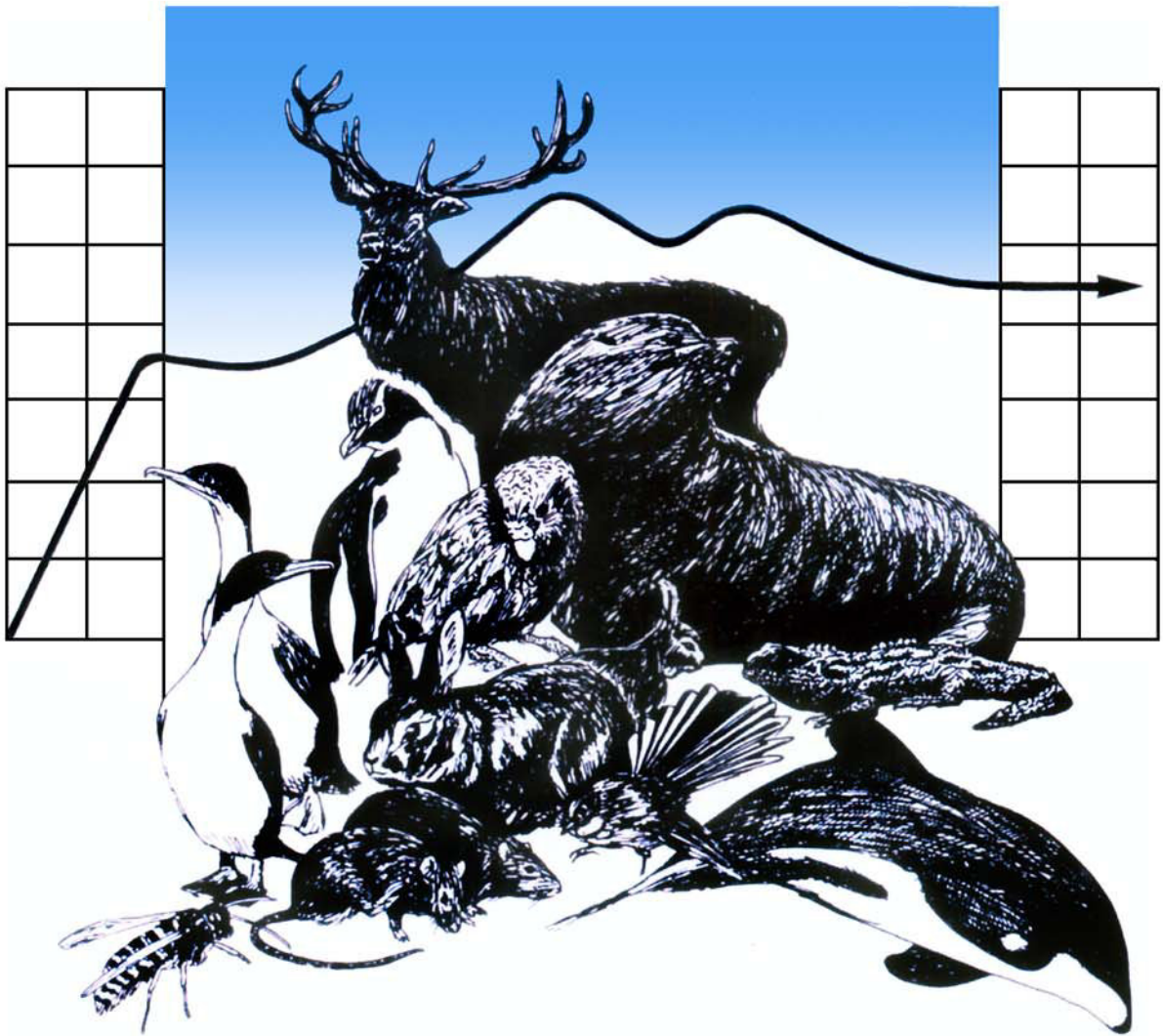




## DEPARTMENT OF ZOOLOGY



## WILDLIFE MANAGEMENT

**A study into the mosquito and  
avian fauna at Nelson Lakes  
National Park (NLNP): A  
preliminary assessment for  
avian malaria management**

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## **A study into the mosquito and avian fauna at Nelson Lakes National Park (NLNP): A preliminary assessment for avian malaria management**

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## **Abstract**

Emerging infectious diseases are an important aspect of wildlife conservation. New Zealand's native wildlife are highly susceptible to emerging diseases due to immunological naivety. Avian malaria is one such disease that has crippled native bird populations in Hawai'i and has been discovered in New Zealand. Avian malaria, caused by protozoans of the genus *Plasmodium*, is a mosquito-borne disease that affects a variety of bird species. A preliminary survey of mosquito fauna at Nelson Lakes National Park was conducted to evaluate the species present and their potential roles as vectors. In conjunction, birds were mist-netted at different elevations to determine if the proportions of native to exotic birds caught changed along an elevational gradient or if separating silvereyes caught into an out-group influenced trends observed.

The most common adult mosquito species present was *Culex pervigilans* (24 positively identified specimens of 55 total caught) followed by *Ochlerotatus antipodeus* (one positively identified specimen) and one individual of another distinct unidentified species. Predominantly desiccation damage prevented positive identifications (29/55). Mosquitoes were captured at ground level at 650 and 800 meters elevation. *Culex pervigilans* larvae were sampled at one location (eight identified of total 30 collected). There were non-significant positive correlations observed for the proportion of native birds caught in respect to elevation (native+silvereye:  $P = 0.0939$ , native:  $P = 0.067$ ). In contrast, there were non-significant negative correlations observed for the proportion of exotic birds caught in respect to elevation (exotic:  $P = 0.205$ , exotic+silvereye:  $P = 0.105$ ). Silvereyes showed no correlation (silvereye:  $P = 1.000$ ).

Artificial water containers may increase mosquito capture rates and provide population estimates. Point-count or transect acoustic/observational surveys and mark-recapture constant effort mist-netting may increase accuracy and confidence of true population estimates.

