

R-Square	Coeff Var	Root MSE	LnPara Mean
0.256355	-61.48047	1.555832	-2.530612

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Season	2	13.66301437	6.83150718	2.82	0.0700
Technique	1	26.13006588	26.13006588	10.79	0.0020

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
winter vs sp and au	1	2.17851913	2.17851913	0.90	0.3479

Least Squares Means

Season	LSMEAN
AU	-1.82015526
SP	-2.89763788
WI	-2.93149582

Season	LSMEAN	95% Confidence Limits	
AU	-1.820155	-2.542604	-1.097707
SP	-2.897638	-3.566993	-2.228282
WI	-2.931496	-4.045167	-1.817824

Location	LSMEAN
F	-3.28877969
MN	-1.81074629

Location	LSMEAN	95% Confidence Limits	
F	-3.288780	-3.927181	-2.650378
MN	-1.810746	-2.513811	-1.107682

Chapter 4.

Mosquito abundance, composition, and disease prevalence in silvereyes (*Zosterops lateralis*) at forest edge and forest interior sites in Waharau Regional Park, North Island, New Zealand

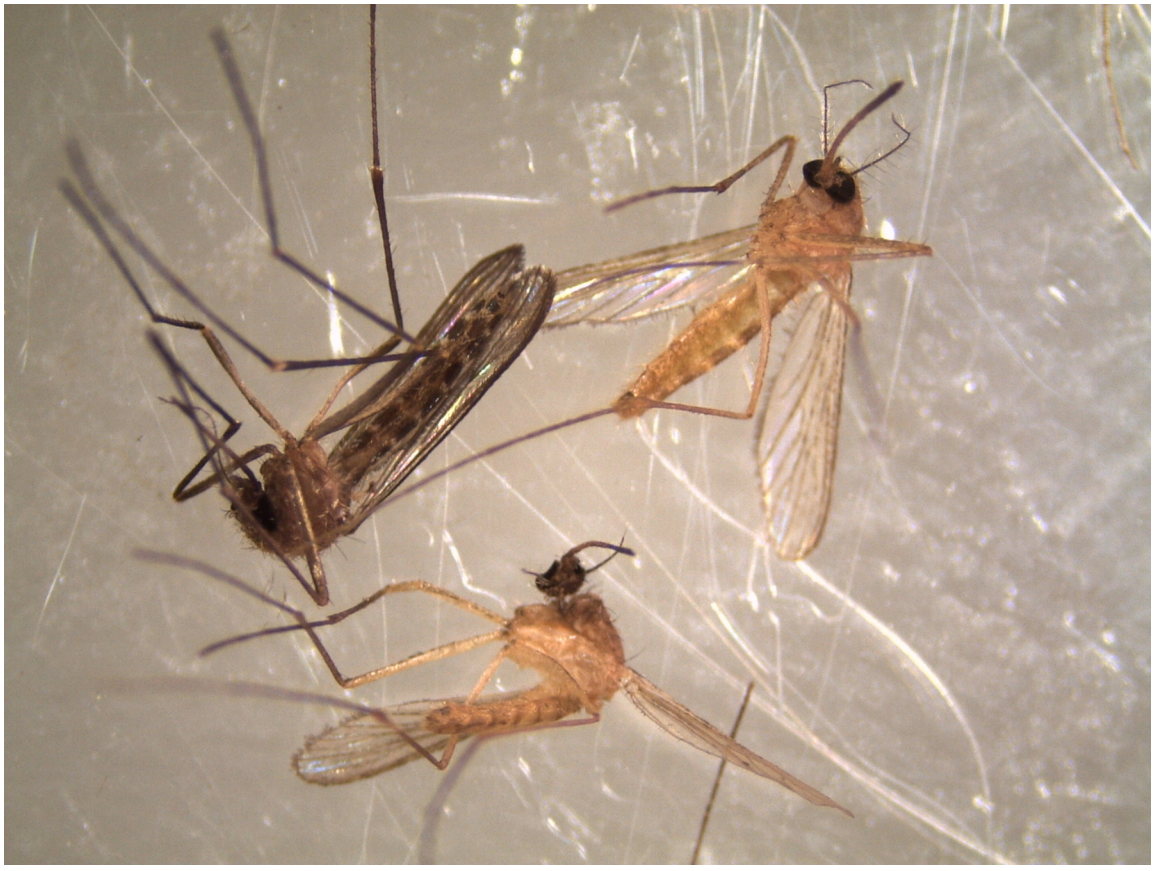


Figure 4.1. Two vector species of avian malaria in New Zealand. *Culex pervigilans* is the darker species on the left and *C. quinquefasciatus* the two lighter individuals on the right**.** Photograph by D.J. Gudex-Cross.

ABSTRACT

Deforestation and forest fragmentation are two anthropogenic landscape alterations affecting the disease dynamics of malarial parasites, largely through their effect on mosquito vectors. A major outcome of these landscape alterations is the creation of greater amounts of forest edge habitat, which can potentially lead to increased mosquito densities infiltrating the forest from surrounding modified landscapes (*e.g.* agriculture) and increased exotic species invasions. Yet to date, no study has investigated differences in mosquito vector abundance, species composition, and avian malaria prevalence at spatially explicit forest edge and interior sites. Thus, I compared the relative abundance of mosquitoes and their species composition at two forest edge and two forest interior sites in Waharau Regional Park, North Island, New Zealand. Potential differences in mosquito feeding patterns were explored by trapping mosquitoes at two heights: at the ground and in the canopy. Finally, avian malaria prevalence in the most abundant passerine species in this forest, silvereyes (*Zosterops lateralis*), was also surveyed at each site. Using CO_2/light traps baited with dry ice, I captured five mosquito species, two exotic (*Aedes notoscriptus* and *Culex quinquefasciatus*) and three native (*Aedes antipodeus*, *Culex asteliae* and *Culex pervigilans*). The vast majority of mosquitoes collected were native (535/580). The relative abundance of exotic species captured at edge sites was significantly higher than the interior (P = 0.0002), with relatively low abundance at the edge but almost complete absence in the interior (1/45). However, when exotic species were analysed individually, this difference was only significant for *Cx. quinquefasciatus* (P = 0.03). When pooled, significantly more exotic species were also caught at ground level than in the canopy (P = 0.022), although individually this

difference was only significant for *Ae. notoscriptus* ($P = 0.04$). Conversely, the relative abundance of native mosquitoes was higher in the interior sites than at the edge ($P = 0.0213$). However, further analysis showed this result was due to one site in the interior, which consisted of a more mature forest type than the other sites and one native species in particular (*Ae. antipodeus*). Similarly, native species were more likely to be trapped at ground level ($P = 0.0316$), but this again was due to the high numbers of *Ae. antipodeus* caught at ground level in one interior site. None of the individual native species showed an edge *versus* interior abundance pattern. However, *Cx. asteliae* was caught in significantly higher numbers in the canopy *versus* ground level ($P = 0.0097$). Using light microscopy, avian malaria was found in 22 of the 240 (9.17%) silvereyes sampled in this study, the majority of infections ($N = 20$) being a parasite consistent with *Plasmodium* (*Huffia*) *elongatum*. The other two individuals harboured a parasite consistent with *P.* (*Haemamoeba*) *relictum*. However, avian malaria prevalence did not significantly differ between sites ($P = 0.801$). These results suggest that forest edge and interior sites can differ in terms of mosquito abundance and species composition, but that this does not necessarily always equate to differences in avian malaria prevalence. Some possible mechanisms underlying this situation are discussed. In addition, further research examining this forest edge-interior interaction with more sampling sites, larger mosquito sample sizes, and a greater number of malarial parasite and avian host species is recommended.

4.1 INTRODUCTION

There is accumulating evidence that anthropogenic landscape changes, such as deforestation and forest fragmentation, are altering the disease dynamics of malarial parasites, largely through their effects on mosquito vector communities (Patz et al. 2000, Harvell et al. 2002, Sehgal 2010). However, much of this evidence is based on human malaria and its associated *Anopheles* (family: Culicidae) mosquito vectors (see Guerra et al. 2006 for a review). The effects of deforestation and forest fragmentation on malarial parasites are complex, as alterations in vector communities and host-parasite dynamics are largely dependent on subsequent land use (Yasuoka and Levins 2007). For example, Yasuoka and Levins (2007) reviewed 60 studies regarding the effects of deforestation followed by agricultural development on human malaria and anopheline vectors and found: 1) changes in vector densities vary by geographic location and type of agricultural development (*e.g.* crop plantations, rice cultivation, etc.) and 2) deforestation often leads to increased anopheline densities in areas where local vector species are adapted to open spaces and high levels of sunlight, but this does not always translate into increased malaria incidence. On the other hand, the effects of landscape variation and change on avian malaria, which is vectored mainly by culicid mosquitoes of the genera *Culex*, *Culiseta* and *Aedes* (Valkiunas 2005), are only beginning to be explored (Sehgal 2010).

Similar to the aforementioned human malaria research, recent studies on avian malaria prevalence at a landscape level are revealing complex patterns. For example, Wood et al. (2007) found avian malaria prevalence in blue tits (*Cyanistes caeruleus*) varied on a local scale within a single forest patch in the United Kingdom. In addition, different parasite lineages exhibited differing spatial distributions within the forest, and

overall prevalence (pooled lineages) decreased moving away from the Thames River. The authors suggested individuals within a host population may not have the same likelihood of infection due to several factors (*e.g.* time of infection and subsequent dispersal, breeding site characteristics, etc), and parasite prevalence and lineage distribution is likely linked to vector activity or abundance.

At a larger scale in terms of deforestation and forest fragmentation, Ribeiro et al. (2005) found higher avian malaria prevalence in larger *versus* smaller Brazilian forest fragments when pooling across species. However, when the authors accounted for prevalence variation by focusing on a single host species, the relationship was not significant. Similarly, Bonneaud et al. (2009) found higher prevalence in pristine rainforest sites *versus* disturbed agroforests in Cameroon when pooling across species. However, a follow-up study conducted by Chasar et al. (2009) comparing prevalence in two widespread African bird species in disturbed *versus* undisturbed Cameroon forests found a more complex infection pattern: the prevalence of one parasite lineage (*Plasmodium megaglobularis*) was higher in undisturbed *versus* disturbed areas and *vice versa* for a different lineage (*P. lucens*). A study of these same two African bird species by Loiseau et al. (2010) in Ghana found higher prevalence in sites with more forest cover than those with less. The authors of each of these studies suggested vector abundance and species composition may be driving prevalence differences, with either pristine or less disturbed forests alternatively providing more suitable habitat for mosquito vectors. Finally, Sehgal (2010) notes that spatial differences in prevalence, particularly the lineage-specific ones, may be driven by vector specificity for a particular parasite lineage. Thus, changes in vector habitat would ultimately drive alterations in host prevalence only

for those parasites that the vector(s) are capable of transmitting. In studies with extensive avian malaria parasite and vector data, competent vector abundance and/or distribution has been positively correlated with host prevalence (van Riper III et al. 1986, Freed et al. 2005, Tompkins and Gleeson 2006). However few avian malaria studies have included vector sampling in their research protocols, and the vector competence of many mosquito species remains unknown (Valkiunas 2005).

One of the main outcomes of deforestation and forest fragmentation is the creation of more edge habitat as the ratio of edge to interior forest decreases (Center et al. 1995). Edge habitats are often more susceptible to invasion by exotic species (Center et al. 1995), especially mosquitoes, which can cause alterations in existing native vector communities and disease dynamics (summarised in Derraik and Slaney 2007). In New Zealand, over 70% of the original forest cover prior to human settlement has been lost, and the remaining forest is highly fragmented, particularly on the North Island where many of the previously forested areas have been converted to agriculture (Ewers et al. 2006). Mosquito studies conducted on the North Island have shown exotic species (*Aedes notoscriptus* and *Culex quinquefasciatus*) may be replacing natives in disturbed habitats (Derraik 2005, Derraik et al. 2005b), and deforested landscapes are supporting higher numbers of mosquito larvae *versus* forested areas (Leisnham et al. 2004, Leisnham et al. 2005). For avian malaria, the exotic vector *Cx. quinquefasciatus* is of particular concern in New Zealand, since its distribution has been correlated with the occurrence of *Plasmodium relictum* in exotic birds (Tompkins and Gleeson 2006). In addition, forest fragmentation and agricultural development have been shown to increase the abundance of this mosquito vector in Hawaii (Reiter and LaPointe 2007). However, research has not

yet been conducted to investigate the relationship between mosquito abundance, species composition, and avian malaria prevalence in forest edge *versus* forest interior sites.

Given this background information, I conducted a study on the effects of spatial habitat location (*e.g.* edge vs. interior) on the relative abundance and species composition of mosquitoes, and avian malaria prevalence, in Waharau Regional Park on the North Island of New Zealand. To control for potential variation in host prevalence, I used only one bird species (silvereye *Zosterops lateralis*) in the comparison.

4.2 NULL HYPOTHESES

I tested three null hypotheses regarding the relative abundance of mosquitoes and malaria prevalence in silvereyes in Waharau Regional Park:

1) There is no difference in the relative abundance of exotic mosquitoes between forest edge and forest interior sites.

2) There is no difference in the relative abundance of native mosquitoes between forest edge and forest interior sites.

3) There is no difference in avian malaria prevalence between birds caught at forest edge sites and those caught at forest interior sites.

4.3 METHODS

4.3.1 Study site and sampling techniques

This study was carried out from late February to late April 2010 at Waharau Regional Park (37°02′95″S, 175°17′98″E), North Island, New Zealand (see Fig. 2.2). The park consists of a large tract of forest from the higher elevation eastern foothills of the

Hunua Ranges which grades into sheep grazing pastures at lower elevations near the Firth of Thames (see Fig. 2.4). The forested area decreases in width from the foothills to lower pastures, giving a bottlenecked appearance, with pastureland also surrounding the eastern and western borders of the park. The lower elevation forest abutting the pastureland has a history of grazing and, as commonly found on the edges of New Zealand forest, thus is dominated by early successional or second-growth canopy trees, namely celery pine (*Phyllocladus glaucus*), kanuka (*Kunzea ericoides*) and manuka (*Leptospermum scoparium*). The low-mid elevation forest interior consists of these species mixed with older kauri (*Agathis australis*), totara (*Podocarpus totara*), tawa (*Beilschmiedia tawa*) and rimu (*Dacrydium cupressinum*). Finally, the high elevation forest abutting the Hunua Ranges consists of mature canopy species, including large mixed beech (*Nothofagus* spp.), puriri (*Vitex lucens*), kauri and rimu.

