PACIFIC COOPERATIVE STUDIES UNIT
UNIVERSITY OF HAWAIʻI AT MĀNOA
Dr. David C. Duffy, Unit Leader
Department of Botany
3190 Maile Way, St. John #408
Honolulu, Hawai`i 96822

Technical Report 201

Evaluating the risk of avian disease in reintroducing the endangered Kiwikiu (Maui Parrotbill: *Pseudonestor xanthophrys*) to Nakula NAR, Maui, Hawaiʻi

March 2019

Christopher C. Warren[1,4], Laura K. Berthold[1], Hanna L. Mounce[1], Jeffrey T. Foster[2], Loren C. Sackett[3]

[1]Maui Forest Bird Recovery Project, Makawao, HI*

[2]Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ; Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, NH

[3]Department of Integrative Biology, University of South Florida, Tampa, FL; Center for Conservation and Evolutionary Genetics, Smithsonian Institution, Washington, DC

[4]chris@mauiforestbirds.org

*Maui Forest Bird Recovery Project is a cooperative effort between the Pacific Cooperative Studies Unit, University of Hawai'i Manoa and the Division of Forestry and Wildlife, Hawai'i Department of Land and Natural Resources

Organization Contact Information:

Maui Forest Bird Recovery Project, 2465 Olinda Rd., Makawao, HI 96768. www.mauiforestbirds.org. Office: (808) 573-0280.

Recommended Citation:

Warren, C. C., L. K. Berthold, H. L. Mounce, J. T. Foster, and L. C. Sackett 2018. Evaluating the risk of avian disease in reintroducing the endangered Kiwikiu (Maui Parrotbill: *Pseudonestor xanthophrys*) to Nakula NAR, Maui, Hawaii. Pacific Cooperative Studies Unit Technical Report XXX. University of Hawai'i at Mānoa, Department of Botany. Honolulu, HI. XX pg.

Keywords:

Avian malaria, mosquitoes, *Plasmodium relictum*, *Culex quinquefasciatus*, Maui, translocation

Table of Contents

Table of Figures

Table of Tables

Abstract

Avian malaria and other introduced diseases have had profound negative effects on Hawaiian honeycreepers, contributing to numerous extinctions and severely limiting the ranges of the remaining species. These diseases, concordant with habitat loss, are thought to restrict many species to narrow ranges at high elevations where cooler climates restrict reproduction of both the malaria parasite, *Plasmodium relictum*, and its mosquito vector, *Culex quinquefasciatus*. The Kiwikiu (Maui Parrotbill, *Pseudonestor xanthophrys*) is a critically endangered honeycreeper that formerly existed throughout Maui and Moloka‘i but now occupies roughly 30 km^2 above 1400 m above sea level (asl) on the windward slopes of Haleakalā volcano. The species is thought to be highly susceptible to avian malaria based on its limited range and reported mortality in related species. The primary conservation action proposed for Kiwikiu is to expand the species' range by reintroducing Kiwikiu to high elevation native forests on the south-facing leeward slope of Haleakalā. As part of an assessment of the suitability of the proposed release site, Nakula Natural Area Reserve, we sought to evaluate the risk of avian disease (i.e., avian malaria and pox) to the future Kiwikiu population. To do this, we trapped adult mosquitoes and surveyed for larvae throughout the release area in 2015–2016. We also tested blood samples from common bird species in Nakula using quantitative polymerase chain reaction analyses to estimate disease prevalence within the current bird population at the release site. To compare disease prevalence to habitat currently occupied by Kiwikiu, we also trapped mosquitoes and tested avian blood samples from common species in The Nature Conservancy's Waikamoi Preserve in 2016. Unexpectedly, we captured adult and larval *C. quinquefasciatus* at much higher rates in Nakula than those reported from similar locations at comparable elevations (1530-1620 m asl) throughout Hawai‘i but did not capture *C. quinquefasciatus* in Waikamoi (1675-1700 m asl). Although leeward slopes receive far less rainfall than windward slopes, the drainages in Nakula contain small pools of water that can provide suitable breeding habitat for the mosquitoes. The frequency of high-flow periods in streams in Waikamoi may regularly "flush out" pools, reducing larval habitat. In contrast, the warmer temperatures and long periods between high-flow events may allow mosquitoes to persist year-round in Nakula. In contrast to mosquito capture rates, analysis of blood samples revealed similar or lower rates of avian malaria in two common honeycreeper species in Nakula compared to similar sites. We also found several individuals of two common honeycreeper species (i.e. Hawai‘i ‘Amakihi [*Chlorodrepanis virens*] and ‘I‘iwi [*Drepanis coccinea*]) captured above 1900 m asl in Waikamoi to be positive for avian malaria. These results suggest that 1) although the persistence of mosquitoes represents an increased risk of infection in Nakula, the *Plasmodium* parasite may still

be physiologically limited by environmental conditions at the release site, 2) the management of mosquitoes (e.g. biopesticides) is advisable to reduce infection risk, and 3) Kiwikiu may be at higher risk in its current range than previously considered. While creating a second population of Kiwikiu in Nakula is critical to safeguarding this species from extinction, mitigating the threat of avian malaria on a larger scale will be the only way to achieve island-wide recovery.