## 1.0 – Introduction and Background Information

Biological interactions at many different ecological levels (such as host-vector interactions) have become increasingly common which has led to transmission of diseases with severe implications for biodiversity (Daszak et al. 2000; Woodworth et al. 2004). The importance of managing emerging diseases in wildlife conservation has gained interest over the last century to reduce large scale declines, especially those of immunologically naive populations (Daszak et al. 2000). Specifically, New Zealand's native wildlife has evolved in isolation of many vector-borne diseases and is therefore comprised of a majority of vulnerable immunologically naive species (Schoener et al. 2013). Avian malaria for example, discovered in New Zealand, is one such emerging disease of concern (Tompkins & Gleeson 2006; Krams et al. 2013) that is a vector-borne (Diptera: Culicidae) haemoparasitic disease caused by lineages of the protozoan genus *Plasmodium* and specifically infects an avian (bird) host (Ortiz-Catedral et al. 2011).

Pathogenesis of avian malaria has been found to include two post-infection stages: a short acute phase with devastating health impacts which if survived, develops into a life-long chronic phase where acute relapses can occur (Derraik et al. 2008a, Schoener et al. 2013). Susceptibility to infection varies across species where immunologically naïve species (natives) often develop acute infections while resistant species (exotics) survive the acute phase and develop chronic infections (Derraik et al. 2008a; Schoener et al. 2013). Chronically infected birds consequently act as reservoirs for infection which leads to spill back of the disease into naïve populations (Derraik et al. 2008a; Gudex-Cross 2011). For example, with the introduction of the invasive mosquito, *Culex quinquefasciatus*, and *Plasmodium reticulum* to Hawai'i, many native and endemic bird species faced population declines and extinctions as a result of acute avian malaria infections (Derraik et al. 2008a). Models and observational studies have also discovered patterns in the distribution of the disease. Samuel et al. (2011) found upwards elevational shifts in species of native Hawaiian honeycreepers (family: Drepanidinae) likely in response to high abundances of infective mosquitoes and high avian malaria infections at lower elevations. The discovery of the same invasive mosquito and avian malaria infections in New Zealand is therefore a cause for concern as New Zealand bird populations and avian malaria dynamics may mimic those observed in Hawai'i (Ortiz-Catedral et al. 2011; Krams et al. 2013).

To determine the widespread effects of avian malaria in New Zealand, identification of the mosquito vectors involved and their ecology is of particular importance due to the strong interactions between the epidemiology of avian malaria and vector ecology and behaviour (Massey et al. 2007; Glaizot et al. 2012; Schoener et al. 2013). New Zealand has relatively low mosquito diversity with only 12 indigenous (genus: *Culex*, *Culiseta*, *Coquillettidia*, *Opifex*, *Ochlerotatus*, *Maorigoeldia*) and four introduced species (genus: *Culex*, *Ochlerotatus*) (Derraik 2004a; Snell 2005) with little known about their ecology (Derraik 2009) or the transmission roles played by different mosquito species in New Zealand (Glaizot et al. 2012; Schoener et al. 2013). The invasive mosquito species, *Cx. quinquefasciatus*, has been identified in New Zealand and known to transmit *Plasmodium* spp. between bird species (Tompkins & Gleeson 2006; Ewen et al. 2012). Possibly even more concerning, research indicates that a New Zealand native mosquito species (*Cx. pervigilans*) is also a vector for avian malaria (Massey et al. 2007; Ortiz-Catedral et al. 2011). Unfortunately, this discovery indicates that transmission pathways to native avian species may be amplified leading to an increased opportunity for *Plasmodium* spp. transmission and avian malaria infection (Massey et al. 2007). One particular area of concern in New Zealand is the Nelson Lakes Nation Park (hereafter: NLNP) where avian malaria has been identified in various bird species (C. Niebuhr, unpublished data). Consequently, an ongoing study into avian malaria in the area is currently taking place to fully understand the dynamics of this devastating disease in a natural environment.

Furthermore, the NLNP area is an alpine environment where avian malaria can be investigated on an elevational gradient. Evidence from Hawai'i suggests immunologically naïve bird species may experience a distributional shift towards higher elevations to escape vector-borne diseases such as avian malaria (Freed et al. 2005; Samuel et al. 2011) as cool temperatures at higher elevations prevent effective malaria development in mosquitoes (Freed et al. 2005). While many of New Zealand's native bird species evolved in the absence of exposure to avian malaria, the silvereye (*Zosterops lateralis*), relatively recently self-introduced to New Zealand (Diamond 1984; Webb & Kelly, 1993; Robertson & Hackwell 1995), may have evolved in the presence of avian malaria. If this assumption is correct, silvereyes may be classed as exotic species rather than a native species for the purposes of studying avian malaria in

New Zealand. These patterns have not yet been studied in New Zealand, however, it is possible trends seen in Hawai'i may also be present in New Zealand.

### 1.1 – Aims and Hypotheses

The aim of this study was therefore to conduct a species survey to determine which mosquito species are present in the NLNP area. It is hypothesised that introduced mosquito species such as *Cx. quinquefasciatus*, will be present in high abundance as in Auckland, whereas New Zealand native species such as *Cx. pervigilans* will be found at lower abundances (Derraik 2004a). As this study was conducted as part of the much larger avian malaria study involving bird sampling at different elevations to determine bird species affected and the role elevation has to play on infection prevalence, this study also aimed to determine the proportions of native and exotic birds caught at different elevations. It is hypothesised that there is a positive correlation between proportions of native birds caught and elevation. Conversely, it is hypothesised that there is a negative correlation between the proportion of exotic birds caught and elevation as has occurred in Hawaii (Samuel et al. 2011). Furthermore, this study also aimed to determine if the correlation between the proportions of native and exotic birds caught and elevation changes when silvereyes were included in each group or as an out-group. It is hypothesised that the correlation between native and exotic proportions caught will change with respect to each other and on an elevational gradient.