Mosquito sampling was conducted from late summer (February 20th) to early autumn (March 8th) when abundance and biting activity remain high (J.G.B. Derraik, Massey University, pers. comm.). Adult female mosquitoes were sampled using All-Weather Encephalitis Vector Survey (EVS) light traps (Bioquip Products, Rancho Dominguez, CA, USA) baited with dry ice. This type of trap was chosen for its relatively high capture efficiency across a wide range of mosquito species (Silver 2008), and similar CO_2-light traps have been used in previous vector studies in New Zealand (*e.g.* Leisnham et al. 2004, Derraik et al. 2005b, Massey et al. 2007). Since most of the mosquito species present in New Zealand are crepuscular and/or nocturnal feeders (Derraik et al. 2005a), the traps were set approximately 2 hours before sunset (~7:00p.m.) and emptied approximately 2 hours after sunrise (~7:00am) for a total of 12 trapping

hours per sampling bout. Captured mosquitoes were later identified in the lab under a dissecting microscope with the aid of Snell (2005).

Bird sampling was conducted within a single nine-week period, from March 1st until April 26th (austral autumn), to control for potential seasonal variation in avian malaria prevalence. Birds were mist-netted from early morning (~8:00am) to dusk. Playback recordings were used as lures in an effort to maximise capture rates. Upon capture, each bird was placed in a cloth bag, weighed, and then banded to prevent re-sampling. Morphometric measurements including tarsus, wing, tail and head/beak lengths were taken to the nearest 0.1 mm. A small blood sample (maximum of ~20 uL) was extracted from the brachial vein, and a thin blood smear prepared (see Section 1.2.1, 2nd paragraph). Thin blood smears were later scanned for parasite presence using light microscopy (see Section 1.2.1, 3rd paragraph and 2.2.3, 1st paragraph). Parasites were identified following Valkiunas (2005), Garnham (1966), and with the aid of R.K. Barraclough, Massey University. Prevalence was calculated as the proportion of infected individuals to the total number sampled and expressed as a percentage.

4.3.2 Experimental design

I sampled two forest edge and two forest interior sites (Fig. 4.2), which were semi-randomly chosen within the limits of the park following the criteria: 1) edge sites had to be less than 50m from the edge; 2) interior sites had to be at least 500m away from any edge and all sites at least 500m away from each other; and 3) sites had to be relatively near a walking track. The first criterion was based on Young and Mitchell (1994), who showed strong microclimate and vegetation differences up to 50m from the

forest edge in New Zealand podocarp-broadleaf forest. The second criterion was set to minimise the potential for bird movement between sites. The distance minimum (500m) reflected a compromise between the park boundaries, which are only approximately 1km wide at lower elevations, and what was logistically feasible for me to sample. Logistic feasibility was also the main basis for the third criterion, as there was no vehicular access to the forested areas of the park and much of the interior consists of deep valleys with thick vegetation. Thus distances between the sites were partially determined by the use of marked walking tracks for site access, in order for mosquito trap emptying and mist netting to be feasible within each day. The locations of the two interior sites were determined by the necessity to maximise distance between the sites, whilst remaining in proximity to walking tracks. The distances between each site and their locations are shown in Figure 4.2. Finally, it should be noted that no canopy trees, and little understory vegetation, are modified along walking tracks. The tracks consist of a simple trail of cleared ground cover, sign-posted by reflective tags attached to trees. Furthermore, they are remote and receive little foot traffic. Thus, the presence of walking tracks was not expected to bias mosquito or bird survey results.

Each site consisted of a 50m x 100m grid, with both mosquito and bird sampling carried out within those boundaries (Fig. 4.2). Table 4.1 summarises key characteristics of each site. In brief, the edge sites (E1 and E2) were predominantly characterised by successional canopy species. The first interior site (I1) was characterised by a mix of mature trees, but also included an element of successional species (Table 4.1). The second interior site (I2) had no history of grazing, and thereby primarily consisted of taller canopy trees with a complete understory. Thus, these two interior sites reflect some

of the variation common within forests of this region, largely due to different grazing histories (Table 4.1). In addition, the park also has a number of streams running through it. However, proximity to water was avoided as a potential source of bias for the mosquito sampling, as the nearest stream distances were relatively similar across sites (Table 4.1). Since the study area abuts the Hunua Ranges, and most forest modification and loss in New Zealand has occurred in lowland tracts (Ewers et al. 2006), the interior sites were higher in elevation than the edge sites (Table 4.1). There was a maximum of 60 metres altitude difference between I1 and the lowest edge site E1. However, although I2 was situated 90 metres higher than this at 270 metres, this relatively low altitude in the greater Auckland region is not characterised by cold temperatures and therefore was not expected to bias mosquito abundance results. Mosquito distribution studies in other temperate climates have shown major relative abundance and species composition shifts rarely occur below 1,000m, except in species specifically adapted to higher elevations (*e.g.* Devi and Jauhari 2004, Eisen et al. 2008).

Finally, I paired the mosquito sampling of edge and interior sites to control for possible temporal variation in relative abundances. Thus, E1 and I1 were sampled from February 20th-27th, and E2 and I2 from March 2nd-9th. Data loggers (Hobo Pro, Onset Computer Corp., Pocasset, MA, USA) were placed at each site throughout both sampling periods to monitor potential differences in relative humidity and temperature, which have been shown to affect mosquito abundances in other parts of the world (Yao et al. 2009, Murty et al. 2010). For sampling, two traps were placed on a single tree, one at ground level (~2m) and one in the canopy (~10m). This was done on two trees, which were separated by at least 20m to prevent over-sampling one small area of each site, for a total

of four traps per site for each weeklong sampling bout (Fig. 4.2). The ground and canopy trap placement was done to discern vertical distribution patterns for each species, which may be related to feeding patterns (Derraik et al. 2005b). Hence, species caught in the canopy may show a preference for feeding on roosting birds. Bird sampling was rotated between sites every two weeks (from E1 to I1, then E2 to I2) to minimise potential temporal variation in malaria prevalence, beginning March 1st through to the end of the study.

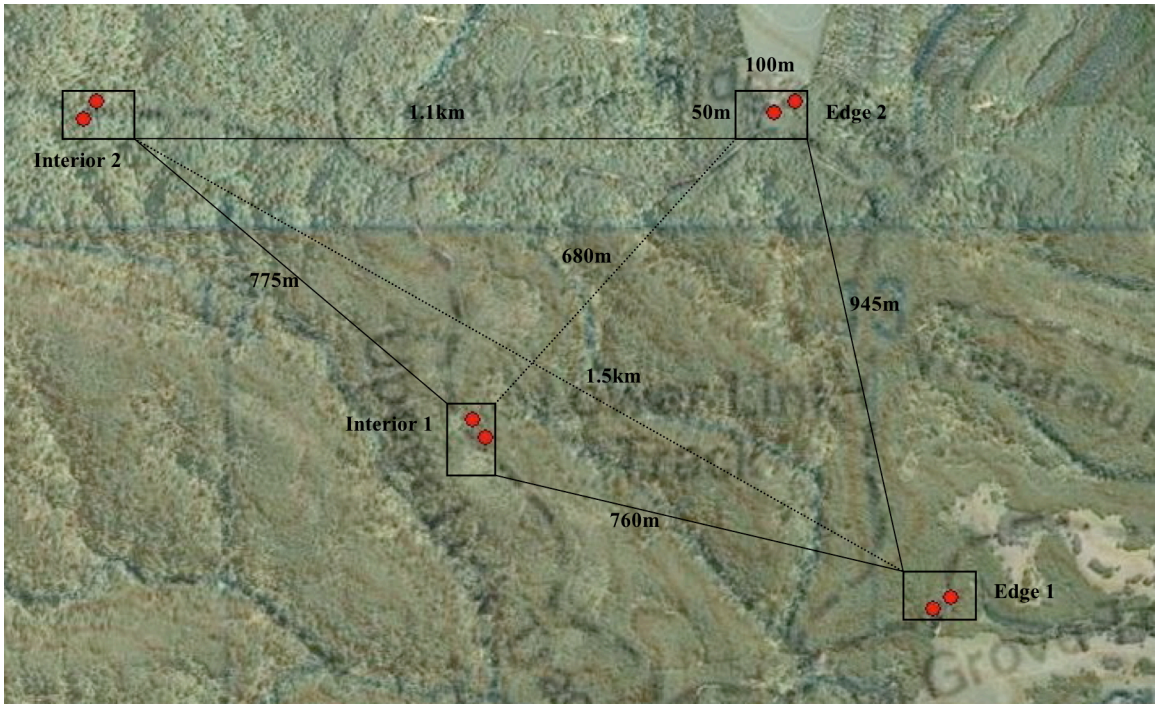


Figure 4.2. Map of the Waharau Regional Park forest edge and forest interior sites. Shown is site size (by Edge 2), distance between sites, and location of the mosquito traps (red dots).

Table 4.1. Key characteristics of each Waharau Regional Park site. The distance to edge and distance to stream measurements represent those to the nearest mosquito trap.

Site	Elevation (m)	Dist. to Edge	Dist. to Stream (m)	Canopy Species
E1	~120	20m	18	*Phyllocladus glaucus, Kunzea ericoides, Leptospermum scoparium*
E2	~150	24m	23	*Phyllocladus glaucus, Agathis australis, Kunzea ericoides, Leptospermum scoparium*
I1	~180	703m	27	*Dacrydium cupressinum, Agathis australis, Vitex lucens*, *Phyllocladus glaucus, Kunzea ericoides*
I2	~270	1.12km	31	*Nothofagus* spp., *Vitex lucens*, *Agathis australis*, *Dacrydium cupressinum*

4.3.3 Statistical analyses

4.3.3.1 Avian malaria prevalence

I used the same statistical procedure to analyse site differences in avian malaria prevalence as described in Section 3.3.3.1. I ran a log-linear model using a maximum likelihood estimator (PROC CATMOD, Statistical Analysis Systems, version 9.1, SAS Institute Inc., Cary, North Carolina, USA 27511) to test for prevalence differences (response variable 0/1) in silvereyes between sites (main effect). Each site was analysed separately to avoid confounding location (edge/interior) with time sampled.

4.3.3.2 Mosquito relative abundances

I analysed the environmental variables (relative humidity and temperature) in two stages. Within sites I used two-way ANOVAs (PROC GLM, Statistical Analysis Systems, version 9.1, SAS Institute Inc., Cary, North Carolina, USA 27511) to compare the effects of location (edge/interior) and height (ground/canopy) on relative humidity and temperature. If these factors were significant then the differences in relative humidity

and/or temperature between the in edge/interior or ground/canopy were calculated. These differences were then correlated against mosquito abundance. Paired t-tests were used to compare locations (one–tailed for humidity as relative humidity is always predicted to be higher within the forest, and two-tailed for temperature). Differences in humidity values between interior/edge locations and the difference in interior/edge abundance of mosquitoes were also correlated (Spearman's rank) across all sampling days.

The effects of location (edge/interior) and height (ground/canopy) on the relative abundance of mosquitoes were tested using a two-way ANOVA (PROC GLM, Statistical Analysis Systems, version 9.1, SAS Institute Inc., Cary, North Carolina, USA 27511). Samples from each trap were pooled across all nights sampled (1 week) to avoid pseudo-replication. Thus each trap had a single value for statistical analyses (i.e. 2 ground and 2 canopy traps per site). The data was not normally distributed and a square-root transformation of relative abundance was used to normalise the data. Model residuals were checked for normality and equal variance to meet the assumptions of ANOVA (all assumptions were met). A α-value of ≤ 0.05 was used as the standard for statistical significance.

Finally, as in the malaria prevalence analysis, each site was analysed separately to avoid confounding due to sites being sampled at different times. To test for differences between edge and interior within each site I ran planned contrasts (orthogonal) as part of the two-way ANOVA model (*i.e.* E1 vs. E2, I1 vs. I2). Additionally, since I2 appeared to be a distinct habitat type compared to the others, I ran a planned (orthogonal) contrast between I2 and the average of all other sites. Tukey's studentized range test was also included when main effects (location, height) were significant. All graphs and text

include reference to the untransformed values for ease of biological interpretation. A significance level of α-value of ≤ 0.05 was used for all statistical tests.

4.4 RESULTS

4.4.1 Exotic and native mosquitoes

A total of 580 mosquitoes comprised of five different species (two exotic and three native) were trapped (see Appendix 4.1 for species identification photographs and descriptions) in Waharau Regional Park. Table 4.2 shows the total number of individuals caught per and across sites by species and origin (exotic or native). Native species represented 92% (535/580) of the total capture and from most to least numerous were: *Aedes antipodeus*, *Culex pervigilans* and *Culex asteliae*. Together, the two most abundant species, *Aedes antipodeus* and *Cx. pervigilans*, comprised 83% (481/580) of the total capture. However, most *Ae. antipodeus* were caught in one site, I2, whereas *Cx. pervigilans* was captured at similar rates across all four sites. The exotic species caught in this study were *Aedes notoscriptus* and *Culex quinquefasciatus*. The total captures for both of these combined was lower than that for any of the native species, and only a single individual (*Ae. notoscriptus*) was trapped at a forest interior site (I1). Thus, the raw capture data showed three general trends: 1) native mosquitoes far outnumbered exotics in total captures within and across sites; 2) exotic mosquitoes were almost entirely absent from the forest interior site captures; and 3) I2 had by far the highest total captures, the vast majority of which were *Ae. antipodeus*. Finally, there was no difference in the average daily temperature between the edge and interior sites ($P = 0.156$), yet the average daily relative humidity was significantly higher in the interior *versus* the edge ($P =$

0.004). However, this variable was not significantly correlated with overall relative mosquito abundance in either the edge ($P = 0.513$) or interior ($P = 0.281$).

Table 4.2. The relative abundance of mosquito species captured at two forest edge and two forest interior sites in Waharau Regional Park. The nightly mean ± standard error is given per and across sites for each species and origin below the total number captured.

Species	**Site**				**Study Total**
	E1	**E2**	**I1**	**I2**	**Pooled Sites**
Aedes antipodeus	24	9	27	220	280
	3.43 ± 0.92	1.29 ± 0.57	3.86 ± 1.44	31.43 ± 6.96	20 ± 5.27
*Aedes notoscriptus**	16	10	1	0	27
	2.29 ± 1.09	1.43 ± 0.81	0.14 ± 0.14	0	1.93 ± 0.72
Culex asteliae	24	9	15	6	54
	3.43 ± 1.57	1.29 ± 0.57	2.14 ± 0.74	0.86 ± 0.55	3.86 ± 1.17
Culex pervigilans	58	58	44	41	201
	8.29 ± 2.83	8.29 ± 2.78	6.29 ± 2.07	5.86 ± 2.74	15.36 ± 3.52
*Culex quinquefasciatus**	11	7	0	0	18
	1.57 ± 0.97	1 ± 0.38	0	0	1.29 ± 0.51
Pooled Species	133	93	87	267	580
	19 ± 4.16	13.29 ± 2.99	12.43 ± 3.52	38.14 ± 7.58	41.43 ± 6.77
Origin					
Exotic	27	17	1	0	45
	3.86 ± 1.24	2.43 ± 0.61	0.14 ± 0.14	0	3.21 ± 0.70
Native	106	76	86	267	535
	15.14 ± 3.48	10.86 ± 2.43	12.29 ± 3.49	38.14 ± 7.58	38.21 ± 6.69

* = Exotic species

Both GLM models examining the dependent variable ‘abundance’ for exotic and native mosquitoes (against the factors of site, height, and site*height interaction) explained a statistically significant proportion of variance in the data (Table 4.3). Significant effects were found in both the site and height variables (Table 4.3, see Appendix 4.2 for full statistical outputs). However, the interaction term site*height in the

native model was also significant, and thus the effects of site and height on overall native mosquito abundance were not independent.