Introduction

Arguably, the greatest threat to the long-term persistence of Hawaiian finches (Passeriformes Fringillidae) is introduced avian disease (Warner 1968, van Riper et al. 1986, Pratt 1994, van Riper et al 2002). These diseases collectively represent one of the largest factors driving abundance and distribution of native forest bird species throughout Hawai'i. The introduction of mosquitoes in 1826 and, subsequently, the avian malaria parasite (*Plasmodium relictum*, henceforth *Plasmodium*) and avian pox virus (*Avipoxvirus spp.*) in the early 20th century coincided with a wave of native bird extinctions and range contractions throughout Hawai'i. These effects were most acute in the immunologically naïve Hawaiian finches, also known as honeycreepers, Carduelinae (Henshaw 1902, van Riper et al. 1986, Lounibos 2002). After these diseases became established, honeycreepers became conspicuously absent from lowland areas (below 1500 m above sea level [asl]), even from intact habitat (Warner 1968, LaPointe 2007). The general hypothesis first put forth by Warner (1968) was that above a certain elevation (he proposed 600 m), the primary vector for avian malaria, the southern house mosquito (*Culex quinquefasciatus*, henceforth *Culex*), is physiologically limited and native birds can persist. Researchers later determined that the *Plasmodium* parasite itself is physiologically constrained by the comparatively cool temperatures at high elevation (van Riper et al. 1986, Atkinson and LaPointe 2009). Honeycreepers have been found to carry higher parasite loads than non-native species and to have high mortality rates once infected (van Riper et al. 1986, Atkinson et al. 2005). Today, the extant populations of honeycreepers are found primarily in higher elevation habitats and the elevational extent of each species is thought to be largely driven by disease tolerance and mosquito/parasite distribution (van Riper et al. 1986, LaPointe et al. 2012, Samuel et al. 2015).

The Kiwikiu (Maui Parrotbill, *Pseudonestor xanthophrys*) is a honeycreeper endemic to the islands of Maui and Moloka'i and is listed as endangered under the US Endangered Species Act and the International Union for the Conservation of Nature (IUCN) Red list. Kiwikiu have a small range and a small population size (USFWS 1967, USFWS 2006, Camp et al. 2009, IUCN 2017) currently estimated at fewer than 312 individuals (mean estimate 157 ± 67, 47-312 95% CI; Judge et al. 2019). The Kiwikiu exhibits a demographic pattern consistent with *K*-selected species including high adult survival, low fecundity, and an extended period of parental care (Mountainspring 1987, Simon et al. 1997, Mounce et al. 2014). The species is insectivorous and uses its large beak to extract larvae from the stems and fruits of a variety of native plant species (Mountainspring 1987, Simon et al. 1997, Stein 2007). These specialized feeding habits appear to drive large home range establishment and low population densities (Pratt et al. 2001, Warren et al. 2015). Kiwikiu are

limited by the amount of suitable habitat available, and currently occupy high elevation wet, montane native forests on windward Haleakalā Volcano on eastern Maui, representing only 5% of its original range (Scott et al. 1986). The wet montane forest currently occupied by the Kiwikiu is possibly suboptimal habitat and is only one of many forest types where the species once existed (Olson and James 1982). The heavy rainfall events that are characteristic of this area decrease Kiwikiu breeding success (Simon et al. 2000, Becker et al. 2010). Subfossil deposits indicate that, prior to human contact, the species inhabited a wide range of habitats including low and mid-elevation koa (*Acacia koa*) forests on leeward Haleakalā. However, deforestation has made much of this habitat unsuitable (James and Olson 1991, Simon et al. 2000). Invasive mammals pose an additional threat to Kiwikiu in its current range through direct predation and habitat modification (Becker et al. 2010, Mounce et al. 2014).

The population dynamics of the avian malaria parasite and avian pox virus as well as the disease vector (*Culex*) vary by season, year, and elevation (Goff and van Riper 1980, van Riper et al. 1986, Atkinson et al. 2009, Hobbelen et al. 2012, Samuel et al. 2018). The steep slopes of the Hawaiian Islands and corresponding abrupt changes in temperature mean these dynamics are highly variable across space and time (Samuel et. al. 2011). At lower elevations, the warm, moist environment facilitates disease transmission year-round, while at middle elevations transmission varies with seasonal, annual, and site-specific weather patterns. Presently, high elevations are considered the only refugia for native forest birds where disease rates are low (Atkinson et al. 2009, Hobbelen et al. 2012). Patterns of disease transmission are driven by the effect of temperature and rainfall on the pathogen and the disease vector. These factors influence the incubation period of *Plasmodium*, adult mosquito survival, and availability of larval mosquito habitat (Rueda et al. 1990, LaPointe et al. 2010, Samuel et al. 2011, Ciota et al. 2014). Disease dynamics below 1500 m asl are primarily driven by the distribution and density of *Culex*. High elevation (above 1500 m asl), dry sites are predicted to have the lowest prevalence of avian malaria because larval habitat may be reduced and development of *Plasmodium* is limited.

Climate warming is predicted to expand the geographic range and abundance of vector-borne diseases like avian malaria and pox by increasing transmission rates, epizootic development, and vector distribution (Benning et al. 2002, Atkinson and LaPointe 2009, Fortini et al. 2015). Liao et al. (2015) demonstrated that the greatest changes in malaria transmission will occur at mid-and high-elevations as a result of climate change. Development of *C. quinquefasciatus* is severely limited below 10 °C (Ciota et al. 2014) and *Plasmodium* development is limited in temperatures below 16 °C (Chao and Ball 1962, Noden et al. 1995, LaPointe et al. 2010). Modeling the 2 °C increase in

temperature that is predicted by 2050 means that in Hanawi NAR, a core Kiwikiu breeding area, the habitat with a low risk of disease transmission may be reduced by at least 57% (Atkinson and LaPointe 2009). The species has already disappeared from several areas below 1400 m asl in Hanawi NAR where it was observed in the late 20th century (Judge et al. 2019). Considering the Kiwikiu population is already limited by suitable habitat, a reduction of this proportion would make the species highly susceptible to a stochastic event (e.g. hurricane) or inbreeding depression that may lead to extinction (USFWS 2006).