## 2.0 - Methods

### 2.1 - Study Site Location and Selection

Sampling took place at Nelson Lakes National Park in the South Island of New Zealand (41°49'07"S, 172°52'25"E) where bird populations have previously been found to be infected with avian malaria (C. Niebuhr, unpublished data). The NLNP area is maintained by the local Department of Conservation (DoC) office which has a cottage for volunteers, trainees and temporary workers to stay and a workshop (an "urban" area at 650 metres elevation). There are many forested walking tracks in the area, particularly the Saint Arnaud (from 650-1600 metres elevation), Black Valley Stream (650 metres elevation) and Honey Dew (650 metres elevation) tracks. The vegetation is dominated by Beech (*Nothofagus*) species; Red (*N. fusca*) and Silver (*N. menziesii*) beech grow at lower elevations while Mountain (*N. solandri* var. *diffortioides*) beech is



found at higher elevations. Opportunistic mosquito sampling was conducted at five different locations: The DoC workshop, Black Valley stream and bird mist-netting sites off the Saint Arnaud track at 800 metres, 1000 meters and 1400 metres elevation. To determine the influence of elevation on the proportion of native and exotic birds and silvereyes caught, birds were mist-netted at four different elevations: the DoC cottage, the Honey Dew track and three bush sites off the Saint Arnaud track (800 metres, 1000 metres and 1400 metres elevation).

## 2.2 - Mosquito Collection

Mosquito sampling occurred from November to March whenever possible during the mosquito breeding season when biting and abundances were thought to be high (Gudex-Cross 2011; Stone & Foster 2013). At each selected study location, mosquitoes were caught using Centres for Disease Control and Prevention (CDC) traps baited with CO<sub>2</sub> (dry ice) and light similar to the BioQuip dry ice traps (Russell & Hunter 2010; Panella et al. 2011; BioQuip n.d., Figure 1). Since many mosquito species in New Zealand are crepuscular or nocturnal (Gray et al. 2011), traps were set before sun-set (approximately 19:00h) and collected after dawn (approximately 07:00h). Six traps (where applicable) were hung on randomly selected tree branches at each selected location. The height of each trap was measured from the ground to the base of the fan to obtain an indication of sampling height.

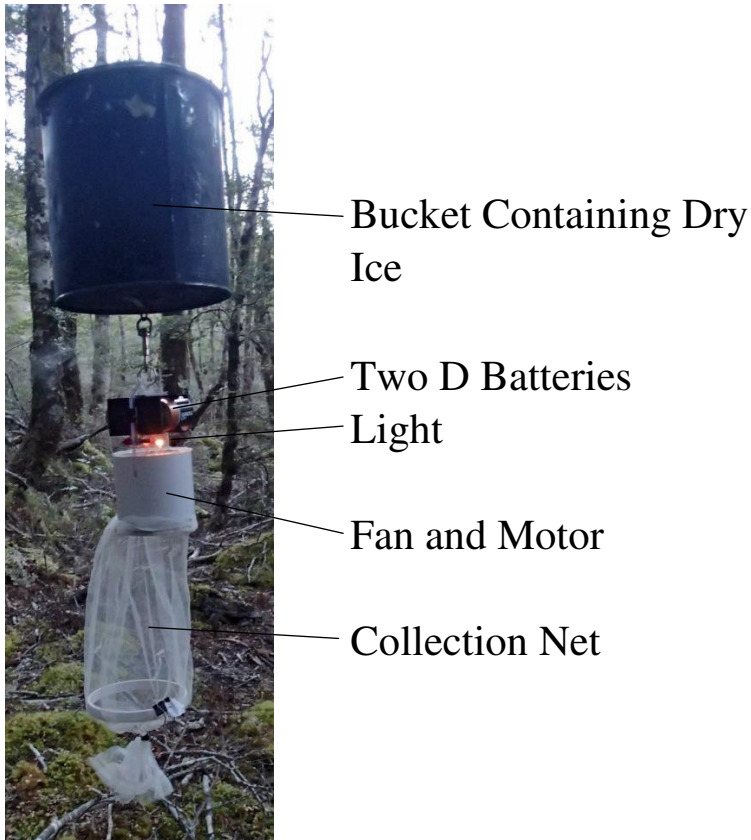


Figure 1: One of the six CDC traps used for mosquito collection.

Each study site was also searched for any pools of standing water that could be suitable habitat for mosquito larvae. Larvae were only found in one pool in an old tyre at the DoC workshop (Figure 2).



Figure 2: Mosquito CDC trap and tyre containing mosquito larvae habitat.

### 2.2.1 – Mosquito Preservation

For preservation in the field, nets and captures were frozen with dry ice then nets were checked for any mosquito captures using tweezers on a clean, white surface. Any mosquitoes found were placed into separate 1.5 ml microtubes and labelled based on trap number and date (e.g. 11/12 indicates the night of 11<sup>th</sup> and morning of 12<sup>th</sup>) to correspond to the site and trap height. All captures were counted and any non-mosquitoes were discarded. Microtubes were placed in the freezer until identification.

Any larvae collected were killed in just below boiling water and preserved in 70% ethanol.

### 2.2.2 – Mosquito Identification

Adult mosquitoes were identified to species level where possible using a dissecting microscope and a morphologic key (Snell 2005). If specimens were too damaged to identify to species level, specimens were identified to genus or family. Each mosquito was pinned using a size 0 pin on a block of Styrofoam through the thorax in the transverse plane for observation and manipulation on a viewing platform. Each mosquito was labelled according to the label on the microtube and specimen number (e.g. CO<sub>2</sub>1 11/12 01) before identification. Photos of each mosquito were taken as a precaution against desiccation and damage once pinned and a lamp was used as an extra light source. Any damage of an individual was noted during identification. Mosquito larvae were identified to species level where possible using a dissecting microscope and a morphologic key (Snell 2005).