Table 4.3. GLM results for differences in exotic and native relative mosquito abundances per site and capture height. All values except R^2 are *P*.

Origin	Model		Categorical Variables		Interaction
	P	**R^2 (%)**	**Site**	**Height**	**Site*Height**
Exotic	<0.001	91.8	<0.001	0.022	0.167
Native	0.004	88.1	0.021	0.032	0.003

Further analysis of the site difference for the exotics showed relative abundance was significantly higher at the forest edge (E1, E2) *versus* the interior (I1, I2) (CONTRAST *P* = <0.001, and see Fig. 4.3). Thus, my null hypothesis of no difference in the relative abundance of exotic mosquitoes between forest edge and forest interior sites was rejected. I2 was also significantly different from the other sites because no exotic mosquitoes were caught there (CONTRAST *P* = <0.001); however the Tukey's analysis grouped the edge sites together (A: $mean_{E1}$ = 3.52, $mean_{E2}$ = 2.86) and the interior sites together (B: $mean_{I1}$ = 1.25, $mean_{I2}$ = 1). The significant height difference for the exotics was due to more individuals captured at ground level than in the canopy (Fig. 4.4.).

Further analysis of the site differences for the native species was more complex than that of the exotics. While there was no difference in the edge site means (CONTRAST *P* = 0.358), the interior sites were significantly different from each other (CONTRAST *P* = 0.010). This result was primarily due to the large number of *Ae. antipodeus* captures in I2 (Table 4.2, Fig. 4.3). Thus, significantly more natives were caught in I2 than the rest of the sites (CONTRAST *P* = 0.004). The forest edge *versus*

interior sites did not significantly differ (CONTRAST P = 0.053). Thus, my null hypothesis of no difference in the relative abundance of native mosquitoes between forest edge and forest interior sites could not be rejected. The Tukey's analysis placed I2 in a separate group (A: $mean_{I2}$ = 8.27) from I1 and E2 (B: $mean_{I1}$ = 5.61, $mean_{E2}$ = 5.28), with E1 overlapping all the other sites (BA: $mean_{E1}$ = 6.06). Finally, the significant interaction between site and height is mainly attributable to I2, where a large number were captured at ground level, as opposed to the other three sites where the native capture was consistently higher in the canopy (Fig. 4.4). Again, most of these ground captures were of *Ae. antipodeus* (see Sec. 4.4.3 below). Each mosquito species is analysed in depth separately in Sections 4.4.2-4.4.6 below.

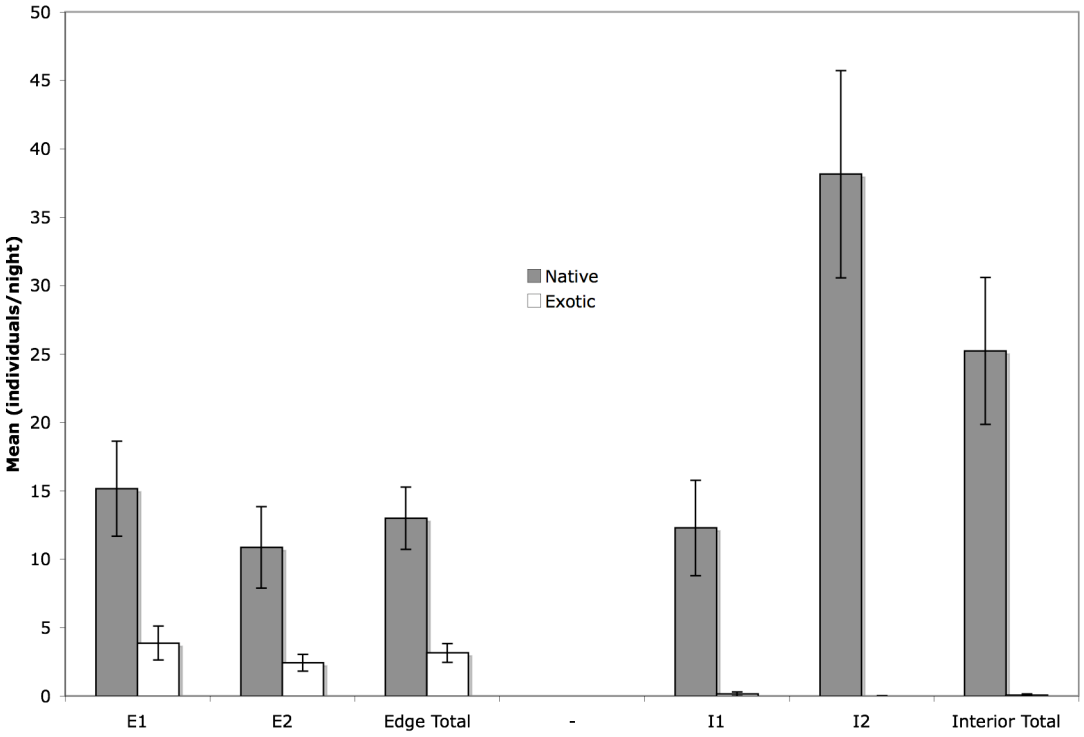


Figure 4.3. The mean number of exotic and native mosquitoes captured per night for each site and habitat location. Error bars represent the standard error of the mean.

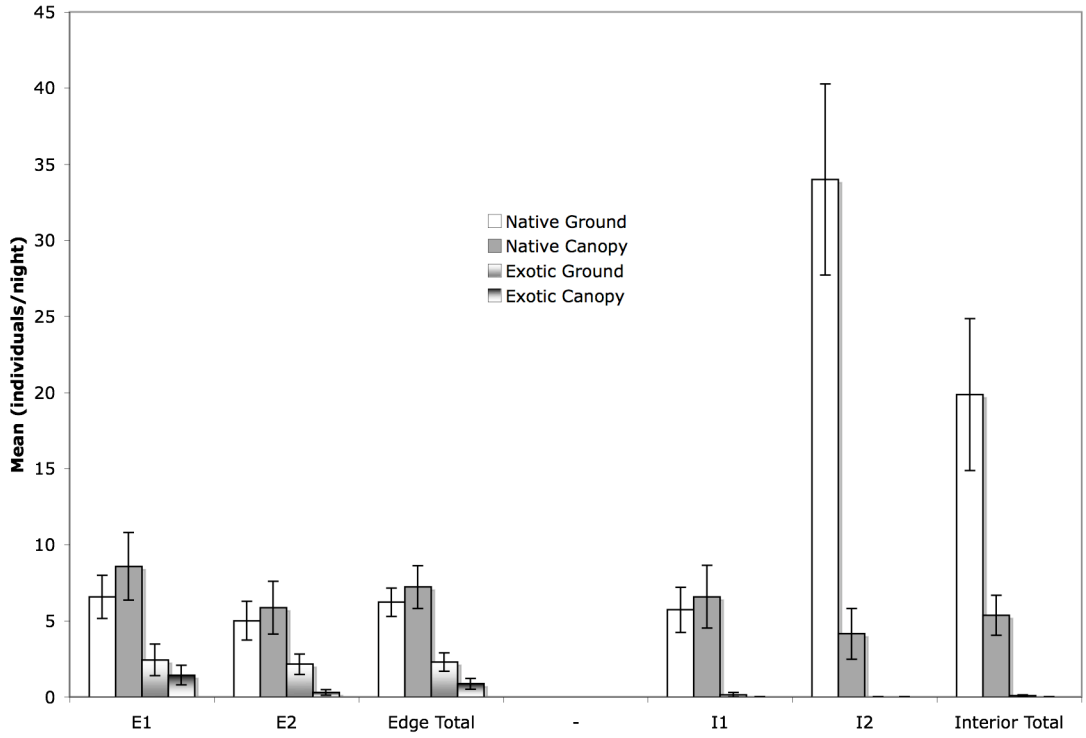


Figure 4.4. The mean number of exotic and native mosquitoes captured per night at canopy and ground level for each site and habitat location. Error bars represent the standard error of the mean.

4.4.2 *Aedes antipodeus*

The GLM model for *Aedes antipodeus* abundance explained a highly significant (P = <0.0001) proportion of variance in the data (R^2 = 96.4%, see Appendix 4.2 for full statistical outputs). Both the site (P = <0.001) and height (P = <0.001) relative abundances were significantly different. More individuals were caught in the interior, particularly at I2, than at the edge (Fig. 4.5). *Ae. antipodeus* was also consistently caught in higher numbers at ground *versus* canopy level across sites (Fig. 4.6). However, the interaction between site and height was also significant (P = <0.001), and thus these two variables were not independent of one another. This result was due to the large number of individuals captured at ground level in I2 (Fig. 4.6). Further analysis of the site differences showed the edge and interior sites were significantly different (CONTRAST P = <0.001). However, while the edge sites were not significantly different from each other (CONTRAST P = 0.053), the interior sites were (CONTRAST P = <0.001), and I2 was significantly different from all the other sites (CONTRAST P = <0.001). Again, the large number of individuals captured in I2 likely influenced each of the significant site results (Fig. 4.5). This is supported by the fact that the Tukey's analysis grouped I2 separately (A: $mean_{I2}$ = 6.62) from the rest of the sites (B: $mean_{E1}$ = 3.35, $mean_{I1}$ = 3.17, $mean_{E2}$ = 2.02).

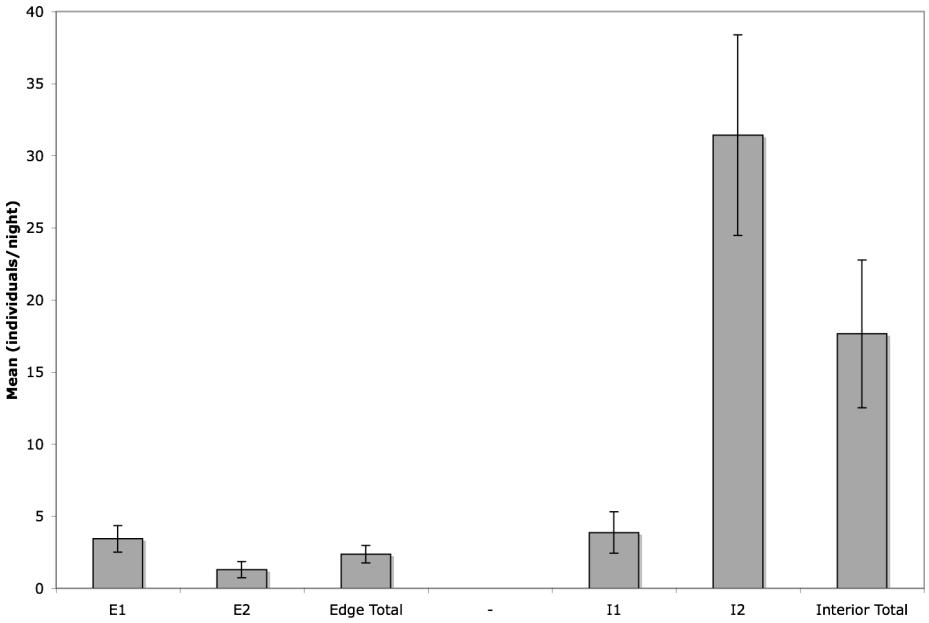


Figure 4.5. The mean number of *Aedes antipodeus* captured per night for each site and habitat location. Error bars represent the standard error of the mean.

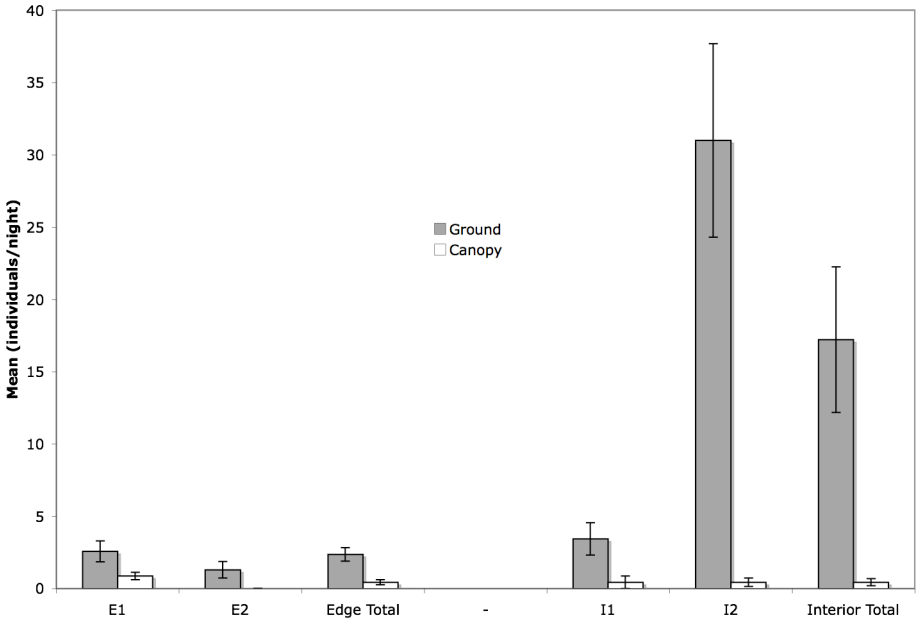


Figure 4.6. The mean number of *Aedes antipodeus* captured per night at canopy and ground level for each site and habitat location. Error bars represent the standard error of the mean**.**

4.4.3 *Aedes notoscriptus*

The GLM model for *Aedes notoscriptus* abundance explained a highly significant (P = 0.0014) proportion of the data variation (R^2 = 90.7%, see Appendix 4.2 for full statistical outputs). Both the site (P = <0.001) and height (P = 0.04) relative abundances were significantly different. Only one individual was caught in an interior site (I1), and the nightly mean captures were low in the edge sites throughout the study (Fig. 4.7). However, although *Ae. notoscriptus* was caught in higher numbers at ground *versus* canopy level in E2 the opposite trend was observed in E1 (Fig. 4.8). This led to a significant interaction between site and height (P = 0.013), and thus these two variables were not independent of one another. The two edge sites also differed significantly (CONTRAST P = 0.017, Fig. 4.7). This was supported by the Tukey's analysis, which also put E1 and E2 in a separate group (A: $mean_{E1}$ = 2.98, BA: $mean_{E2}$ = 2.06). Since no *Ae. notoscriptus* were caught in I2 and only one was trapped in I1, these interior sites were not significantly different from each other (CONTRAST P = 0.438). Moreover, the planned contrast between edge and interior sites indicated that these were significantly different (CONTRAST P = 0.0002). However, the Tukey's grouping indicated an overlap between E2 and the interior site I1 (BC: $mean_{I1}$ = 1.25). Thus, within the available sample size, the variation in relative abundances meant that there was no consistently supported significant edge *versus* interior habitat association pattern for *Ae. notoscriptus* *per se*, beyond its absence from I2.