Researchers have proposed several management options to ameliorate the impacts of climate change and avian disease on the Kiwikiu. The natural evolution of disease resistance appears to be occurring in several Hawaiian honeycreeper species (Foster et al. 2007, Krend 2011). Facilitating this evolution may be incorporated into management strategies (Kilpatrick 2006, Hobbelen et al. 2012). This may be a viable solution for common species like ʻAmakihi (*Chlorodrepanis* spp.). However, endangered species may not have enough genetic diversity to facilitate evolution of disease resistance (USFWS 2006, Hobbelen et al. 2012, Mounce et al. 2015). Local reduction of mosquito populations through methods that have established regulatory pathways, such as the sterile male technique (Vreysen et al. 2007), present promising options to address these diseases on a large scale. A technique is currently being evaluated using *Wolbachia* incompatibility (Laven 1967) to reduce mosquito abundance in Hawaiʻi and is likely to be implemented in the wild within the next few years (DLNR 2017). A further step that has potential to be a more permanent solution is the introduction of transgenic mosquitoes to reduce the effectiveness of *Culex* as a *Plasmodium* vector or eliminate these mosquitoes from the landscape entirely. However, presently there is no logistical or regulatory structure in place for such a strategy (Atkinson and LaPointe 2009).

Establishing a second population of Kiwikiu where avian disease poses low risk, is considered essential for the species recovery (USFWS 2006). Nakula Natural Area Reserve (NAR) was selected as the first intended release site for reintroduction of Kiwikiu to its historic range on the leeward slope of Haleakalā. Ensuring the factors that contributed to the extirpation of the species are no longer threats is essential to the success of a reintroduction (Armstrong and Ewen 2002). Nakula has been selected in part because it is on the drier leeward slopes of Haleakalā compared to the windward habitat currently occupied by Kiwikiu. It was hypothesized that this drier forest, besides providing potentially better habitat for Kiwikiu nesting and foraging, may support fewer mosquitoes than wet forest and thus, limit transmission of avian disease. Prior to the release of Kiwikiu to this area, we sought to better understand the extent of the mosquito population and disease prevalence within the release site.

Methods

Study Areas

We studied disease prevalence at two high-elevation sites on Haleakalā Volcano in east Maui, Nakula NAR (1330 m-1810 m asl) and The Nature Conservancy's Waikamoi Preserve (1670-1925 m asl; henceforth Nakula and Waikamoi). Comparison between these sites will allow evaluation of the disease threat between the intended Kiwikiu reintroduction site (Nakula) and an area the species currently occupies (Waikamoi). We conducted this study in a section of Waikamoi west of the Koʻolau Gap in a transition zone between wet and mesic native forest with a canopy composed of ʻōhiʻa (*Metrosideros polymorpha*) and, to a lesser extent, koa. This preserve also contains a diverse mid- and understory of native shrubs, vines and ferns. The dominant plant species in Nakula are very similar to those in Waikamoi although the canopy in Nakula is dominated by koa with a smaller proportion of ʻōhiʻa and other tree species. The forest in Nakula has been greatly denuded by over a century of unchecked browsing by feral ungulates. Feral ungulates had a particularly negative impact on the understory in the reserve and understory is largely missing from much of the area, creating a savanna-like habitat throughout the site. Mature forest patches still exist in steep gulches that are out of reach of ungulates. The lower elevation of the Nakula site is bordered by pastureland and little to no habitat exists for forest birds below 1100 m asl, with much of the habitat well above that. Unlike many other locations where disease prevalence has been studied in Hawaiʻi, any malaria or pox detected in Nakula is likely endemic, given the habitat limitations and very little elevational movement is likely in these forest bird populations.

Waikamoi is on the windward (north) slope of the volcano and Nakula is on the leeward (south) slope. Due to predominant weather patterns, Waikamoi receives roughly twice the annual rainfall as Nakula, receiving over 200 cm per year on average while Nakula receives closer to 100 cm (Giambelluca et al. 2014). Running water occurs in streams during heavy rainfall at both sites and standing pools of water can develop between rainfall events. Flowing water is much more common in Waikamoi and streambeds often remain free of vegetation. In Nakula, most of the streams flow semi-annually and are often filled in with non-native grasses. At this site, waterfalls often carve out pools below them but the streambed downstream is raised, restricting water flow below the pool. Additionally, the exposed lava rock in streambeds contains many natural depressions that may fill with water. Even moderate rainfall events often fill pools within the drainages that quickly become stagnant. We installed an Onset© weather station within the Nakula site and programmed the station to log precipitation and temperature on a 30-min cycle. No onsite

weather station was installed in Waikamoi. Broad-scale weather patterns and site comparisons were examined using the Rainfall Atlas of Hawai'i (Giambelluca et al. 2014).

Mosquito sampling

Adult sampling: We sampled for adult mosquitoes at five stations in Nakula and Waikamoi. In Nakula, we placed these stations in mature forest patches at approximately 1530–1620 m asl (20.674, -156.234; Figure 1). In Waikamoi, we used an existing trail system in an area used as part of a long-term study of Kiwikiu to establish mosquito sampling stations. The Waikamoi stations were established on the lowest trails of the study site and were placed slightly higher in elevation, between 1675–1700 m asl (20.781, -156.215; Figure 1). Two types of non-lethal mosquito traps were installed at each station, totaling ten traps at each site. We used the same Centers for Disease Control and Prevention (CDCP) light traps and CDCP gravid traps that have been used throughout Hawai'i for mosquito sampling (Figure 2). The CDCP light traps, "CO_2 traps", were baited with dry ice, which emitted carbon dioxide to attract females seeking a blood meal. The gravid traps were baited with a bucket of stagnant water to attract females looking to lay eggs after collecting a blood meal. In both traps, a battery-powered fan blows the mosquitoes into a net where they remain overnight until they can be collected.