### 2.3 - Bird Sampling/Mist-Netting

Birds were mist-netted using 12 metre x 2.6 metre or nine metre x 2.6 metre standard general purpose mist-nets each with four shelves and 38 mm mesh size. At the 650 metre sites, altogether seven 12 metre and one nine metre mist-nets were used. At the 800 metre site, five 12 metre and one nine metre mist-nets were opened. At the 1000 metre site five 12 metre and one nine metre mist-nets were opened. At the 1400 metre site only four 12 metre mist-nets were opened.

Mist-nets were checked at roughly 20 minute intervals. Any captures (birds) were banded, bled if possible and recorded for species, weight, age (adult or juvenile) and sex if possible. Captures were then released at the capture site. Sampling effort at

each elevation differed as sampling was dependent on weather conditions. Net hours were recorded for each mist-net.

#### 2.4 - Statistical Analysis – Bird Capture Proportions

Depending on the analyses and species classification, birds caught were classed as either native, exotic or silvereye. Before any analyses took place, captures were standardized into proportions of birds caught for each group at each elevation relative to the total caught at each elevation. Three separate analyses were conducted on the bird proportion data: 1) silvereyes classed as native (hereafter: native+silvereyes), 2) silvereyes classed as exotic (hereafter: exotic+silvereyes), 3) silvereyes classed as a separate group (silvereyes). Multiple mixed-effects logistic regressions specifying Net Identification and Net hours as random variables were run in version 3.0.2 of the statistical program R. For each analysis, models were ran on each of the groups in relation to elevation. Graphical representations of each analysis were produced in Microsoft Excel which displayed the proportion of natives caught to the proportion of exotics caught per 100 net hours in respect to each elevation sampled for both native+silvereyes and exotic+silvereyes. Additionally, the silvereye graph displays native, exotic and silvereyes as separate groups.

### 3.0 - Results

#### 3.1 - Mosquito Survey

*Culex pervigilans*, a native New Zealand mosquito was the most common adult mosquito identified (24 positively identified individuals, Table 1). Only one individual of *Ochlerotatus antipodeus* was caught which was at 650 metres elevation (Table 1). Only one other distinct currently unidentified species was caught at 800 metres (Table 1). Due to damage, preventing further identification, many individuals were only identifiable to family or genus (Table 1, Figure 2). In total, 55 mosquitoes were caught over the trapping season (Table 1). Most mosquitoes were caught at 650 metres elevation, two were caught at 800 metres elevation and while trapping did occur at 1000 and 1400 metres elevation none were caught (Table 1). All mosquitoes were caught at ground-level (below 2 metres) (Table 1). Only *Cx. pervigilans* larvae were collected and identified in one stagnant pool of water. Eight of approximately 30 larvae collected were positively identified as *Cx. pervigilans*.

Table 1: Family, genus, species, trap height, elevation (m) and count of adult mosquitoes caught at Nelson Lakes National Park. **Species?** indicates a tentative identification of the species. **spp.** indicates individuals were too damaged to identify further than genus and blank cells indicate individuals were too damaged to identify past family.

Family	Genus	Species	Trap Height	Elevation (m)	Count
Culicidae	Culex	pervigilans	Ground-level	650	24
Culicidae	Culex	pervigilans?	Ground-level	650	7
Culicidae	Culex	pervigilans?	Ground-level	800	1
Culicidae	Culex	spp.	Ground-level	650	14
Culicidae	Ochlerotatus	antipodeus	Ground-level	650	1
Culicidae			Ground-level	650	7
Culicidae			Ground-level	800	1
				<b>Total</b>	55