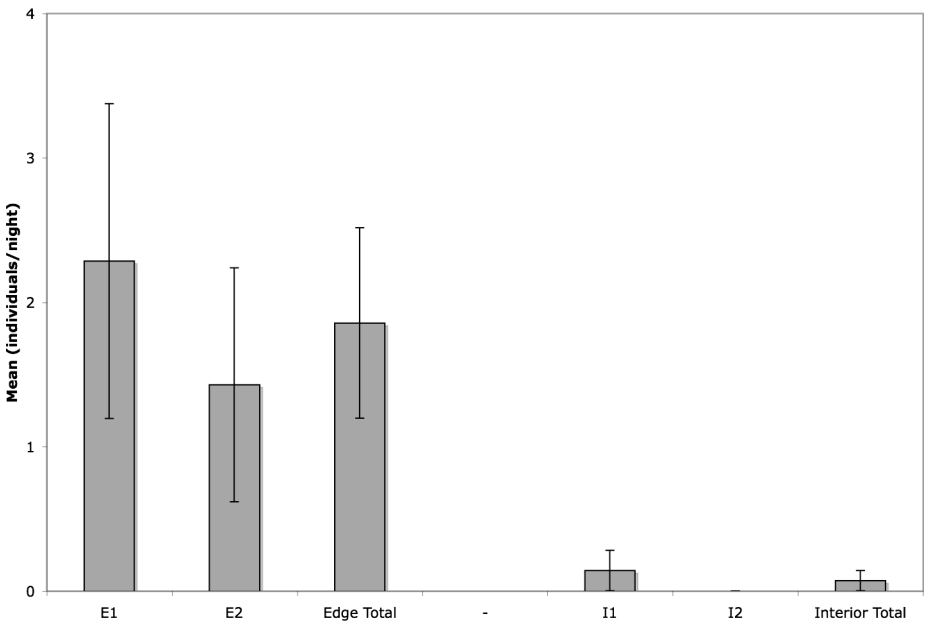


Figure 4.7. The mean number of *Aedes notoscriptus* captured per night for each site and habitat location. Error bars represent the standard error of the mean.

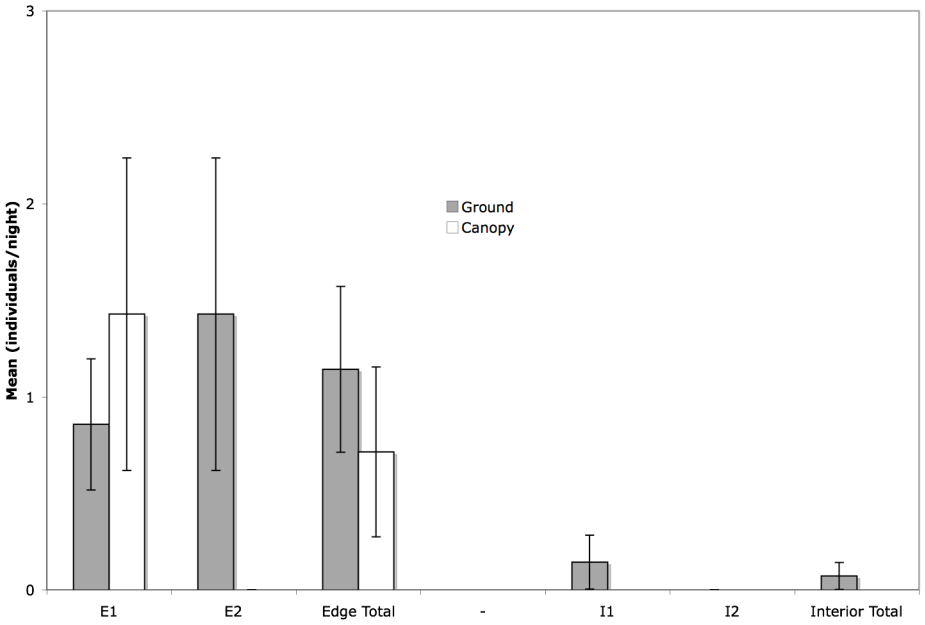


Figure 4.8. The mean number of *Aedes notoscriptus* captured per night at canopy and ground level for each site and habitat location. Error bars represent the standard error of the mean.

4.4.4 *Culex asteliae*

The GLM model for *Culex asteliae* abundance explained a moderate amount (R^2 = 65.6%), but not statistically significant proportion (P = 0.149), of the data variation (see Appendix 4.2 for full statistical outputs). The site relative abundances were not significantly different (P = 0.414). *Cx. asteliae* was caught in the highest numbers in E1, but at relatively low nightly means across all sites (Fig. 4.9). In addition, none of the planned site comparisons significantly differed, and the Tukey's analysis grouped all the site means together. However, height was significant in the model (P = 0.04). *Cx. asteliae* was caught in higher numbers in the canopy than at ground level across all sites (Fig. 4.10). Finally, the interaction between site and height was not significant (P = 0.887), and thus these two variables were independent of one another.

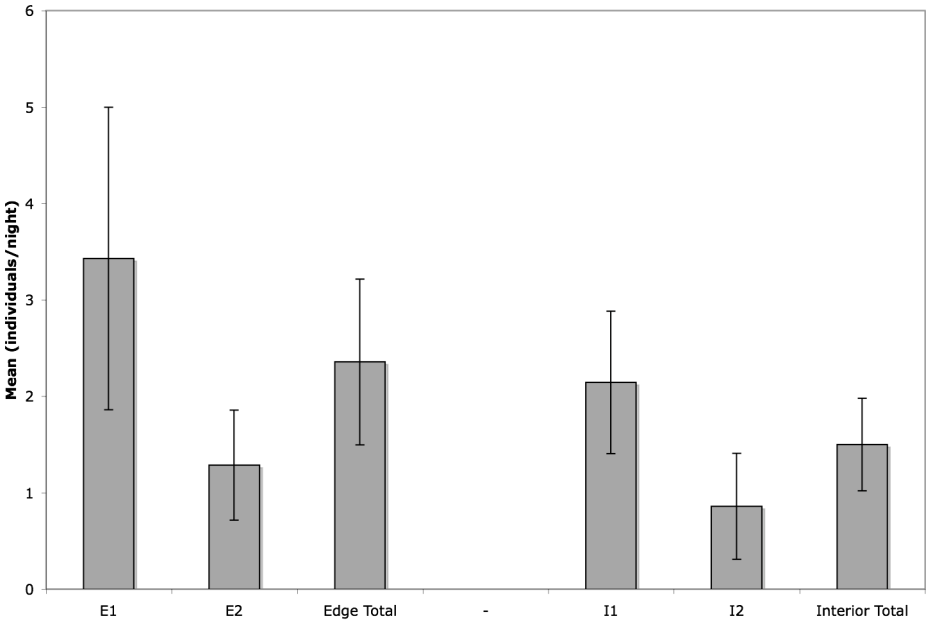


Figure 4.9. The mean number of *Culex asteliae* captured per night for each site and habitat location. Error bars represent the standard error of the mean.

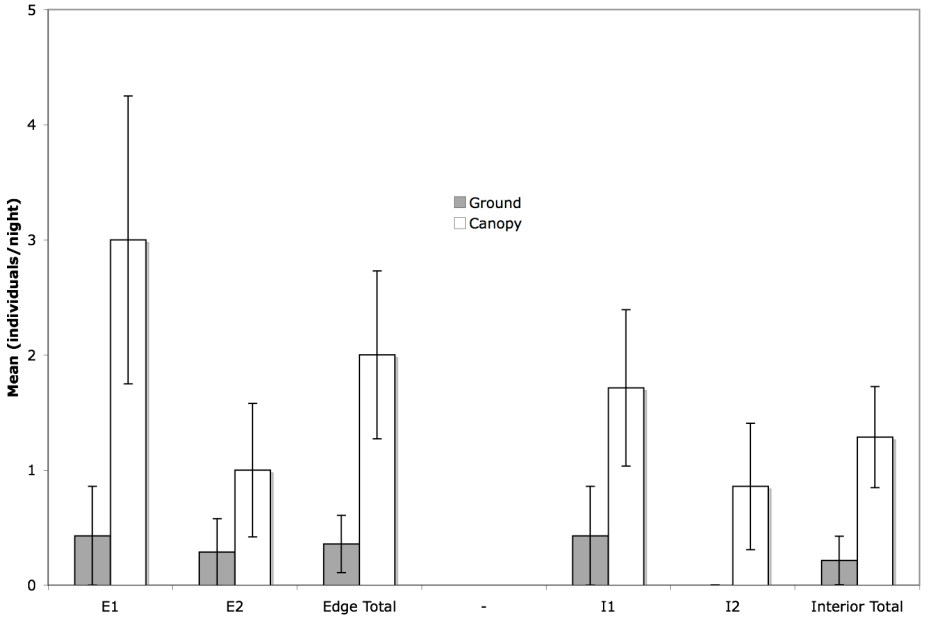


Figure 4.10. The mean number of *Culex asteliae* captured per night at canopy and ground level for each site and habitat location. Error bars represent the standard error of the mean.

4.4.5 *Culex pervigilans*

The GLM model for *Culex pervigilans* abundance failed to explain a statistically significant proportion of the data variation ($P = 0.637$, $R^2 = 39.8\%$, see Appendix 4.2 for full statistical outputs). Thus, neither the factors site ($P = 0.649$) and height ($P = 0.228$), nor the interaction between them ($P = 0.619$), adequately explained the relative abundance trends for *Cx. pervigilans*. This is unsurprising considering this species was caught in relatively high numbers throughout the sites (Fig. 4.11) and at both trapping heights (Fig. 4.12). In addition, none of the planned site comparisons significantly differed, and the Tukey's analysis grouped all the site means together. There was a consistent trend of edge sites capturing more individuals than interior sites (Fig. 4.11), and canopy traps capturing more individuals than ground traps (Fig. 4.12), but each had substantial amounts of standard error overlap.

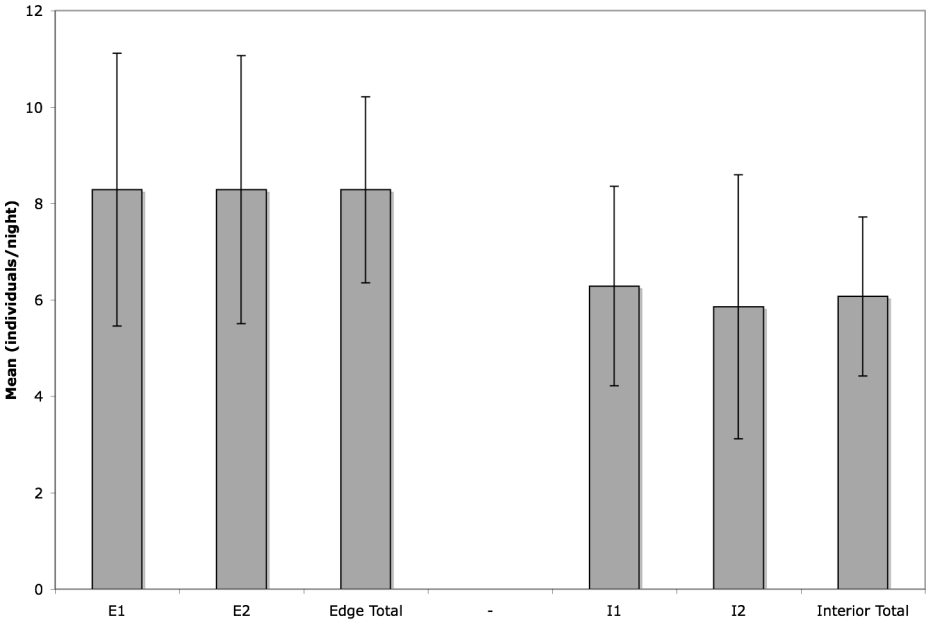


Figure 4.11. The mean number of *Culex pervigilans* captured per night for each site and habitat location. Error bars represent the standard error of the mean.

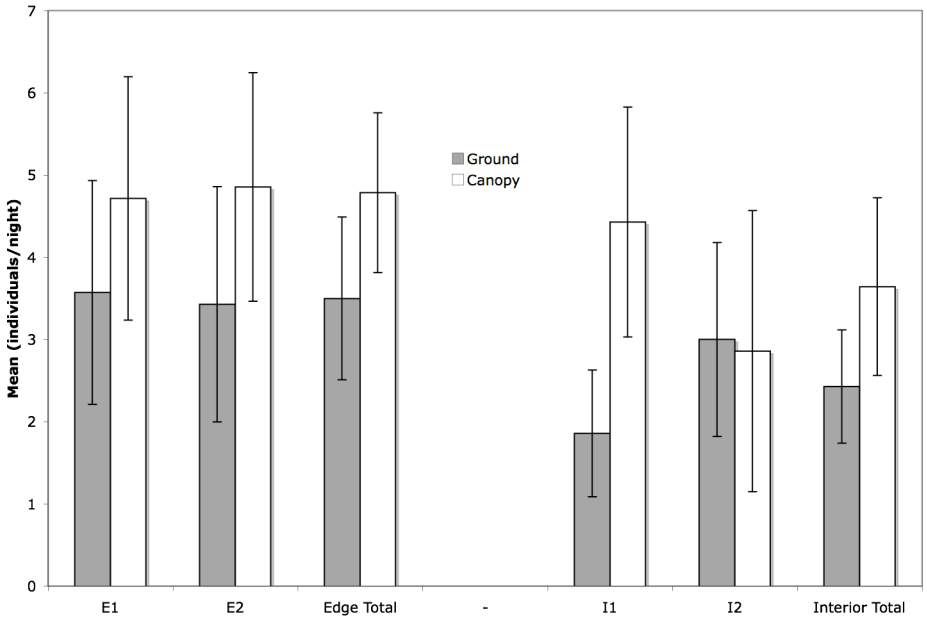


Figure 4.12. The mean number of *Culex pervigilans* captured per night at canopy and ground level for each site and habitat location. Error bars represent the standard error of the mean.

4.4.6 *Culex quinquefasciatus*

The GLM model for *Culex quinquefasciatus* abundance explained a significant (P = 0.035) proportion of the data variation (R^2 = 77.8%, see Appendix 4.2 for full statistical outputs). The site relative abundances were significantly different (P = 0.030), but not the height (P = 0.062). No *Cx. quinquefasciatus* were caught in the interior, and the nightly mean captures were low in the edge sites throughout the study (Fig. 4.13). Though *Cx. quinquefasciatus* was caught in higher numbers at ground level than in the canopy at the edge sites (Fig. 4.14), this difference was likely non-significant due to the small sample sizes. The interaction between site and height was not significant (P = 0.113), and thus these two variables were independent of one another. Unsurprisingly, edge and interior sites were significantly different (CONTRAST P = 0.005). None of the other planned comparisons were significant. Finally, the Tukey's analysis also put the edge sites in a separate group (A: mean_{E1} = 2.25, mean_{E2} = 2.04) from the interior sites (C: $\text{mean}_{I1\&I2}$ = 1). Thus, *Cx. quinquefasciatus* showed a clear habitat location pattern of relatively low abundances at the forest edge and absence from the interior.