The CO_2 traps were suspended from a tree 1–2 m above the ground. We filled coolers with ~900 g of dry ice before dusk to ensure the trap contained bait throughout the night when *Culex* are most active. We placed the gravid traps on level ground near the paired CO_2 trap. We mixed the water for gravid traps with alfalfa rabbit feed and yeast a week prior to trapping and we did not change this water during each trapping session (2-3 days). We changed fan batteries every two nights and turned them off during the day. After dawn following a night of trapping, we visited stations to collect captured mosquitoes. Mosquitoes were preserved in 70% isopropyl alcohol and identified to species.

We sampled for mosquitoes in three seasons (spring [March-May], summer [June-August], and winter [November-December]) in each of two years in Nakula and one year in Waikamoi. We sampled 1–3 times per season and stations were open for three to four consecutive nights during each session (Table 1). In Nakula, we sampled for adult mosquitoes for a total of 39 nights equaling 359 trapping nights in 2015 and 2016. Waikamoi traps were open for 18 nights in total and 167 trap nights over six three-day sessions in 2016.

We tested all mosquito samples for the presence of *Plasmodium relictum* using quantitative polymerase chain reaction (qPCR) analyses at the University of New Hampshire. After crushing the

entire body of each mosquito sample, we extracted DNA with the Qiagen DNeasy PowerSoil Kit using the manufacturer's instructions. We used GRW4/11F and GRW4/11R primers from Zehtindjiev et al. (2008) for the qPCR analysis. The probe for the qPCR TaqMan assay was modified from an assay developed by Kristina Paxton and the optimization conditions are currently unpublished.

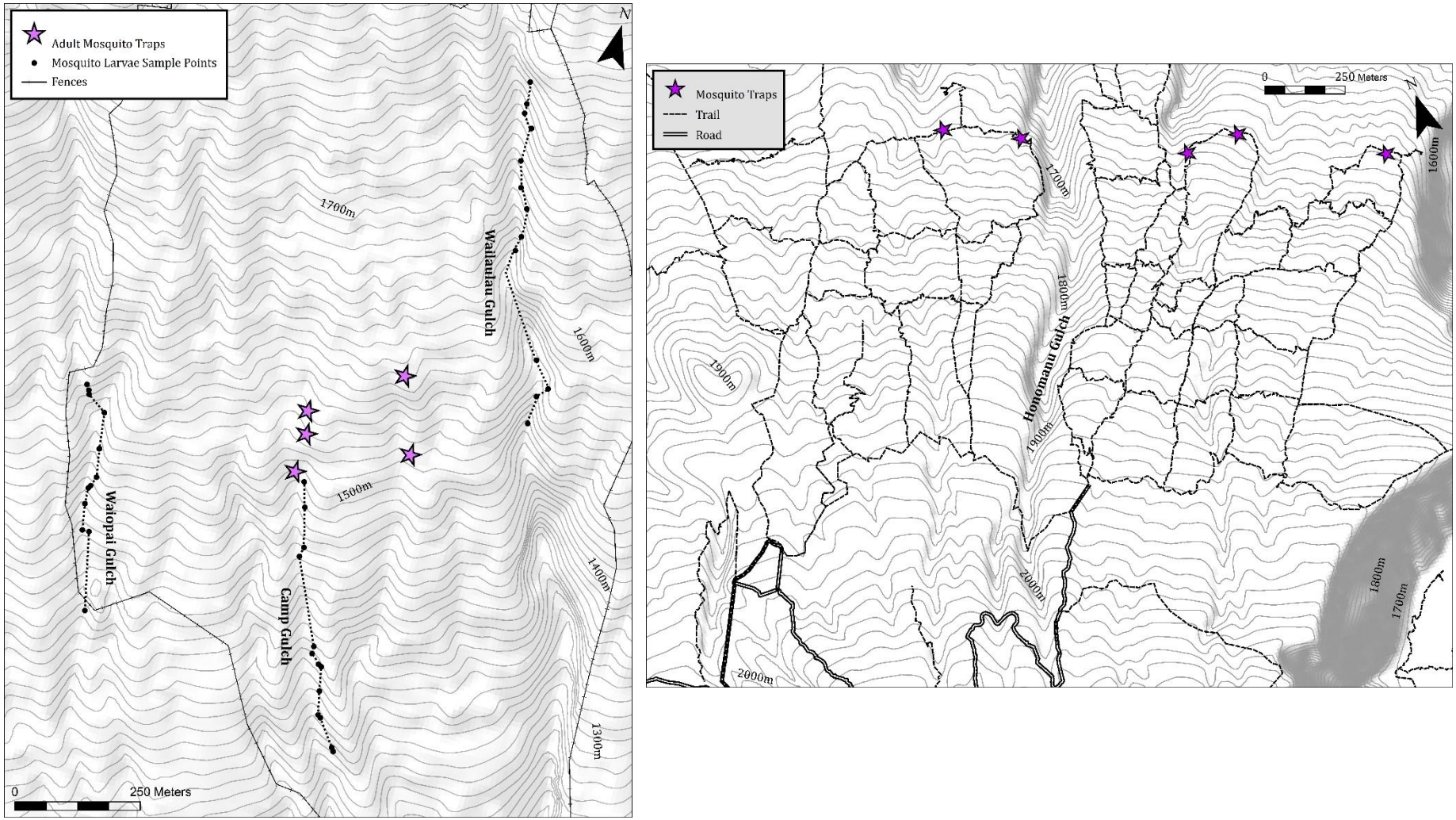

Figure 1. Maps of the Nakula Natural Area Reserve (left) and The Nature Conservancy's Waikamoi Preserve (right) study sites showing the locations of adult and larval mosquito sampling points.