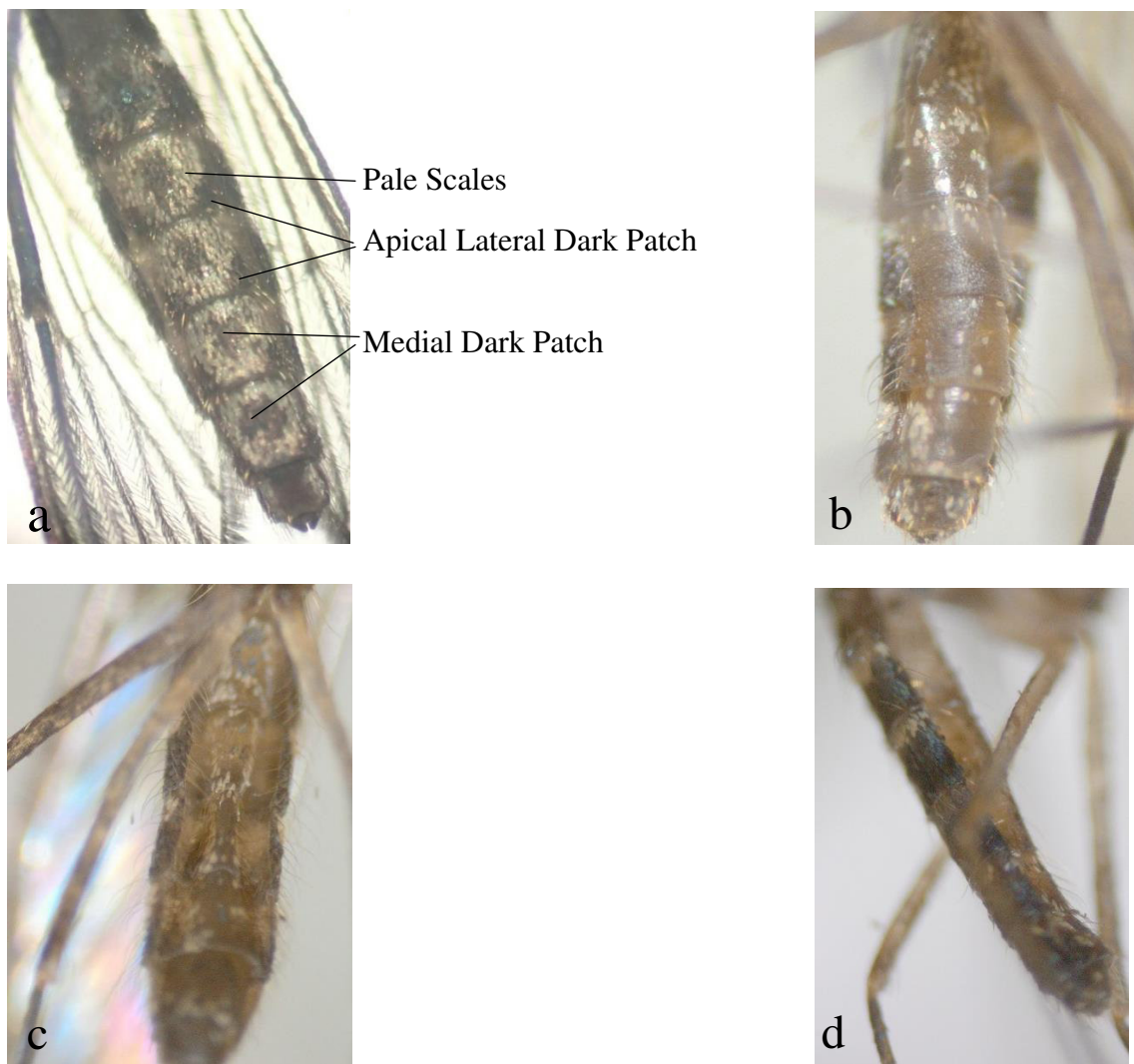


Figure 3: Photographs of the sternites (ventral abdomen) of adult mosquitoes captured in the present study. Note: d) is a lateral view. a) Intact *Culex pervigilans* adult mosquito (before desiccation damage). b) A mosquito adult with rubbed off scales on the sternites. c) - d) *Culex* mosquito adults with 'shrivelled sternites'. Note the inability to detect the medial dark patch and apical lateral patches (in b, c and d) important for differentiating between *Cx. pervigilans* and *Cx. quinquefasciatus*.

### 3.2 - Bird Proportion Results

There was no statistical significance observed with any of the three analyses. The native+silvereeye analysis appeared to show a positive correlation whereby the proportion of natives (+ silvereeyes) caught increased with increasing elevation (Figure 4). However, it is not statistically significant ( $Z = 1.675$ ,  $P = 0.0939$ ). Conversely, there appears to be a negative correlation whereby the proportion of exotic birds caught decreased with increasing elevation (Figure 4). Again this trend was not statistically significant ( $Z = -1.268$ ,  $P = 0.205$ ).

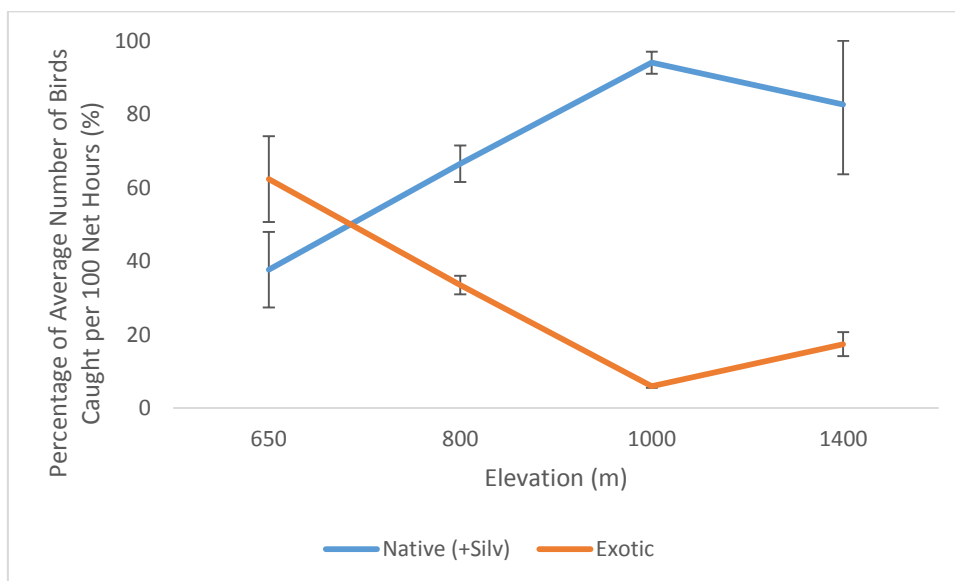


Figure 4: The proportion of native (including silvereeyes) and exotic birds caught by mist-netting at Nelson Lakes National Park in relation to elevation (m). Proportions were standardised based on net hours and calculated from the mean number of birds caught at each elevation. Sample size (number of mist-nets at each site) varies for each elevation (650m:  $n = 8$ , 800m:  $n = 6$ , 1000m:  $n = 6$ , 1400m:  $n = 4$ ). Error bars are the standard error of means.

Similarly with the exotic+silvereeye model, there appears to be a positive correlation between proportion of native birds caught and elevation (Figure 5), however this is not significant ( $Z = 1.833$ ,  $P = 0.067$ ). There also appears to be a negative correlation between exotic+silvereeyes caught and elevation (Figure 5), however again this is not significant ( $Z = -1.619$ ,  $P = 0.105$ ).