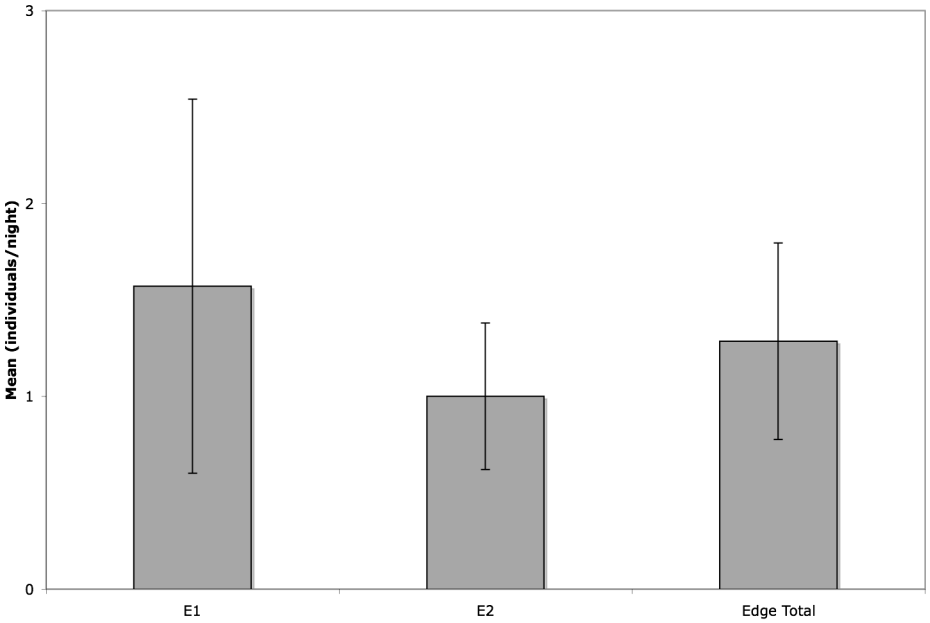


Figure 4.13. The mean number of *Culex quinquefasciatus* captured per night for each edge site. No individuals were caught in the interior sites. Error bars represent the standard error of the mean.

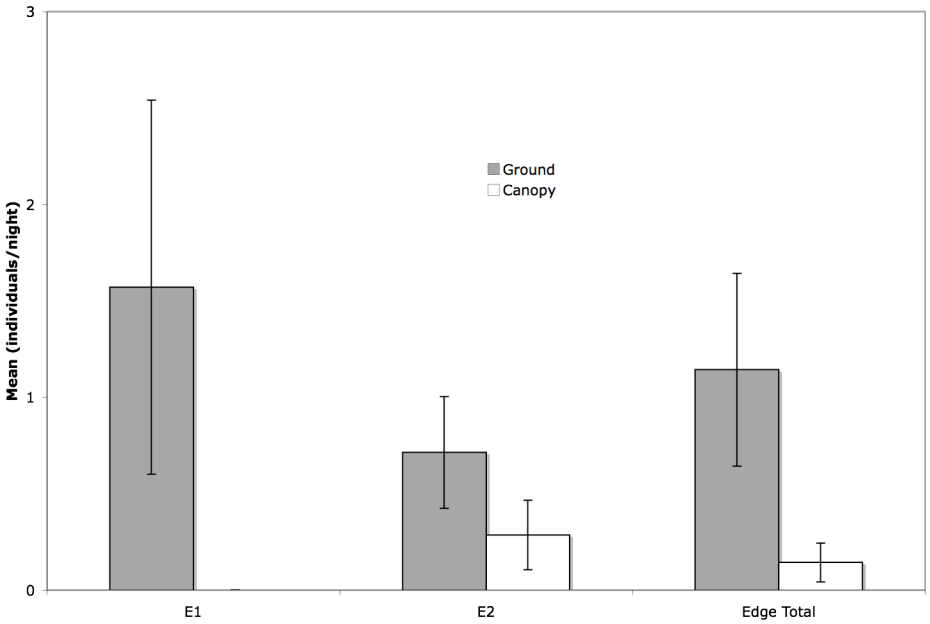


Figure 4.14. The mean number of *Culex quinquefasciatus* captured per night at canopy and ground level for each edge site. No individuals were caught in the interior sites. Error bars represent the standard error of the mean.

4.4.7 Avian malaria prevalence

A total of 22 of the 240 (9.17%) silvereyes captured across all sites were found to be positive for avian malaria (*Plasmodium*) via light microscopy. Table 4.4 summarises the prevalence data per site and habitat location.

Table 4.4. Avian malaria prevalence in silvereyes per site and habitat location in Waharau Regional Park: positive individuals per total sampled (+ve/*n*) and prevalence (prev %).

Site	+ve/n	Prev (%)	Parasite species
E1	4/23	17.39	*Plasmodium elongatum*
E2	7/93	7.53	*P. elongatum* (6), *P. relictum* (1)
Edge Total	11/116	9.48	*P. elongatum* (10), *P. relictum* (1)
I1	4/58	6.90	*P. elongatum*
I2	7/66	10.61	*P. elongatum* (6), *P. relictum* (1)
Interior Total	11/114	9.65	*P. elongatum* (10), *P. relictum* (1)
Study Total	22/240	9.17	*P. elongatum* (20), *P. relictum* (2)

The majority of infected birds (N = 20) harboured a parasite identified as *Plasmodium* (*Huffia*) *elongatum* (see Chapter 2). The other two infections were identified as *P.* (*Haemamoeba*) *relictum* (see Chapter 2), which was found in a single individual from each of sites E2 and I2. A total of 11 birds were recaptured throughout the course of the study, all within the same site in which they were initially sampled. E1, the site where the greatest numbers of exotic mosquitoes were collected also recorded the highest malaria prevalence, but the lowest avian host sample size. Moreover, the site differences in prevalence were not significant ($\chi^2 = 0.06$, $P = 0.801$, see Appendix 4.3 for full statistical outputs). Thus, my null hypothesis of no difference in malaria prevalence in silvereyes between forest edge and forest interior sites could not be rejected.

4.5 DISCUSSION

Parasite prevalence, number of available bird hosts, and the abundance and competency of available mosquito vectors determine the opportunity for avian malaria transmission in any given landscape. Thus, any anthropogenic landscape alteration that influences the species composition and density of potential mosquito vectors would be expected to affect malarial parasite-host dynamics. In New Zealand, deforestation and subsequent agricultural development have altered historically large, contiguous tracts of forest into fragmented patches of varying sizes, especially on the North Island (Ewers et al. 2006). One consistent outcome of such forest fragmentation is the creation of edge habitat, often abutting agricultural lands in New Zealand, which generally increases as forest size decreases (Young and Mitchell 1994). Edge habitats are prone to invasion by exotic species (Center et al. 1995), and this is particularly true for mosquitoes (see Derraik and Slaney 2007 for a review). Thus, this study sought to examine potential changes in mosquito abundance and composition at forest edge sites as compared to sites deeper in the forest interior, and if these potential changes are affecting avian malaria prevalence in the silvereye, an abundant passerine species in the forest. The underlying assumptions of this study were: 1) that the sites sampled were characteristic of the forest edge and forest interior in Waharau Regional Park and 2) that the differing elevation between edge and interior sites was not influencing any potential differences in mosquito abundance and composition (see Methods section 4.3.2, 2nd paragraph as to why this was unlikely). Given these assumptions, the results show differences in mosquito abundance and composition between sites, most notably the presence of exotic species at the forest edge and their almost complete absence from the forest interior. In addition, the large

total number of mosquitoes trapped over a relatively short time frame demonstrates mosquito activity remains high during the early autumn months in New Zealand.

4.5.1 Exotic mosquito species

This study found a significant difference in the total relative abundance of exotic mosquitoes (*Ae. notoscriptus* and *Cx. quinquefasciatus*) between forest edge and forest interior sites in Waharau Regional Park. Relatively low numbers of exotics were caught at edge sites throughout the entire sampling period and only a single individual (*Ae. notoscriptus*) was caught in the interior. However, analysis of the two species individually revealed that the edge *versus* interior result was only significant for *Cx. quinquefasciatus.* This edge-association corroborates the findings of Reiter and LaPointe (2007) in Hawaii, where *Cx. quinquefasciatus* abundance was positively correlated with increased forest fragmentation and the amount of agriculture surrounding a trap site. This species has been shown to utilise man-made containers for breeding in the Auckland Region (Derraik 2004b), and thus livestock water troughs or drainage pipes in the pasturelands surrounding Waharau may be providing increased larval habitat (*e.g.* Leisnham et al. 2005), whereby adult females then invade the forest edge to forage. However, this potential link requires further study.

Ae. *notoscriptus* utilises a variety of breeding habitats in New Zealand, including artificial containers, such as used tyres, empty pots, and drainage pipes commonly found in urban and modified landscapes (Leisnham et al. 2004, Derraik 2005, Derraik et al. 2008b), and tree holes and bromeliads in forested landscapes (Derraik 2005, Derraik 2009). Such breeding versatility has likely facilitated its status as the most widespread

and abundant exotic mosquito in both modified and forested landscapes in New Zealand (Derraik and Slaney 2007). Yet this species was restricted to the two edge sites beyond a single occurrence in the interior in this study, though the edge-association was not significant due to low sample sizes. This result suggests *Ae. notoscriptus* may be utilising artificial containers in the pastureland adjacent to the edge sites for breeding, and adult females are then foraging at the forest edge. The trapping of a single individual in the interior may be a case of wind-assisted dispersal, as a mark-recapture study of *Ae. notoscriptus* in Australia by Watson et al. (2000) estimated the mean travel distance of adult females at less than 200m, with a maximum under 250m. Thus if this species is restricted to the edge in this study as the results suggest, the female trapped in the interior would have travelled over three times the maximum distance estimated by Watson et al. (2000), as the nearest edge site from I1 where the individual was trapped is approximately 680m away. Nonetheless, additional replication and a larger sample size are needed to determine if the distribution trend found in this study is indicative of an edge-association for *Ae. notoscriptus* adult females.

The low relative abundance of exotic mosquitoes caught at the edge sites suggests their densities may be low around Waharau; and their almost complete absence in the interior sites suggests they are either yet to invade the forest interior, or are restricted to modified habitats. This supposition is supported by other vector studies on the North Island that have found exotic mosquitoes are more abundant in urban areas (Derraik 2004b, Derraik 2005, Derraik et al. 2005a, Leisnham et al. 2005), infrequent in modified landscapes (Leisnham et al. 2004, Leisnham et al. 2005, Derraik et al. 2007), and almost completely absent from large tracts of native forest (Derraik et al. 2005a, Derraik et al.

2005b). Alternatively, adult females of the exotic species may be exhibiting an aversion to the CO_2/light traps. While this would not account for the differences between edge and interior since the same traps were used at each site, if *Ae. notoscriptus* and *Cx. quinquefasciatus* are averse to this capture method it could explain the low relative abundances of these species. However, this seems unlikely, since a number of the aforementioned vector studies that support this distribution used larval rather than adult mosquito survey data (*e.g.* Derraik 2004b, Leisnham et al. 2004, Derraik 2005, Derraik et al. 2005b Leisnham et al. 2005).

Finally, when pooled together, the exotic mosquito species were more likely to be captured at ground *versus* canopy level. However when the two species were individually analysed, this result was only significant for *Ae. notoscriptus*. Yet, this result was not consistent among the edge sites, as no individuals were caught in the canopy at E2. Possible explanations for this may be there were fewer hosts present, higher wind exposure, or displacement of *Ae. notoscriptus* by other species in the canopy at E2. The latter seems most likely since a number of native individuals and a few *Cx. quinquefasciatus* were caught in the canopy at E2. However, the capture of several *Ae. notoscriptus* in the canopy at E1, in addition to ground level at both edge sites, supports previous work by Derraik et al. (2005b, 2008b) showing this species has a wide vertical distribution in forested landscapes. It is important to point out that the capture of both *Ae. notoscriptus* and *Cx. quinquefasciatus* in the canopy suggests these species may be taking blood meals from roosting birds in Waharau. *Cx. quinquefasciatus* is a well-known avian feeder and important avian malaria vector (van Riper III et al. 1986). *Ae. notoscriptus* has been captured with a blood meal containing avian malaria in New Caledonia, though its

competency as a vector remains unclear (Ishtiaq et al. 2008) and requires further research.

4.5.2 Native mosquito species

The overall relative abundance of native mosquitoes was higher in the interior *versus* the edge. Yet further analysis showed this result was due to the most abundant species in this study, *Aedes antipodeus*, being caught in very high numbers in site I2. Similarly, the majority of these individuals were caught at ground level, which led to significantly more native individuals at ground *versus* canopy level. Derraik et al. (2005b) found a nearly identical result in a large tract of mature native forest in a different part of the Auckland Region (Waitakere Ranges, West Auckland), with *Ae. antipodeus* making up the majority of captures, mostly at ground level. Site I2 was characterised by the most mature canopy and highly developed understory of all the sites. Given *Ae. antipodeus* breeds in temporary ground pools in areas with partial or dense shade (Belkin 1968), the results of this study and Derraik et al. (2005b) suggest relatively pristine, mature forests in New Zealand may offer ample amounts of such breeding pools, which in turn lead to numerous adult females being caught in ground traps. However, the feeding preferences of *Ae. antipodeus* remain unknown (Belkin 1968, Derraik et al. 2005b), as does its ability to vector avian malaria.

Neither of the other two native species caught in this study, *Culex asteliae* and *Cx. pervigilans*, showed any significant site abundance patterns. However, the vast majority of *Cx. asteliae* were caught at canopy level. This finding corroborates previous work by Derraik et al. (2005b), who collected adult females of this species for the first time in the

wild, predominately in the canopy. Larvae of this species are often found near ground level (Belkin 1968), and thus the collection of adult females at canopy level may reflect their avian host specificity given New Zealand's historic lack of land mammals (Derraik et al. 2005b). Yet, the competency of *Cx. asteliae* as an avian malaria vector remains unconfirmed. In contrast, *Cx. pervigilans*, which was ubiquitous throughout the study sites and caught in relatively high numbers in both canopy and ground traps, is believed to be an efficient vector of avian malaria (Holder et al. 1999, Massey et al. 2007).