Figure 2. Adult mosquito trap set up showing the CDCP oviposition (on ground) and CDCP light "CO_2" (hanging) trap designs.

Larvae sampling: To estimate the spatial and elevational distribution of breeding mosquitoes within Nakula we sampled for the presence of mosquito larvae in the three most prominent drainages in the site. These three drainages, Waiopai, Wailaulau and "Camp" gulches, follow the western and eastern boundaries and center of the site, respectively (Figure 1). We chose these drainages based on their longitudinal and altitudinal distributions within the site. Rocky pools in intermittent streams, like those within these gulches, serve as the primary larval habitat for *Culex* in dry-mesic forests in Hawai'i (Reiter and LaPointe 2009). The section of Wailaulau Gulch sampled was higher than the other two (1530 m to 1810 m) because the other drainages at these higher elevations were too small or remained dry throughout the sampling period. Wailaulau was not accessible below 1530 m. The remaining two drainages covered roughly the same elevation range (1330 m to 1600 m). Collectively, we sampled 1.76 km of these drainages for the presence of mosquito larvae.

During the first survey for mosquito larvae, we marked the location of each sampled pool for future guidance. All of these points were visited during each survey. However, we sampled all pools found within the surveyed section of each gulch. Pools within 10 m of each other were considered a

single point and were often sampled as one unit depending on the size and depth of the pools. At each pool (or small group of pools) we used a dip cup (red Solo® cup attached to a 0.5 m PVC pole) to sample for the presence of mosquito larvae. To sample, we slowly submerged the dip cup, bottom first, into the pool letting the water suction into the top of the cup. Observers were careful to not cast a shadow over the pool or disturb the water before sampling to prevent disrupting larvae and causing them to flee the surface. If a pool was not deep enough to accommodate the entire cup, we turned the cup on its side to obtain as large a sample as possible. We then examined the sampled water on a white plate for larvae. We dipped in each pool five times. At places where multiple pools were located within 10 m of one another, we sampled multiple pools to get a representative sample from the area. At times this meant sampling in several small pools for a combined five dips or more than five if the pools were larger. We did not sample in pools that could not fit the dip cup, generally smaller than 10 cm wide and 5 cm deep. If larvae were found, we collected a representative sample (1–70 larvae), which were tentatively identified to genus in the field, and preserved in isopropyl for positive identification in the lab. We did not survey for mosquito larvae in Waikamoi.

Avian blood sampling: Using established mist-netting and banding methodology, we collected blood samples from birds captured in Waikamoi and Nakula. Banders operated under Federal Bird Banding Permit #08487. All birds were captured between 1440-1755 m asl in Nakula and 1900-1925 m asl in Waikamoi. After capture and banding, we collected a small blood sample from the brachial vein and stored in Queen's Lysis Buffer. To ensure best practices for animal welfare were followed, we did not take blood samples from birds with compromised physical condition (e.g. stressed, very young). This may have biased the results by possibly excluding some individuals suffering from an acute malaria infection. However, with the exception of a few fledglings that were not sampled, we excluded very few birds from blood sampling based on body condition or behavior in this study. We prioritized native species for collecting blood samples and collected from as many suitable individuals as possible. We collected samples from a subset of non-native species, sampling from individuals as time allowed. All birds captured were examined for symptoms of avian pox infection. All birds exhibiting missing nails, partial or whole digits on the feet, swollen digits or joints on the legs were recorded and these symptoms were considered potential signs of past or minor infections (Samuel et al. 2018). Following van Riper and Forrester (2007), we considered symptoms of active avipox infection to be open sores or large swellings usually in the presence of exudate on the legs, feet, or face. No laboratory analysis was conducted to confirm infection of

avipox. All native birds were fitted with a unique combination of color bands so that individuals could be later identified in the field (Figure 3).

In 2015 and 2016, we captured and banded 102 ʻApapane and 122 Hawaiʻi ʻAmakihi and collected blood samples from 163 individuals in Nakula and Waikamoi. We analyzed 15 random samples each of ʻApapane and ʻAmakihi blood for *Plasmodium* infection at the University of New Hampshire. We also analyzed a smaller subset of up to five samples for each non-native species captured in Nakula in 2015. The non-native species included in these analyses were House Finch (*Haemorhous mexicanus,* n =5), Hwamei (*Garrulax canorus*, n = 1), Japanese Bush-Warbler (*Horornis diphone,* n =5), Japanese White-eye (*Zosterops japonicus,* n =5), Red-billed Leiothrix (*Leiothrix lutea,* n =5), and Scaly-breasted Munia (*Lonchura punctulata,* n =5). These samples were analyzed for the presence of *Plasmodium* using the same PCR technique used for adult mosquito samples. We analyzed the additional samples collected from ʻApapane (n = 42), ʻAmakihi (n = 46), and ʻIʻiwi (*Drepanis coccinea*; n = 7) at the University of South Florida via PCR amplification of a portion of the *Plasmodium* cytochrome b gene using primers F2/R2 (Beadell et al. 2004). Amplified fragments were visualized on an agarose gel, and any ambiguous samples were re-run. To validate our primers, we randomly selected 2/3 of the samples to screen using at least one additional primer set (the mitochondrial cytochrome oxidase III subunit; Beadell et al. 2004). As additional research on avian population dynamics in Nakula was being conducted concurrently, the majority of the samples were from Nakula (134 out of 151 samples). We compared the proportion of samples positive for *Plasmodium* between years (2015-2016) in ʻApapane and ʻAmakihi using separate binary generalized linear models (ʻglmʼ) followed by one-way ANOVA (Type III) (ʻcarʼ package; Fox and Weisberg 2011) in R 3.4.2 (R Core Team 2017). We performed similar analyses to investigate the effect of elevation on the proportion of infected birds.