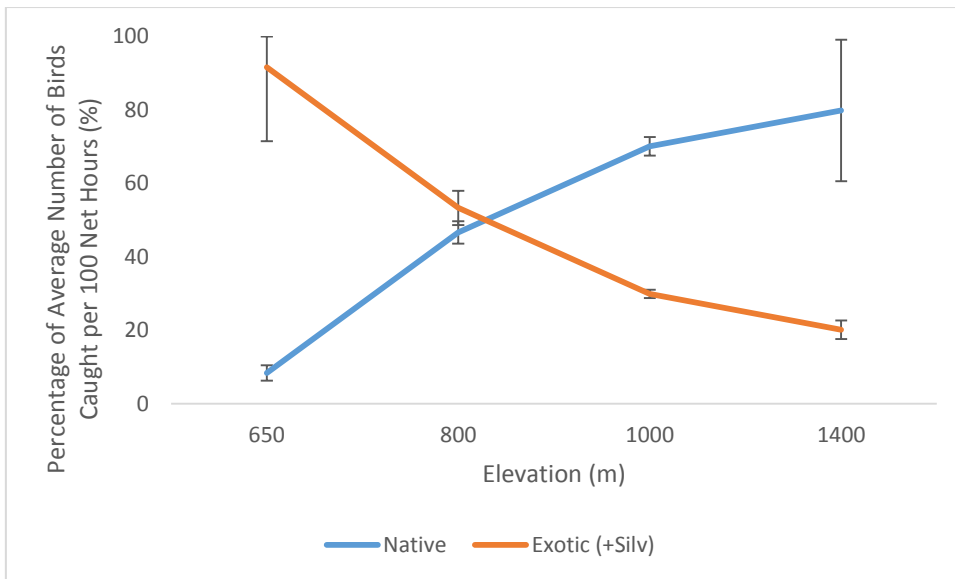


Figure 5: The proportion of native and exotic (including silvereyes) birds caught by mist-netting at Nelson Lakes National Park in relation to elevation (m). Proportions were standardised based on net hours and calculated from the mean number of birds caught at each elevation. Sample size (number of mist-nets at each site) varies for each elevation (650m: n = 8, 800m: n = 6, 1000m: n = 6, 1400m: n = 4). Error bars are the standard error of means.

For the silvereye model, the proportion of native birds caught appears to be positively correlated with increasing elevation (Figure 6). However, is not statistically significant ( $Z = 1.883$ ,  $P = 0.067$ ). The proportion of exotic birds caught appears to be negatively correlated with increasing elevation (Figure 6). However, is not significant ( $Z = -1.268$ ,  $P = 0.205$ ). Silvereyes do not appear to show any particular correlation except a decrease at 1400 metres elevation (Figure 6) which is highly non-significant ( $Z = 0.000$ ,  $P = 1.000$ ).

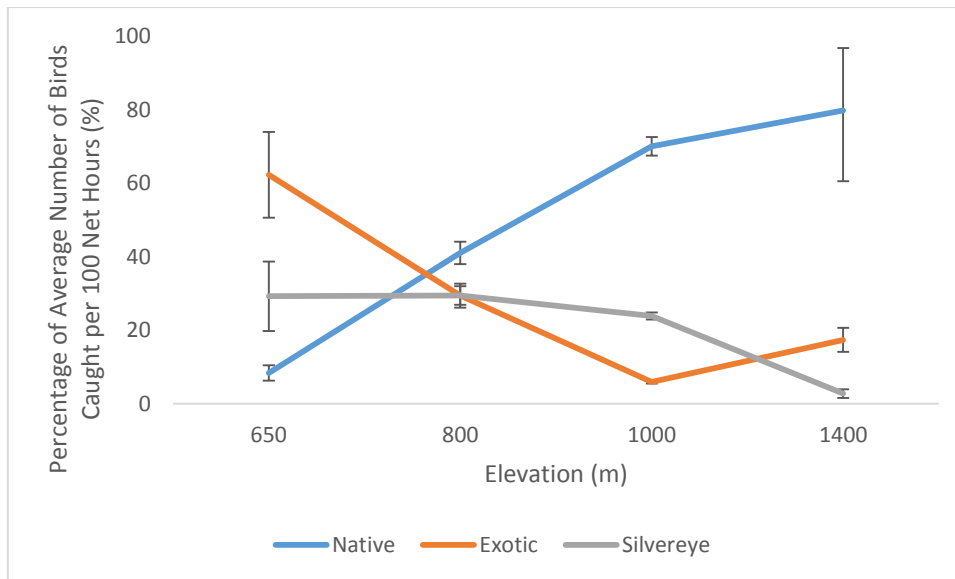


Figure 6: The proportion of native, exotic and silvereeye birds caught by mist-netting at Nelson Lakes National Park in relation to elevation (m). Proportions were standardised based on net hours and calculated from the mean number of birds caught at each elevation. Sample size (number of mist-nets at each site) varies for each elevation (650m: n = 8, 800m: n = 6, 1000m: n = 6, 1400m: n = 4). Error bars are the standard error of means.

#### 4.0 – Discussion

The mosquito survey and avian study are discussed in separate sections. Future directions for each investigation are discussed within these sections.

##### 4.1 - Mosquito Survey

While it was hypothesised that introduced mosquitoes would be the most common species found and in potentially high abundance, this was not the case. The most common species collected was *Cx. pervigilans*, a native species known to have a nationwide distribution (Holder et al. 1999). Furthermore, *O. antipodeus* is also a native species. However, *Cx. quinquefasciatus*, not detected in this study, is becoming one of the most commonly occurring species in New Zealand (MOH 1998). As the cause for such devastating effects in Hawai'i (Grudex-Cross 2011), it is fortunate that *Cx. quinquefasciatus* was not discovered in this survey at NLNP. While the absence of an introduced species should be considered beneficial, previous evidence (e.g. Orana wildlife park in Canterbury (Derraik et al. 2008a) and identification of *Plasmodium* spp. in a *Cx. pervigilans* blood meal (Massey et al. 2007; Ortiz-Catedral et al. 2011)) suggests avian malaria transmission may occur via native mosquitoes such as *Cx.*



pervigilans. At Orana wildlife park, *Cx. pervigilans* was identified as the predominant mosquito species inhabiting areas associated with avian malaria infected birds (Derraik et al. 2008a). With the presence of avian malaria confirmed at NLNP and the absence of the invasive mosquito species such as *Cx. quinquefasciatus*, which is hypothesised to be responsible for the spread and subsequent extinction of endemic Hawaiian birds (Derraik et al. 2008a; Freed et al. 2005; Woodworth et al. 2005), it is highly possible *Cx. pervigilans* and other native mosquito species are vectors for avian malaria (Derraik et al. 2008a) at NLNP. Further evidence indicates *Cx. pervigilans* is primarily a bird feeder, rarely feeding on humans which increases the potential for transmission between birds (Derraik & Slaney 2007). However, as mosquito numbers for this 2013-2014 summer survey were relatively low (cf. e.g. Derraik et al. 2003; Gudex-Cross 2011) and no direct evidence of avian malaria for this sampling season is currently available, this must only be considered an hypothesis. As the present study was a pilot study for mosquito sampling at NLNP, methods can be further refined to increase capture probability and identification rate in future sampling seasons (see below).