4.5.3 Avian malaria prevalence

The overall prevalence of avian malaria (*P. elongatum* and *P. relictum*) in silvereyes was low in this study (22/240, 9.17%) and did not significantly differ between sites. One possible explanation for the lack of prevalence difference is the relatively high abundance of *Cx. pervigilans* throughout the study sites. This species was often caught in the canopy, and thus may be vectoring avian malaria to roosting silvereyes at both the forest edge and interior. Another possibility may be that silvereyes, which are a flocking species (Kikkawa 1961), are potentially roosting in an aggregated fashion common in other flocking species (Emlen, Jr. 1952) and foraging in different areas of the forest during the day. As mosquito vectors would be biting individuals in an aggregated group at night, and these individuals foraging at different areas of the forest by day (some at the edge and others the interior), this situation would lead to the lack of prevalence difference at the sites sampled in this study. However, mass roosting behaviour has not been substantiated in this species. Alternatively, silvereye flocks may be moving throughout the forest during the day, which would violate the assumption that each site is a spatially

explicit area. Thus, individuals would be equally likely to be caught in any given area of the forest, regardless of infection status. However, the fact that 11 different individuals were each recaptured at the same site in which they were originally sampled supports the assumption that flocks are remaining in relatively localised areas.

4.6 CONCLUSION

This study shows that differences in potential vector abundance and composition between forest edge and interior sites do not necessarily equate to differences in avian malaria prevalence. However, this study was limited to a simple correlation of these factors, and there are a number of ways this research could be substantially advanced. For instance, a limitation to this study is that the competency of three of the mosquito species caught (*Ae. antipodeus*, *Ae. notoscriptus* and *Cx. asteliae*) remains unknown. Given that all three were present in the canopy, and *Ae. notoscriptus* and *Cx. asteliae* are confirmed avian feeders, research into their ability to vector a variety of avian malaria species is highly recommended. This would ideally be pursued both in captivity (*e.g.* Kim et al. 2009) and in the field (*e.g.* Ishtiaq et al. 2008, Kim and Tsuda 2010, Kimura et al. 2010). In captivity, adult female mosquitoes of unknown vector competency are exposed to avian malaria-infected birds, and then recaptured after taking a blood meal. In the field, mosquitoes are collected via bird-baited or CO_2/light traps and those carrying a blood meal used for analysis. For both study types, the host blood in the abdomen and head of engorged females is amplified via PCR using malaria-specific markers. Presence of malarial parasites in the salivary glands represents the infective stage of transmission (Atkinson 1999), and thus is used to confirm vector competency (Kim et al. 2009).

It would also be informative to pursue field investigations incorporating a greater range of avian hosts and malarial parasites. The silvereye was self-introduced to New Zealand in the 1800's, and it is unknown if the *P. elongatum* identified in this study arrived with them or was encountered after arrival (R.K. Barraclough, Massey University, pers. comm.). Native and introduced birds of New Zealand carry a range of malarial parasites (see Chapter 2), and a comprehensive study that addresses vector-parasite-host dynamics using both molecular and microscopic techniques would significantly advance our understanding of this system. Landscape and habitat comparisons, similar to the forest edge *versus* interior one used in this study, can be incorporated into comprehensive avian malaria studies of this nature, as researchers have recently done in Africa (*e.g.* Bonneaud et al. 2009, Chasar et al. 2009, Loiseau et al. 2010).

The significantly higher number of *Cx. quinquefasciatus* at the forest edge *versus* interior, albeit at low relative abundances, is of concern with regards to avian malaria. This species was responsible for the avian malaria epizootic in Hawaii (van Riper III et al. 1986), and thus its presence at the forest edge may alter future disease dynamics by increasing transmission of the parasites identified in this study or through the introduction of new parasite strains to Waharau. However, the results of this study indicate *Cx. quinquefasciatus* may not be playing a major role in avian malaria transmission to silvereyes in Waharau at this time, likely due to its low abundance.

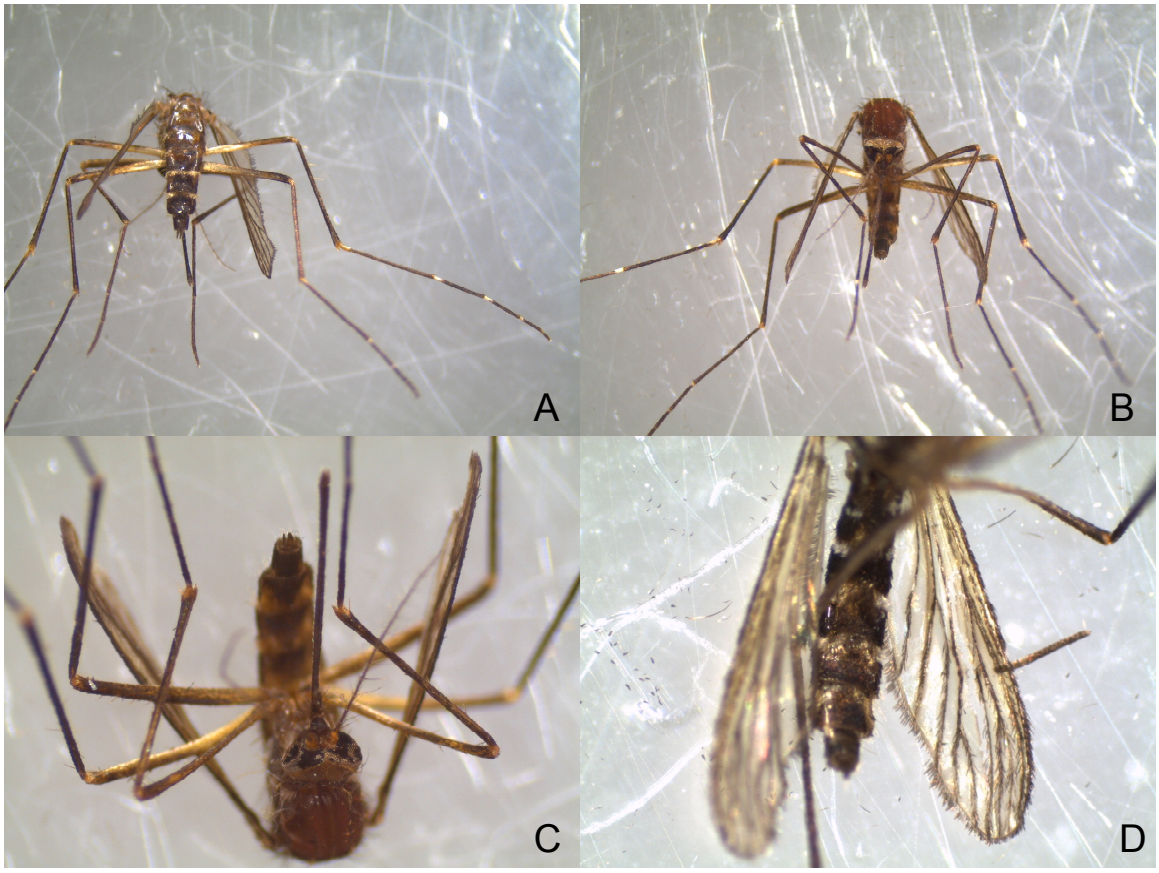
Finally, this study supports the notion that reduction in forest size and its concomitant creation of more edge habitat favours the introduction of exotic mosquitoes. This is an important consideration not only for bird conservation in New Zealand as

discussed throughout this study, but also from a human health perspective. The exotic mosquitoes trapped in this study are known vectors of West Nile Virus (*Cx. quinquefasciatus*) and Ross River Virus (*Ae. notoscriptus*) (see Derraik and Slaney 2007). Thus, the protection and/or restoration of large tracts of forest in New Zealand may mitigate future disease concerns for both wildlife and human health.

Appendix 4.1 Mosquito species identification photographs and descriptions

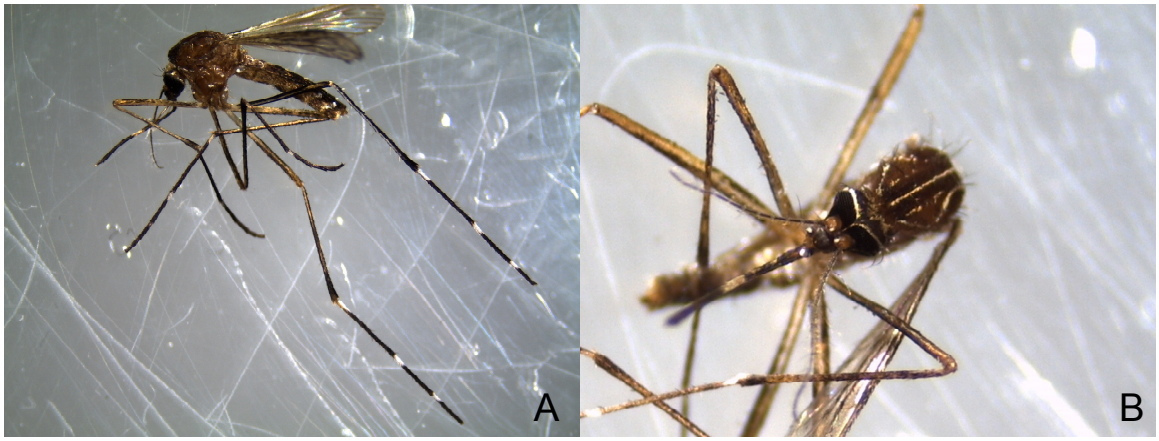
The following descriptions of the mosquito species identified in this study are based on Snell (2005). All photographs are of individuals trapped at Waharau Regional Park and were taken by the author.

Aedes antipodeus



- *Ae. antipodeus* is the only native *Aedes* species present on the North Island; the similar *Ae. subalbirostris* is only found in the south-eastern portion of the South Island
- Distinct pale banding on the tarsi in addition to an inconspicuous knee spot (A, B)
- Hind femur with pale scale on basal half to two-thirds ventral side (A, C)
- Abdominal sternites (underside) dark-scaled with basal lateral patches (C, D)
- Proboscis all dark-scaled (C)

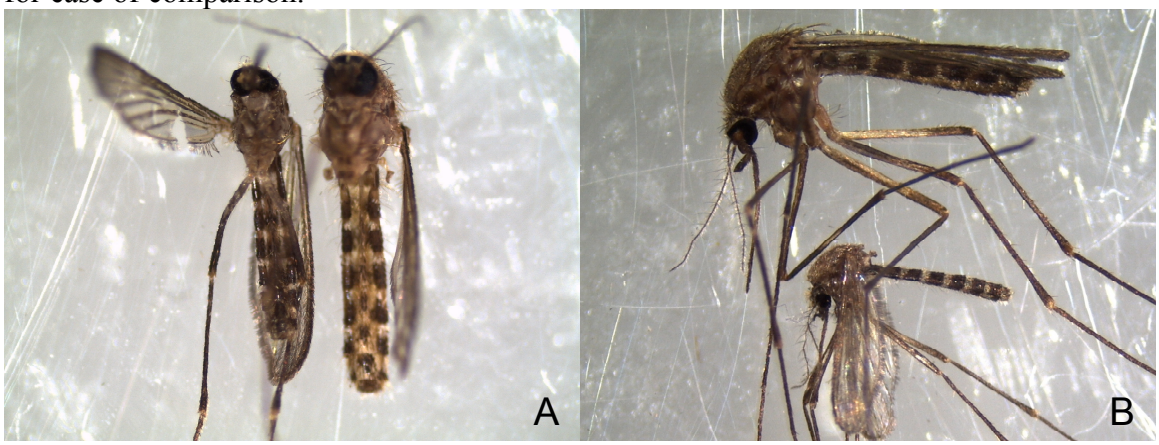
Aedes notoscriptus



- *Ae. notoscriptus* is easily distinguishable from all other mosquito species in New Zealand due to a distinct pale band in the middle of its proboscis (A, B)
- Strong white or pale banding on the tarsi (A)
- "Lyre"-shaped silvery scaling on the scutum (B)

Culex asteliae and *Cx. pervigilans*

These two species are part of the *Culex pervigilans* species complex and can be difficult to distinguish. Thus, I've provided pictures and descriptions of these two species together for ease of comparison.



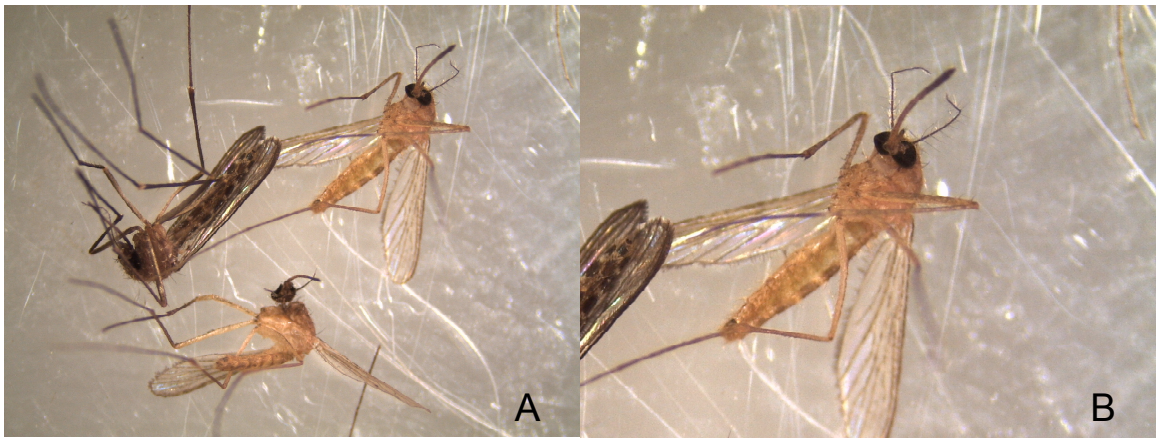
Cx. asteliae (left) and *Cx. pervigilans* (right) *Cx. pervigilans* (top) and *Cx. asteliae* (bottom)

- The best identification character for *Cx. pervigilans* is the distinct medial dark patches on its abdominal sternites (A); *Cx. asteliae* has mostly dark-scaled sternites (A)

- *Cx. asteliae* is smaller in size than *Cx. pervigilans* (A, B), and the pale bands on its abdominal tergites are generally wider than *Cx. pervigilans* (B)

Culex quinquefasciatus

This species is relatively distinctive, however, I've included comparative pictures with *Cx. pervigilans* to accentuate the main characters.