Figure 3. A color-banded Hawai'i 'Amakihi feeding on an 'ōhi'a blossom in Nakula NAR. This individual tested negative for *Plasmodium relictum* and was known to survive through spring 2019.

Results

Mosquito samples

Adult samples: We sampled adult mosquitoes for a total of 361 trap-nights (number of traps times number of nights) in Nakula (155 nights in 2015, 206 nights in 2016) and 167 trap-nights in Waikamoi (Table 1). In total, we captured 235 mosquitoes in Nakula including 222 *Culex* (94% of captures). The remaining 13 mosquitoes captured in traps were *Aedes vexans* (identified by the authors). We also captured seven *A. vexans* and two *A. albopictus* incidentally outside of traps. *Aedes albopictus* are not as effective vectors for *Plasmodium* as *Culex*; vector competency of *A. vexans* is not known (LaPointe et al. 2005). Thus, we restricted our analyses of capture rates to *Culex.* Very few *Culex* were captured in the oviposition traps (0.9%); the majority were captured in the CO_2 traps (99.1%). The overall capture rate of *Culex* in CO_2 traps in Nakula was 1.02 ± 0.38 (SE) mosquitoes/trap night (0.49 ± 0.17 in 2015 and 1.32 ± 0.65 in 2016; Table 2). Overall capture rate within oviposition traps was very low, 0.01 ± 0.007 mq/TN; a single mosquito was captured in this trap type each year. Capture rate within CO_2 traps was fairly uniform across seasons in 2015, although there were fewer captures in the spring compared to other seasons (Figure 4). In 2016, capture rate was similar to that from 2015 in the summer and winter seasons (< 0.5 mosquitoes/trap night). However, the capture rate in spring 2016 (4.41 mq/TN) was more than four times that found in any other season in either year. In fact, the majority of all mosquitoes

captured during this study were captured in the spring of 2016 (63.5% of all captures). A single night in April 2016 produced 66 *Culex,* 29.7% of all *Culex* captures, demonstrating the variability among trap-nights. A total of 13 mosquitoes were captured incidentally outside of traps, including one *Culex*; the rest were *Aedes vexans* and *A. albopictus*. No mosquitoes were captured in traps in Waikamoi. The only mosquito captured in Waikamoi was a single *A. vexans/japonicus* captured incidentally. Definitive identification to species of this specimen was not possible due to its condition.

The onsite weather station in Nakula recorded different rainfall patterns between the years, particularly during the summer months (Figure 5). The wettest period in 2015 was during the summer months showing a spike in both the frequency of rainfall events (defined as > 0 cm rainfall per 30 min) and cumulative rainfall July-September. In a period of two weeks in August 2015, more than 20 cm of rain fell in Nakula, approximately 20% of the annual average precipitation for the site. In contrast, spring 2016 (March-May) experienced the greatest frequency of rainfall events but comparatively little cumulative precipitation fell during this period. Unfortunately, a malfunction of the weather station led to data loss during March-April 2015 so we cannot fully compare the spring months between years. However, May 2015 was much drier in both frequency and intensity of precipitation compared to May 2016. In 2015, adult mosquito capture rates were greatest in the summer and winter months, corresponding with elevated precipitation compared to spring. The pattern in capture rates in 2016 appears to correlate with precipitation rates, greatest in spring and declining into summer and winter. The wet spring in 2016 likely increased the larval habitat in Nakula resulting in a boost in adults, which may not have been sustained during the comparatively dry summer and winter months.

Table 1. Trap nights for each adult mosquito trapping session in Nakula NAR and The Nature Conservancy's Waikamoi Preserve. Trap nights fewer than other traps within a session are the result of equipment failure or lack of bait (CO_2).

				CO_2 Trap ID					Oviposition Trap ID					
NAKULA														
Year	Season	Start	End	A	B	C	D	E	A	B	C	D	E	TOTAL
2015	spring	5/11/2015	5/14/2015	3	4	4	4	4	3	4	4	3	4	37
2015	spring	5/28/2015	5/31/2015	3	4	4	3	4	3	4	4	4	4	37
2015	summer	7/6/2015	7/8/2015	3	3	2	3	3	3	3	3	3	3	29
2015	winter	11/18/2015	11/20/2015	3	3	1	3	3	3	3	3	2	3	27
2015	winter	12/4/2015	12/6/2015	3	3	2	3	2	3	3	1	2	3	25
2016	spring	3/4/2016	3/6/2016	3	3	2	3	3	3	3	2	3	3	28
2016	spring	4/6/2016	4/9/2016	3	4	4	4	3	3	4	4	4	3	36
2016	summer	7/8/2016	7/10/2016	3	3	3	3	3	3	3	3	3	3	30
2016	summer	8/18/2016	8/21/2016	4	4	3	4	4	4	4	3	4	4	38
2016	winter	11/10/2016	11/13/2016	4	4	4	4	4	4	4	4	4	4	40
2016	winter	12/8/2016	12/11/2016	3	3	4	4	3	3	3	4	4	3	34
													Σ_{Nakula}	361
WAIKAMOI														
2016	spring	3/14/2016	3/16/2016	2	3	3	3	3	3	3	3	3	3	29
2016	spring	4/18/2016	4/20/2016	3	3	2	2	3	3	0	3	2	3	24
2016	summer	7/18/2016	7/20/2016	3	3	3	3	3	3	3	3	3	3	30
2016	summer	8/2/2016	8/4/2016	3	2	3	0	2	3	3	3	3	3	25
2016	winter	11/28/2016	11/30/2016	3	3	3	3	2	3	3	3	3	3	29
2016	winter	12/19/2016	12/21/2016	3	3	3	3	3	3	3	3	3	3	30
													$\Sigma_{Waikamoi}$	167

Table 2. Mosquito capture results from Nakula NAR in CO_2 and Oviposition traps and incidental captures. Shown are the number on *Culex quinquefasciatus* mosquitoes (mq) captured per trap night (TN) and the number and percentage of Culex that tested positive for *Plasmodium relictum* (+Plas).