#### 4.1.1 - Damage

Unfortunately, many of the captured mosquitoes were damaged (missing important identifiable characteristics such as hind legs and sternite scales), preventing species level identification. One very important feature, the sternites (ventral surface of the abdomen; Figure 2), of many mosquitoes became desiccated and shrivelled after a freeze-thaw cycle and approximately half an hour after pinning for identification. With this form of damage, mosquitoes could only be identified to genus.

To address the issue of desiccation in the field, I took photographs of each individual mosquito down the microscope to create a library of intact sternites. Fortunately, identification of the desiccation issue occurred early in the identification process therefore, photographs were taken of intact sternites of most individuals. However, the morphologic key (Snell 2005) used in this study does not address this form of damage. Therefore, this was not understood to be an issue before sampling. A paper by Harbach and Harrison (1983) recognises this problem and suggests freeze-drying to keep mosquitoes and sternites intact. Unfortunately, the discovery of this paper was post-identification but may be useful for future sampling seasons. Additionally, most of the mosquitoes caught in this study were identified within 48

hours of capture (38/55), a time frame where sternites were still intact. However, some mosquitoes identified a few weeks after capture (17/55), were unidentifiable past genus due to shrivelled sternites. The exact cause of damage of these later-identified mosquitoes is currently unknown. Nonetheless, perhaps extended freezing periods still leads to shrivelling. It is also possible in that time, the mosquitoes were unintentionally thawed and refrozen leading to damage. Therefore, to protect against this desiccation damage, mosquitoes should be identified and photographed as soon as possible after capture.

#### 4.1.2 – Initial Proposed Study Ideas and Biases

While this study was presented as a survey of the mosquito fauna at NLNP, it was initially intended to be a comparison of the mosquito fauna at different heights within the canopy. While mosquitoes were only caught at ground-level, removing any statistical analysis from the study, it is an important aspect of mosquito ecology at NLNP. This provides evidence that mosquitoes sampled over November – March at NLNP were likely not present or breeding high in the canopy. The absence of mosquito species above ground-level (two metres) cannot be explained by low efficacy of traps as other aerial invertebrates were caught in high traps. Additionally, ground-dwelling mosquitoes will likely only feed on ground-dwelling birds which will in turn increase transmission of avian malaria between ground-dwelling birds (Derraik et al. 2005).

In future sampling seasons, to determine if mosquito breeding does occur high in the canopy, artificial water containers which provide oviposition sites for gravid females could be set at different canopy heights to act as a direct determinant of mosquito breeding and to maximise capture rates (Derraik 2004b; Derraik et al. 2008b). Furthermore, *Cx. pervigilans* and other mosquito species are known to breed in artificial water containers and have been used in previous studies to produce reliable estimates of mosquito relative abundances by measuring egg rafts (Derraik 2004b; Jackson 2004; Lalubin et al. 2013).

Another initial proposed research area for this study aimed to identify the difference between the relative abundance of mosquito species at different elevations. Again due to low numbers found at low elevations and high effort to transport traps to high elevations, very little trapping was conducted at elevations higher than 800 meters and was focussed at lower elevations to maximise sampling effort. However, previous

studies indicate cooler temperatures and reduced larval habitats at higher elevations prevent mosquito lifecycle completion consequently providing evidence for the absence of mosquitoes at higher elevations in the present study (Woodworth et al. 2005; Samuel et al. 2011).

Further, on some mornings, some motors were no longer running due to low battery power. However, aerial invertebrates were still collected in these traps. To reduce battery failure, new, more efficient CDC dry ice traps were purchased from BioQuip (BioQuip n.d.). If battery failure occurs for a population estimate study, these traps and captures should be removed from the study. Nevertheless, further reducing trapping nights, dry ice, the predominant attractant for the dry ice traps could unfortunately only be transported to the field site in batches of 20-30 litres when possible. Consequently, not all traps could be set every night. Moreover, to preserve dry ice, each trap was only  $\frac{1}{4}$  filled with dry ice each trapping night.  $\frac{1}{4}$  full was sufficient dry ice to last a single night with enough left over in the morning for initial freezing of captures in the field.

#### 4.2 - Bird Proportion Study

In comparison, sufficient birds were mist-netted at different elevations to perform statistical tests. While there is no statistical significance in any of the three analyses, there appeared to be a positive correlation between the proportions of native birds caught in relation to increasing elevation in each of the three analyses. Conversely, there appeared to be a negative correlation between the proportions of exotic birds caught in relation to increasing elevation. In the second analysis (exotic+silveryeyes) the correlation between native and exotic bird proportions appeared to be more extreme, with a greater proportional difference at lower elevations where exotic birds have a much higher proportion than native birds. At the higher elevation however, this correlation is inverted. With no statistical significance and no direct evidence of avian population dynamics and avian malaria infections, only inferences and suggestions can be made as to the explanation of these apparent patterns. These patterns are less likely explained due to competition between native and exotic birds as whole groups (Mountainspring & Scott 1985), but more likely habitat preference (Robertson & Hackwell 1995; van Heezik et al. 2008). Another explanation may be the effect of avian malaria forcing native birds to shift to upper elevations to escape life-threatening acute

infections, while exotic birds are capable of coping with infections at lower elevations where completion of the mosquito lifecycle and *Plasmodium* spp. development occurs (Woodworth et al. 2005; Samuel et al. 2011). If supported, this hypothesis indicates silvereyes may have co-evolved with avian malaria infection, contract chronic infections and act as reservoirs for avian malaria. If avian malaria is having the same impact on the avian species populations at NLNP as in Hawai'i, these hypotheses may be supported. However, this study is only preliminary and any conclusions drawn must be tentative.