- *Cx. quinquefasciatus* is a lighter brown than the other *Culex* spp. present in New Zealand (A)

- Abdominal sternites are largely pale-scaled (B)

- Proboscis is three-fourths pale-scaled basally on the ventral side, and the rest dark-scaled (B)

Appendix 4.2. Statistical outputs for the mosquito data

EXOTIC SPECIES MODEL

The GLM Procedure

Class Level Information

Class	Levels	Values
Site	4	E1 E2 I1 I2
Height	2	Canopy Ground

Dependent Variable: Abun_1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	21.60570244	3.08652892	12.72	0.0009
Error	8	1.94141850	0.24267731		
Corrected Total	15	23.54712094			

R-Square	Coeff Var	Root MSE	Abun_1 Mean
0.917552	22.82841	0.492623	2.157938

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Site	3	18.06031319	6.02010440	24.81	0.0002
Height	1	1.94811806	1.94811806	8.03	0.0220
Site*Height	3	1.59727119	0.53242373	2.19	0.1665

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
E1 vs E2	1	0.86395513	0.86395513	3.56	0.0959
I1 vs I2	1	0.12500000	0.12500000	0.52	0.4934
E1 E2 vs I1 I2	1	17.07135806	17.07135806	70.35	<.0001
I2 vs all	1	7.15103602	7.15103602	29.47	0.0006

Tukey's Studentized Range (HSD) Test for Abun_1

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.242677
Critical Value of Studentized Range	4.52880
Minimum Significant Difference	1.1155

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Site
A	3.5195	4	E1
A	2.8623	4	E2
B	1.2500	4	I1
B	1.0000	4	I2

NATIVE SPECIES MODEL

The GLM Procedure

Class Level Information

Class	Levels	Values
Site	4	E1 E2 I1 I2
Height	2	Canopy Ground

Dependent Variable: Abun_1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	74.36340644	10.62334378	8.43	0.0037
Error	8	10.07828550	1.25978569		
Corrected Total	15	84.44169194			

R-Square	Coeff Var	Root MSE	Abun_1 Mean
0.880648	17.79736	1.122402	6.306563

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Site	3	21.78446219	7.26148740	5.76	0.0213
Height	1	8.51910156	8.51910156	6.76	0.0316
Site*Height	3	44.05984269	14.68661423	11.66	0.0027

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
E1 vs E2	1	1.19970050	1.19970050	0.95	0.3577
I1 vs I2	1	14.11664113	14.11664113	11.21	0.0101
E1 E2 vs I1 I2	1	6.46812056	6.46812056	5.13	0.0532
I2 vs all	1	20.57617352	20.57617352	16.33	0.0037

Tukey's Studentized Range (HSD) Test for Abun_1

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	1.259786
Critical Value of Studentized Range	4.52880
Minimum Significant Difference	2.5416

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Site
A	8.2708	4	I2
B A	6.0580	4	E1
B	5.6140	4	I1
B	5.2835	4	E2

AEDES ANTIPODEUS MODEL

The GLM Procedure

Class Level Information

Class	Levels	Values
Site	4	E1 E2 I1 I2
Height	2	Canopy Ground

Dependent Variable: Abun_1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	150.0243230	21.4320461	30.76	<.0001
Error	8	5.5739740	0.6967467		
Corrected Total	15	155.5982970			

R-Square	Coeff Var	Root MSE	Abun_1 Mean
0.964177	22.03428	0.834714	3.788250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Site	3	47.01673100	15.67224367	22.49	0.0003
Height	1	59.50579600	59.50579600	85.41	<.0001
Site*Height	3	43.50179600	14.50059867	20.81	0.0004

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
E1 vs E2	1	3.56979200	3.56979200	5.12	0.0534
I1 vs I2	1	23.90169800	23.90169800	34.30	0.0004
E1 E2 vs I1 I2	1	19.54524100	19.54524100	28.05	0.0007
I2 vs all	1	42.82740833	42.82740833	61.47	<.0001

Tukey's Studentized Range (HSD) Test for Abun_1

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.696747
Critical Value of Studentized Range	4.52880

Minimum Significant Difference 1.8901

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Site
A	6.6220	4	I2
B	3.3510	4	E1
B	3.1650	4	I1
B	2.0150	4	E2

AEDES NOTOSCRIPTUS MODEL

The GLM Procedure

Class Level Information

Class	Levels	Values
Site	4	E1 E2 I1 I2
Height	2	Canopy Ground

Dependent Variable: Abun_1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	14.64259175	2.09179882	11.16	0.0014
Error	8	1.49969800	0.18746225		
Corrected Total	15	16.14228975			

R-Square	Coeff Var	Root MSE	Abun_1 Mean
0.907095	23.74222	0.432969	1.823625

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Site	3	9.63993475	3.21331158	17.14	0.0008
Height	1	1.12042225	1.12042225	5.98	0.0403
Site*Height	3	3.88223475	1.29407825	6.90	0.0131

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
E1 vs E2	1	1.70570450	1.70570450	9.10	0.0166
I1 vs I2	1	0.12500000	0.12500000	0.67	0.4378
E1 E2 vs I1 I2	1	7.80923025	7.80923025	41.66	0.0002
I2 vs all	1	3.61791008	3.61791008	19.30	0.0023

Tukey's Studentized Range (HSD) Test for Abun_1

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.187462
Critical Value of Studentized Range	4.52880
Minimum Significant Difference	0.9804

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Site
A	2.9840	4	E1
B A	2.0605	4	E2
B C	1.2500	4	I1
C	1.0000	4	I2

CULEX ASTELIAE MODEL

The GLM Procedure

Class Level Information

Class	Levels	Values
Site	4	E1 E2 I1 I2
Height	2	Canopy Ground

Dependent Variable: Abun_1

Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	7	14.15743644	2.02249092	2.18	0.1492
Error	8	7.43882650	0.92985331		
Corrected Total	15	21.59626294			

R-Square	Coeff Var	Root MSE	Abun_1 Mean
0.655550	39.79629	0.964289	2.423063

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Site	3	2.98874319	0.99624773	1.07	0.4141
Height	1	10.58363556	10.58363556	11.38	0.0097
Site*Height	3	0.58505769	0.19501923	0.21	0.8869

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
E1 vs E2	1	0.97371013	0.97371013	1.05	0.3361
I1 vs I2	1	1.42636050	1.42636050	1.53	0.2506
E1 E2 vs I1 I2	1	0.58867256	0.58867256	0.63	0.4492
I2 vs all	1	2.01105469	2.01105469	2.16	0.1796

Tukey's Studentized Range (HSD) Test for Abun_1

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.929853
Critical Value of Studentized Range	4.52880
Minimum Significant Difference	2.1835

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Site
A	2.9638	4	E1
A	2.6535	4	I1
A	2.2660	4	E2
A	1.8090	4	I2

CULEX PERVIGILANS MODEL

The GLM Procedure

Class Level Information

Class	Levels	Values
Site	4	E1 E2 I1 I2
Height	2	Canopy Ground

Dependent Variable: Abun_1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	4.60990075	0.65855725	0.76	0.6372
Error	8	6.96630900	0.87078863		
Corrected Total	15	11.57620975			

R-Square	Coeff Var	Root MSE	Abun_1 Mean
0.398222	21.01299	0.933161	4.440875

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Site	3	1.49562525	0.49854175	0.57	0.6488
Height	1	1.48474225	1.48474225	1.71	0.2279
Site*Height	3	1.62953325	0.54317775	0.62	0.6194

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
E1 vs E2	1	0.00082012	0.00082012	0.00	0.9763
I1 vs I2	1	0.02343612	0.02343612	0.03	0.8738
E1 E2 vs I1 I2	1	1.47136900	1.47136900	1.69	0.2298
I2 vs all	1	0.68115675	0.68115675	0.78	0.4023

Tukey's Studentized Range (HSD) Test for Abun_1

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	*0.05*
Error Degrees of Freedom	*8*
Error Mean Square	*0.870789*
Critical Value of Studentized Range	*4.52880*
Minimum Significant Difference	*2.113*

Means with the same letter are not significantly different.

Tukey Grouping	*Mean*	*N*	*Site*
A	*4.7543*	*4*	*E2*
A	*4.7340*	*4*	*E1*
A	*4.1918*	*4*	*I1*
A	*4.0835*	*4*	*I2*

CULEX QUINQUEFASCIATUS MODEL

The GLM Procedure

Class Level Information

Class	*Levels*	*Values*
Site	*4*	*E1 E2 I1 I2*
Height	*2*	*Canopy Ground*

Dependent Variable: Abun_1

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	*7*	*9.91473175*	*1.41639025*	*3.99*	*0.0353*
Error	*8*	*2.83712200*	*0.35464025*		
Corrected Total	*15*	*12.75185375*			

R-Square	*Coeff Var*	*Root MSE*	*Abun_1 Mean*
0.777513	*37.86769*	*0.595517*	*1.572625*

Source	*DF*	*Type I SS*	*Mean Square*	*F Value*	*Pr > F*
Site	*3*	*5.33417075*	*1.77805692*	*5.01*	*0.0304*
Height	*1*	*1.66539025*	*1.66539025*	*4.70*	*0.0621*
*Site*Height*	*3*	*2.91517075*	*0.97172358*	*2.74*	*0.1131*

Contrast	*DF*	*Contrast SS*	*Mean Square*	*F Value*	*Pr > F*
E1 vs E2	*1*	*0.08778050*	*0.08778050*	*0.25*	*0.6322*
I1 vs I2	*1*	*0.00000000*	*0.00000000*	*0.00*	*1.0000*
E1 E2 vs I1 I2	*1*	*5.24639025*	*5.24639025*	*14.79*	*0.0049*
I2 vs all	*1*	*1.74879675*	*1.74879675*	*4.93*	*0.0571*

Tukey's Studentized Range (HSD) Test for Abun_1

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	*0.05*
Error Degrees of Freedom	*8*
Error Mean Square	*0.35464*
Critical Value of Studentized Range	*4.52880*
Minimum Significant Difference	*1.3485*

Means with the same letter are not significantly different.

Tukey Grouping	*Mean*	*N*	*Site*
A	*2.2500*	*4*	*E2*
A	*2.0405*	*4*	*E1*
B	*1.0000*	*4*	*I1*
B	*1.0000*	*4*	*I2*

Appendix 4.3. Statistical outputs for the malaria prevalence data

The CATMOD Procedure

Data Summary

Response	RESP	Response Levels	2
Weight Variable	FREQ	Populations	4
Data Set	WAHA	Total Frequency	240
Frequency Missing	0	Observations	8

Population Profiles

Sample	Site	rep	Sample Size
1	E	1	23
2	E	2	93
3	I	1	58
4	I	2	66

Response Profiles

Response	RESP
1	0
2	1

Maximum Likelihood Analysis of Variance

Source	DF	Chi-Square	Pr > ChiSq
Intercept	1	94.04	<.0001
Site	1	0.06	0.8007
rep	1	0.11	0.7388
Likelihood Ratio	1	2.24	0.1341

Analysis of Maximum Likelihood Estimates

Parameter		Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept		2.2679	0.2339	94.04	<.0001
Site	E	-0.0591	0.2340	0.06	0.8007
rep	1	-0.0812	0.2436	0.11	0.7388

Maximum Likelihood Predicted Values for Response Functions

			------Observed------		------Predicted-----		
Site	rep	Function Number	Function	Standard Error	Function	Standard Error	Residual
E	1	1	1.558145	0.55012	2.127545	0.494978	-0.5694
E	2	1	2.508437	0.393046	2.290011	0.334577	0.218426
I	1	1	2.60269	0.518188	2.245726	0.398639	0.356964
I	2	1	2.131627	0.399758	2.408192	0.399281	-0.27656

Maximum Likelihood Predicted Values for Probabilities

			--------Observed-------		-------Predicted-------		
Site	REP	RESP	Standard Probability	Error	Standard Probability	Error	Residual
E	1	0	0.8261	0.079	0.8936	0.0471	-0.067
		1	0.1739	0.079	0.1064	0.0471	0.0675
E	2	0	0.9247	0.0274	0.908	0.0279	0.0167
		1	0.0753	0.0274	0.092	0.0279	-0.017
I	1	0	0.931	0.0333	0.9043	0.0345	0.0268
		1	0.069	0.0333	0.0957	0.0345	-0.027
I	2	0	0.8939	0.0379	0.9174	0.0302	-0.024
		1	0.1061	0.0379	0.0826	0.0302	0.0235

Chapter 5.

General summary and recommendations for future research

Figure 5.3. North Island tomtit (*Petroica macrocephala toitoi*) in Waharau Regional Park, North Island, New Zealand. Photograph by D.J. Gudex-Cross.

5.1 GENERAL SUMMARY

5.1.1 Avian malaria host-parasite associations in the Auckland Region

This study furthered our knowledge of avian malaria (*Plasmodium* spp.) host prevalence and parasite diversity in the Auckland Region, as well as established new host-parasite associations in New Zealand (Chapter 2). In the Auckland Region, four different parasite morphospecies were identified, including *Plasmodium* (*Haemamoeba*) *relictum*, *P.* (*Huffia*) *elongatum*, *P.* (*Novyella*) *rouxi* and a meliphagid-specific *P.* (*Novyella*) sp. highly prevalent in bellbirds (*Anthornis melanura*) (Barraclough et. al. in press). In addition, a *P.* (*Novyella*) spp., only reported once prior to this study was found in tui (*Prosthemadera novaeseelandiae*). Molecular characterisation and further thin blood smear examples of this parasite are required to define its relationship to the bellbird parasite and other honeyeater malarial lineages (e.g. Beadell et al. 2004). The infected tuis and bellbirds were sampled on Tiritiri Matangi Island; the other species infected at this site included the exotic myna (*Acridotheres tristis*) and blackbird (*Turdus merula*). At Waharau Regional Park on the mainland, infected species included the endemic New Zealand fantail (*Rhipidura fuliginosa placabilis*) and North Island tomtit (*Petroica macrocephala toitoi*), native silvereye (*Zosterops lateralis*), and exotic song thrush (*Turdus philomelos*) and blackbird (*Turdus merula merula*).

P. elongatum was only found on the mainland at Waharau and had the largest host range of the parasites identified in this study. Although the silvereye was already a confirmed carrier of *P. elongatum* via microscopy and molecular sequencing (Barraclough, unpub. data, Howe et al. *under review*), this study provides a rigorous baseline prevalence value of 8.3% for *P. elongatum* in silvereyes for this region, based on

microscopic inspection of samples from 240 birds. This is also the first report of its incidence in the other three species New Zealand fantail, North Island tomtit, and song thrush. Since this parasite has also been found in North Island brown kiwi, North and South Island saddleback and blackbird (Alley et al. 2010, Howe et al. *under review*, Castro et al. *accepted*), it is proving likely to be the most widely spread malarial parasite across native and introduced birds in New Zealand. Moreover, although it has been suggested that this parasite may be native to New Zealand (Alley et al. 2010), more research is required to establish this.