		CO_2					Oviposition					Incidental		
		# *Culex*	TN	mq/TN	+ Plas	% +	# *Culex*	TN	mq/TN	+ Plas	% +	# *Culex*	+ Plas	% +
2015	spring	5	37	0.14	0	0.0%	0	37	0.00	0	-	1	0	0.0%
2015	summer	13	14	0.93	0	0.0%	0	15	0.00	0	-	0	0	-
2015	winter	20	26	0.77	0	0.0%	1	26	0.04	0	0.0%	0	0	-
2016	spring	141	32	4.41	7	5.0%	0	32	0.00	0	-	0	0	-
2016	summer	31	68	0.46	2	6.5%	0	34	0.00	0	-	0	0	-
2016	winter	9	37	0.24	1	11.1%	1	37	0.03	0	0.0%	0	0	-
2015		38	77	0.49	0	0.0%	1	78	0.01	0	0.0%	1	0	0.0%
2016		181	137	1.32	10	5.5%	1	103	0.01	0	0.0%	0	0	-

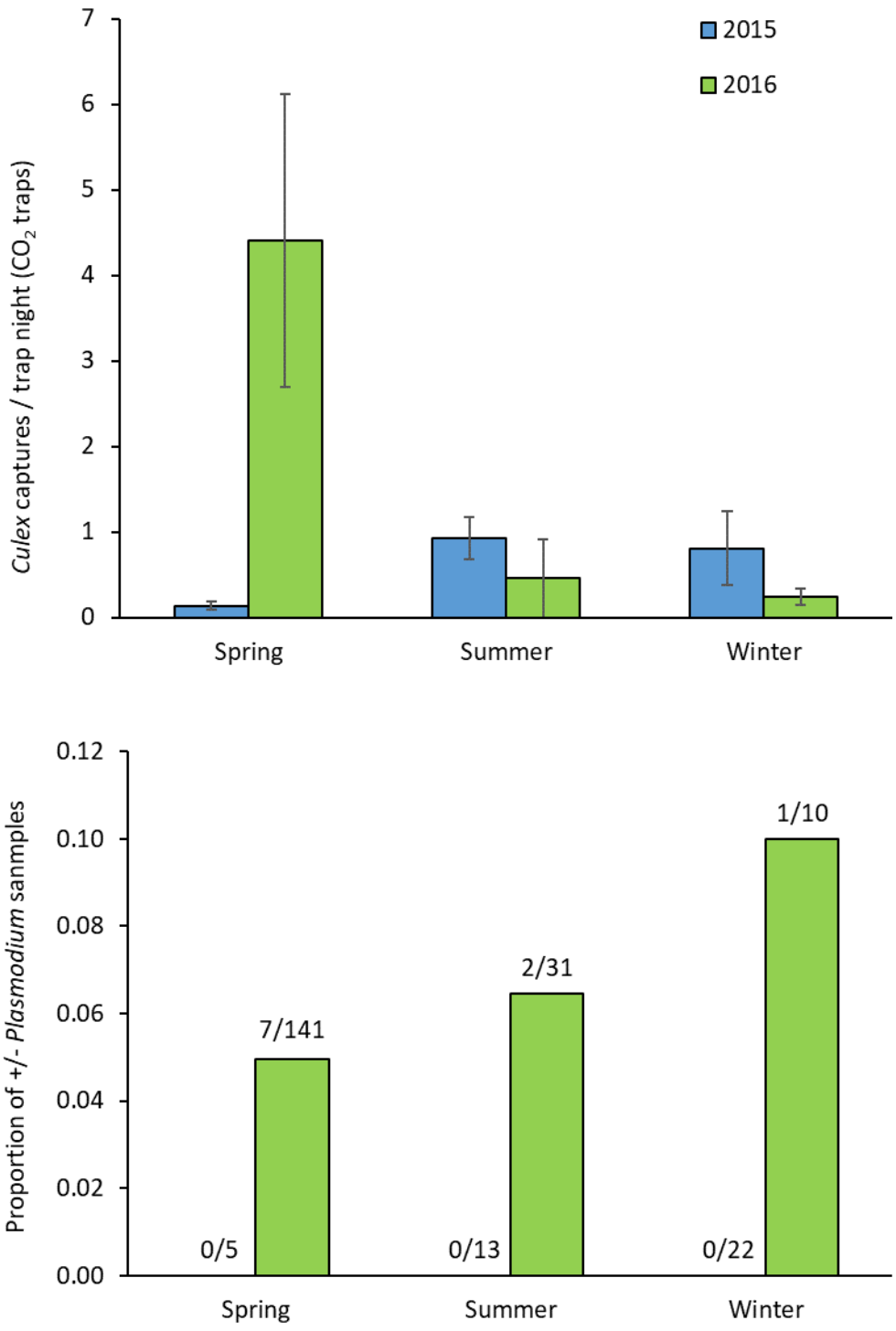

Figure 4. Top) Adult *Culex quinquefasciatus* captures per trap-night by season (± SE) in CO_2 traps in Nakula NAR. Bottom) The proportion of captured adult *C. quinquefasciatus* mosquitoes from Nakula NAR that tested positive for *Plasmodium relictum* by season in both years of study. No mosquitoes captured in 2015 tested positive for *P. relictum*. Values above bars indicated the number of individuals that tested positive and the number of samples tested.