Even though the graphs appear to show significant trends, there was no statistical significance observed in any of the analyses. Possibly, sample size (mist-net number at each site) was too small to detect any statistical significance (Whitlock & Schuller 2009). Furthermore, there were many biases and confounding factors associated with the data that are discussed in the following sections.

#### 4.2.1 – Population Estimate Study Design

As this study was an initial study into the proportions of native to exotic birds and silvereyes at NLNP I specified proportions were of birds caught. By combining another survey method (such as point-count or transect visual/acoustic surveys) with mist-netting and mark-recapture data, more confident and accurate inferences can be made about the populations as a whole. In mist-netting studies alone, rarer, shy or cryptic species may be underrepresented within the sample as they have lower capture probabilities reducing the accuracy of true population estimates (James & Rathburn 1981; Derlindati & Caziani 2005). Similarly, detectability of various species will differ depending on location of sampling (i.e. canopy height, distance from water bodies) (Bibby et al. 2000b; Derlindati & Caziani 2005). Certainly, during sampling larger-bodied species were seen and heard in locations not sampled and were therefore not caught/sampled (e.g. south island weka (*Gallirallus australis*), great spotted kiwi (*Apteryx haastii*), mallard (*Anas platyrhynchos*)) which may all interact with avian malaria differently. Mist-netting mostly targets small-bodied passerine species (e.g. tomtit (*Petroica macrocephala*), song thrush (*Turdus philomelos*) and tui (*Prothemadera novaeseelandiae*) e.t.c) which were caught and sampled. Conversely, mist-netting studies may capture avian species that are unvocal and underrepresented in acoustic surveys (Bibby et al. 2000a). Moreover, mist-nets may be biased towards

healthier individuals where infected individuals may not be active enough to be caught. Consequently, infected individuals are also correlated to increased predation risk reducing capture probability (Moller & Neilson 2007).

#### 4.2.2 – Capture Probability Bias

While captures were standardised based on net hours, one assumption I made was that each mist-net had the same capture probability. However, this was possibly not the case. One of the mist-nets used was a nine metre mist-net while all other mist-nets were 12 metres. The capture probability for the nine metre mist-net would have been lower than for the 12 metre mist-nets. However, without knowing capture probability of the native and exotic species at NLNP, capture probability was assumed to be the same. To determine capture probability, a mark recapture study can be undertaken and used to estimate true population sizes and determine sample size to detect statistical significance (Bibby et al. 2000a); Sutherland 2006; Whitlock & Schluter 2009). Since all birds caught in this study were banded (marked), mark-recapture data could be collected in future sampling seasons if the same locations are sampled. This would also provide a more accurate indication of the true population estimates of native, exotic and silvereye avian fauna (Bibby et al. 2000a; Sutherland 2006). Additionally, to fully understand the population trends of native and exotic birds, combining mist-netting data with a point-count or transect survey may determine the level of detectability of different bird species compared to mist-netting alone (Bibby et al. 2000a; Wang & Finch 2002; Sutherland 2006).

#### 4.2.3 – Constant Effort Schemes

Even with the many biases associated with mist-netting, constant effort schemes (CES) have been undertaken in the UK and North America which attempt to reduce and prevent bias (Silkey et al. 1999; Efford & Dawson 2012). These constant effort schemes use standard lengths, types, locations and netting periods of mist-nets in standard conditions to monitor changes in population level and reduce variation of sampling at different sites and over time (Sutherland 2006; Efford & Dawson 2012). Unfortunately, for the present study, constant effort was unfeasible due to poor weather conditions (excessive sun or excessive rain or cold) and site accessibility. Additionally, seasonal effects were not taken into account in the present study as sampling at each site

occurred at multiple times throughout the season. However, constant effort could be planned and undertaken in future sampling seasons.

#### 4.2.4 – Population Density Estimates

In conjunction with constant effort, many mist-netting studies measure avian populations based on density estimates (e.g. number of birds/ha) (Silkey et al. 1999; Bibby et al. 2000a; Efford & Dawson 2012). As spatial data of net location was not recorded in the present study, density estimates can only be calculated in future sampling seasons. To utilise mist-netting data for density estimates, sampling should occur within a designated area with mist-nets placed either randomly or systematically depending on study design (Bibby et al. 2000a; Silkey et al. 1999). Consequently, constant effort spatial data can be entered into the DENSITY software which requires location/session code, band number, day of mist-netting session, net number and X-Y coordinates of nets to estimate avian species densities (Efford et al. 2004; Efford & Dawson 2012). Furthermore, to fully understand the mosquito-avian species interactions, all mosquito trapping and mist-netting should be conducted at the same locations within a designated area (Gudex-Cross 2011).

### 5.0 - Final Conclusions

Mosquitoes sampled at Nelson Lakes National Park predominantly consisted of the native New Zealand mosquito, *Cx. pervigilans* which were captured at ground level at elevations of 650 metres and 800 metres. Also, an *O. antipodeus* individual was caught. As a result of damage, another distinct species was unable to be identified. Due to damage, many mosquito individuals were not identifiable. However, reduced post capture and identification periods or freeze-drying may prevent desiccation damage. Proportions of exotic birds captured appeared to be higher at lower elevations where mosquito lifecycles are completed and *Plasmodium* spp. develops. At higher elevations, there appeared to be a greater proportion of native birds captured where mosquitoes were not captured. Higher elevations may act as a refuge for immunologically naïve avian species (i.e. native birds). However, these conclusions must only be tentative as there was no statistical significance seen, many biases associated with the data and no evidence for avian malaria infections of birds for the present sampling season. The information presented in this study can therefore be used as a basis to refine methods

and hopefully provide initial data to determine if changes occur from sampling season to season. Based on the present, initial study, New Zealand avian malaria dynamics may be foreshadowed by those observed in Hawai'i. Hopefully, this study has helped to increase the understanding of mosquito ecology and bird population dynamics at NLNP and in New Zealand.

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