P. relictum, the only morphospecies identified in the first New Zealand blood parasite records of Doré (1920) and Fantham and Porter (1944), was only found in silvereyes and blackbirds sampled at Waharau. This study may provide the first incidence of *P. relictum* in a New Zealand silvereye population. Although, a very small percentage (0.8%) of the 240 silvereyes sampled were identified as being infected with this parasite. In contrast, *P. relictum* was detected in all three blackbirds as a co-infection with *P. rouxi*. This result corroborates the molecular study by Tompkins and Gleeson (2006), which found blackbirds to be commonly infected with *P. relictum* in many parts of New Zealand. Given the high pathogenicity of this parasite demonstrated in a number of experimental infection trials (summarised in Valkiunas 2005) and endemic bird populations in Hawaii (van Riper III et al. 1986), the low prevalence in silvereyes sampled in this study may reflect a lower probability of capture for *P. relictum*-infected individuals. However, its pathogenicity in native New Zealand birds is unknown and requires further research. In addition to co-infection with *P. relictum* in all three blackbirds sampled at Waharau, *P. rouxi* was also found in a blackbird and myna on

Tiritiri Matangi. This parasite has previously been found in a different blackbird on Tiritiri Matangi and a New Zealand pigeon (*Hemiphaga novaeseelandiae*) at an Auckland bird rescue centre (Barraclough, unpub. data, Howe et al. *under review*), but this is the first reported incidence of it in a New Zealand myna population.. The presence of both *P. relictum* and *P. rouxi* in exotic species may provide a potential pathway for future spillover into sympatric native birds.

The meliphagid-specific *P.* (*Novyella*) sp. found in Tiritiri Matangi bellbirds was by far the most prevalent parasite *versus* all other host-parasite associations identified in this study. A sample of 123 predominately male bellbirds examined by microscopy yielded 41.5% prevalence on Tiritiri Matangi, and 38.6% in the Auckland Region after this parasite was not found in nine birds sampled at Waharau. Barraclough et al. (*in press*) previously documented this parasite as highly prevalent in Auckland Region bellbirds, and this study corroborates their finding. In addition, a parasite with similar gametocyte, but different schizont, morphology to that of the bellbird was found in tui sampled on Tiritiri Matangi. However, further survey work on this latter species, as well as molecular sequencing of the parasite, is needed to examine their relatedness. Cyt *b* sequences of the bellbird parasite have been found to be most similar to parasites from Australasian and Pacific honeyeater lineages sequenced by Beadell et al. (2004). Thereby the tui *Novyella* parasite may prove to be yet another member in this group.

5.1.2 Does capture technique bias avian malaria survey results? A case study of Tiritiri Matangi Island bellbirds

The first comparison of avian malaria prevalence and parasitaemia from two live-capture techniques, mist netting and capture at supplementary feeders, was conducted in this study (Chapter 3). In the highly parasitised Tiritiri Matangi bellbird population, adult males caught in mist nets suffered from significantly higher parasitaemia than those captured at supplementary feeders. Thus supporting the notion set forth by Valkiunas (2005) and Zehtindjiev et al. (2008) that healthier individuals are overrepresented in samples from live capture techniques targeting active birds. In addition, there was a weak seasonal trend of higher parasitaemia in autumn followed by spring and winter (no birds were sampled in summer) in both techniques, but again with mist nets yielding higher parasitaemia than feeders. The same trends were found for avian malaria prevalence but without statistical significance, which supports the point noted by O'Brien et al. (2009) that much higher sample sizes are required for detecting significance in prevalence *versus* parasitaemia. Finally, the majority of male bellbirds caught using both capture techniques exhibited low parasitaemia, which is consistent with most avian malaria surveys (e.g. Booth and Elliott 2003, Valkiunas 2005, Bensch et al. 2007, Ricklefs and Sheldon 2007).

I was unable to ascertain the causal mechanisms behind the significant parasitaemia difference between capture techniques, due to the confounding factors of bellbird social hierarchy and competition for access to high quality food at the feeders. Nevertheless, this study shows that the use of different live-capture techniques in avian malaria surveys can yield very different parasitaemia representations in a single host

population. Thus care must be taken when interpreting avian malaria data obtained by differing live-capture techniques. Lastly, the significantly higher parasitaemia found in mist netted adult male bellbirds is important from a translocation perspective, as Tiritiri Matangi bellbirds are often used as a source population and captured using this technique (K. Parker, Massey University, pers. comm.). The results of this study indicate mist netted individuals, particularly those caught in autumn, may pose a higher risk of mortality if immunosuppressed during translocation due to high levels of avian malaria infection.

5.1.3 Vector abundance, composition, and avian malaria prevalence at forest edge and interior sites in Waharau Regional Park

This study provided the first simultaneous investigation of mosquito communities and avian malaria prevalence in a New Zealand forest. In comparing these variables between forest edge and forest interior habitats, it also contributed a new aspect to the emerging study of anthropogenic deforestation and forest fragmentation effects on avian malaria dynamics. The exotic mosquitoes *Aedes notoscriptus* and *Culex quinquefasciatus* were caught almost exclusively at the forest edge in this study, with only one *Ae. notoscriptus* individual trapped at a forest interior site. However, the edge-association was only significant for *Cx. quinquefasciatus*, as *Ae. notoscriptus* abundance varied between edge sites. In addition, significantly more exotic mosquitoes were captured at ground *versus* canopy level. However when analysed individually, this result was only significant for *Ae. notoscriptus*. Further replication and higher sample sizes, particularly

for *Ae. notoscriptus*, are needed to corroborate the edge- and height-associations found in this study.

No significant edge- or interior-association was found for any of the three native mosquito species trapped in Waharau Regional Park: *Aedes antipodeus*, *Culex asteliae* or *Cx. pervigilans. Cx. pervigilans* was trapped in relatively high numbers across all sites and both heights. However, a significantly higher number of *Ae. antipodeus* were trapped at ground level in the interior site with a more mature forest type than the other sites. Similarly, Derraik et al. (2005b) trapped a large number of *Ae. antipodeus* at ground level in a relatively pristine forest in the Waitakere Ranges, West Auckland. Taken together, the results of this study and Derraik et al. (2005b) suggest *Ae. antipodeus* reaches higher abundances in mature forest *versus* other forest types. Further research into the habitat associations and feeding preferences of *Ae. antipodeus* is needed. Finally, *Cx. asteliae* was trapped almost exclusively in the canopy across all sites. This result corroborates the finding Derraik et al. (2005b), who caught *Cx. asteliae* for the first time in the wild via canopy traps. Given New Zealand's historical lack of land mammals, these results likely reflect an avian host specialisation in the foraging ecology of *Cx. asteliae*.

Avian malaria prevalence in silvereyes did not significantly differ between forest edge and interior sites. Overall prevalence determined by microscopy was low (9.17%) in 240 individuals examined. The majority of infections (N = 20) were identified as *P. elongatum*, and the other two *P. relictum*. One possible explanation for the lack of prevalence difference between edge and interior is the ubiquitous distribution and high abundance of *Cx. pervigilans* in Waharau. This species is likely an efficient avian malaria vector (Holder et al. 1997, Massey et al. 2007), and thus may be transmitting the parasites

to silvereyes throughout the forest. In contrast, the other known competent vector trapped in this study, *Cx. quinquefasciatus*, may be playing a limited role in avian malaria transmission in Waharau due to low abundance. However, this study is limited by the fact that the competency of the three other mosquito species to vector avian malaria remains unconfirmed. Such vector competency research is direly needed in New Zealand.

Other possible explanations for a lack of prevalence difference between edge and interior are the roosting behaviour and/or daily movements of silvereyes. This species forages in flocks, and other flocking species have been shown to exhibit mass roosting behaviour (Emlen, Jr. 1952). Thus each individual would have an equal likelihood of being bitten by mosquito vectors foraging at night. However, the roosting behaviour of silvereyes is unknown and requires further research. Alternatively, silvereye flocks may have been foraging throughout the forest during the day. Thereby a parasitised individual would have had an equal likelihood of being caught at the forest edge as in the interior. However, this possibility appears less likely; as the recapture of 11 individual birds in the same site in which they were originally sampled supports the assumption silvereye flocks were remaining in relatively localised areas throughout the study. Future studies can address these issues by: 1) increasing the distance between edge and interior sites; 2) incorporating more host species into the edge/interior comparison; and 3) increasing host sample sizes.

5.2 RESEARCH RECOMMENDATIONS

5.2.1 Host-parasite associations and vector competency in New Zealand

The presence of avian malaria parasites from three different subgenera (*Haemamoeba*, *Huffia* and *Novyella*) in endemic birds sampled in this study suggests malarial diversity may be high in the Auckland Region. More survey work is needed to determine the extent of parasite diversity, prevalence, and distribution in New Zealand host populations, particularly endemic species on the mainland. Future studies of these aspects should incorporate molecular techniques such as PCR and sequencing, along with microscopy, into their research protocols. PCR amplification of parasite DNA (*e.g.* cyt *b* gene) using malaria-specific primers allows for rapid, accurate identification of infected hosts (Hellgren et al. 2004). Parasite gene sequences obtained in New Zealand can then be used to explore taxonomic relatedness and evolutionary relationships with parasites from other parts of the world (see Ricklefs et al. 2004, 2005, Beadell et al. 2004, 2009). Microscopy would serve as a check against the prevalence values obtained via PCR, help identify multiple infections, determine parasitaemia, and allow for the morphological description of any unique parasite lineages (see Valkiunas et al. 2007, 2008a). It would also enable detection of other blood parasites not targeted by malaria specific primers. Such studies would greatly advance our understanding of avian malaria host-parasite associations in New Zealand.

A crucial link to understanding avian malaria dynamics in New Zealand, in need of further research, is the vector competency of a number of mosquito species. New Zealand has a poor mosquito fauna, with only 12 native and four exotic species present in the country (Derraik 2004a). Yet, avian malaria vector status has been confirmed in only

one native (*Cx. pervigilans*, Holder et al. 1999, Massey et al. 2007) and one exotic (*Cx. quinquefasciatus*, van Riper III et al. 1986) species to date. In mosquitoes, the ability to transmit malaria occurs when sporozoites invade salivary glands (Atkinson 1999). Thus, PCR amplification of the thorax indicates that mosquitoes are feeding on infected birds, whilst amplification of the head for parasite DNA in mosquitoes is used to confirm vector competency (Kim et al. 2009). Ideally, future studies investigating vector competency in New Zealand would employ both captive- and field-based research protocols (see Kim et al. 2009). In captivity, adult female mosquitoes of unknown vector competency are exposed to avian malaria-infected birds, recaptured after taking a blood meal, and tested as described above (*e.g.* Kim et al. 2009). Similarly in the field, mosquitoes are collected via bird-baited or CO_2/light traps and those carrying a blood meal used for further competency analysis (see Ishtiaq et al. 2008, Kim and Tsuda 2010, Kimura et al. 2010). Determining the vector competency of the mosquito species present in New Zealand would elucidate host-parasite associations, as well as possible native or exotic host-parasite-vector systems. It would also provide management strategies on islands or in conservation reserves if an avian malaria epizootic were to affect endemic host populations.

5.2.2 Host-parasite dynamics in Tiritiri Matangi Island bellbirds and the effects of supplementary feeders

Gaining an understanding of any negative impacts that malaria infection may have on its bellbird host would better inform future management of the Tiritiri Matangi population. Furthermore, the high prevalence of avian malaria in this host population

makes it an ideal candidate for further research on the effects of these parasites in the wild. Studies of chronically infected host species have revealed population-level fitness consequences of infection, namely through detrimental effects on individual survival rates (Möller and Nielsen 2007, Marzal et al. 2008), reproductive success (Knowles et al. 2010) and sexual selection traits (Johnson and Boyce 1991, Gilman et al. 2007). That many bellbirds on Tiritiri Matangi are already colour-banded for individual identification facilitates future studies into these potential effects.

Firstly, monitoring individual survival rates of uninfected *versus* infected bellbirds would require long-term data on an individual's infection status and capture-recapture events (see Marzal et al. 2008). Considering bellbirds are highly vagile (S. Baillie, Massey University, pers. comm.) and are used in translocations from Tiritiri Matangi to other parts of New Zealand, such long-term data may be difficult to obtain. Nevertheless, research on the survival consequences of malaria infection is fundamental to our understanding of its impact on this endemic host species. In addition, potential infection effects on sexual selection could be investigated by comparing plumage colouration of uninfected *versus* infected male bellbirds (*e.g.* Sundberg 1995, Hõrak et al. 2001), song consistency and output (*e.g.* Gilman et al. 2007), and/or copulation frequency (*e.g.* Johnson and Boyce 1991). Finally potential effects on reproductive success could be tested by using anti-malarial drugs to clear or reduce infection in breeding females, and then monitoring reproductive output and fledgling survival in treated individuals *versus* untreated parasitised individuals, and a control group of non-parasitised individuals (see Merino et al. 2000, Marzal et al. 2005, Knowles et al. 2010).

Lastly, further research is needed to determine the effects of constant access to supplementary feeders on Tiritiri Matangi bellbird immune response. This study demonstrated adult male bellbirds caught in mist-nets had higher malaria parasitaemia than those caught at supplementary feeders. A possible explanation for this difference is feeder-caught individuals may be gaining an immunological boost from the feeder food. This could be tested through quantifying and monitoring T-cell-mediated immune response (see Moller et al. 2003) in parasitised individuals at the feeders *versus* parasitised individuals outside the feeders, with a non-parasitised control group. Such information would help inform future management decisions on the use of supplementary feeding on Tiritiri Matangi, as well as other locations where this management tool is being considered.

5.2.3 Land use, habitat fragmentation, and avian malaria disease dynamics

The burgeoning study of disease ecology in relation to anthropogenic land use changes and habitat fragmentation offers a number of future research avenues in New Zealand. Future studies, expanding on this research, could improve upon the forest edge *versus* interior comparison in a number of ways, including: 1) adding further replication through sampling more sites and prolonging the study period 2) increased sampling of the number of avian host species and individuals to allow for a more comprehensive edge interior malaria comparison 3) incorporating molecular techniques to aid parasite identification and 4) comparing malaria parasitaemia in addition to prevalence in hosts. Another area of future research that would address a similar forest fragmentation question would be to compare avian malaria dynamics in a number of similar forest types of

different fragment sizes, or in similar forest types with differing amounts of canopy cover (see Loiseau et al. 2010). These studies are particularly relevant in areas of high conservation value, such as regional and national parks and island sanctuaries. Determining the effects of forest fragmentation on avian malaria dynamics would provide managers with important information regarding the design, maintenance, and/or restoration of bird conservation areas in New Zealand.

Finally, it would also be worthwhile to examine the effects of urbanisation on avian malaria dynamics in New Zealand, especially in endemic species that frequently inhabit cities (*e.g.* tui, New Zealand fantail, grey warbler *Gerygone ignata*, etc.). Recent studies conducted in the American Southwest (Fokidis et al. 2008) and across Europe (Evans et al. 2009) found reduced avian malaria prevalence in conspecific host populations inhabiting urban *versus* rural environments. However in New Zealand, two exotic mosquito species (*Ae. notoscriptus* and *Cx. quinquefasciatus*) and one native (*Cx. pervigilans*) dominate urban environments (Derraik and Slaney 2007). *Cx. pervigilans* and *Cx. quinquefasciatus* are known vectors of avian malaria in the country, and thus avian populations inhabiting cities may face higher exposure than those in rural areas. Considering urbanisation will only increase in the future, research into the avian malaria dynamics of cities *versus* rural habitats would be valuable to the conservation of endemic bird populations in New Zealand.

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