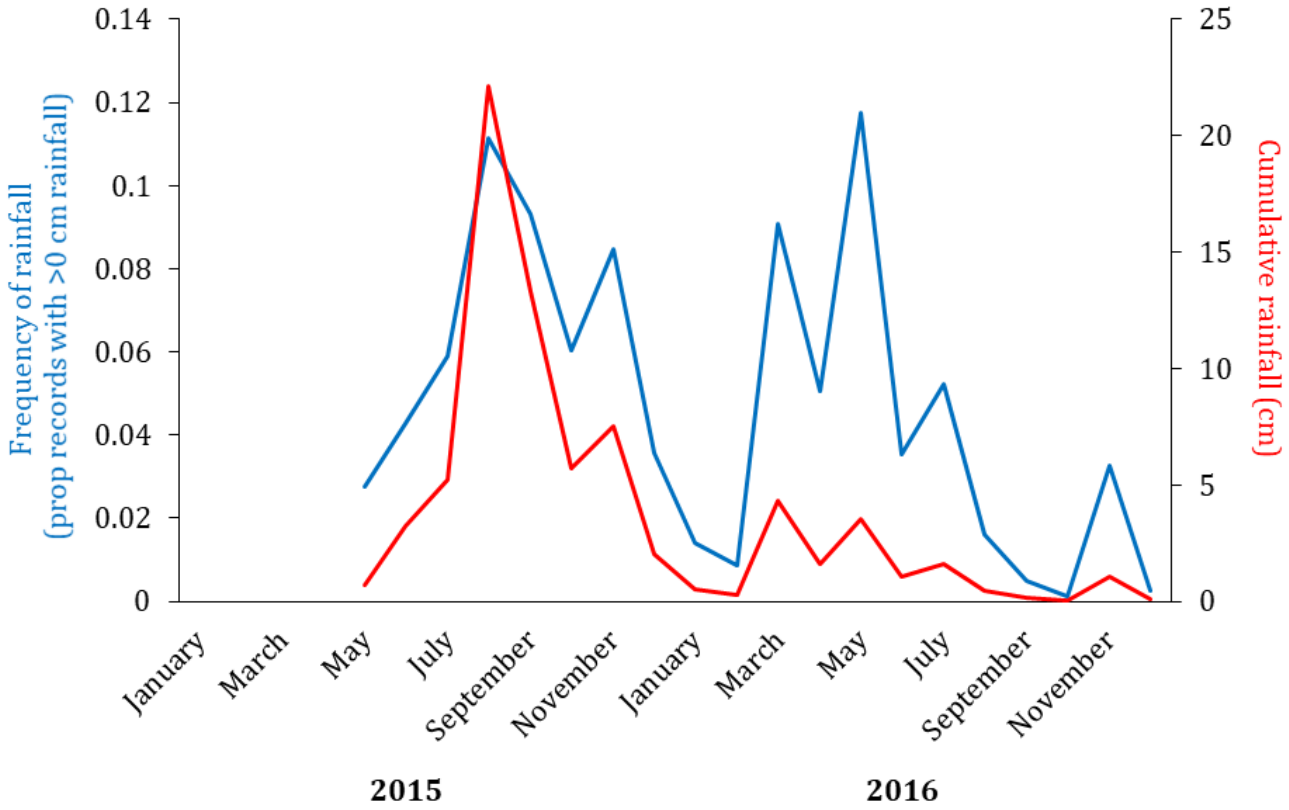

Figure 5. Frequency and intensity of rainfall in Nakula from data collected using an Onset© weather station set to recorded every 30 minutes. Frequency of rainfall events is shown as the proportion of records (30-minutes) recorded with > 0 cm rainfall. Cumulative rainfall is presented as total rainfall recorded (cm) per month.

Larvae samples: We found mosquito larvae in Nakula in each season in both years of study (larvae surveys were not conducted in Waikamoi). When larvae were found, a representative sample was collected for identification. Of the 455 larval mosquito samples collected, 419 were *Culex* sp. (*C. quinquefasciatus* are the only species of this genus recorded on Maui) and 36 were *Aedes* sp. (not identified to species). The number of pools available to sample ranged from 3–42 per transect depending on the amount of rainfall that occurred prior to sampling. However, the mean number of pools sampled per season did not differ between years (t = 1.31, p = 0.321). In total, 8.3% of sampled pools contained *Culex* larvae and 3.4% contained *Aedes* larvae. The mean proportion of pools that contained *Culex* larvae did not differ between years (t = 2.64, p = 0.119). However, there was some seasonal variation in the proportion of pools that contained *Culex* larvae. The proportion of pools with *Culex* larvae increased between spring and summer of 2015 but then declined approximately half between the summer and winter months (Table 3). An increase in precipitation between spring and summer corresponds with the increase in larvae captures between these seasons (Figure 5). This is in concordance with the pattern of adult capture rates during this year as well. Several large rainfall events occurred between the summer and winter sampling periods that likely greatly increased the flow rate of the drainages and may have "flushed out" pools resulting in fewer larvae despite the similar number of pools present. Larvae capture rates were also much higher in the spring of 2016 compared to spring 2015. This also is reflected in the differences in precipitation during the spring seasons in the two years (Figure 5).

Table 3. Mean proportion of sampled pools containing *Culex quinquefasciatus* larvae (± SE), the mean number of pools sampled, and the number of sessions per season.

Year	Season	Pools	Sessions	Prop(*Culex* larvae)
2015	Spring	48.3 ± 10.5	3	0.04 ± 0.02
	Summer	43	1	0.12
	Winter	36 ± 6.0	2	0.06 ± 0.03
2016	Spring	19.5 ± 4.5	2	0.12 ± 0.05
	Summer	33.5 ± 4.5	2	0.15 ± 0.02
	Winter	38.5 ± 2.5	2	0.09 ± 0.09

Larvae were captured most consistently at middle elevations (between 1560-1580 m asl) on all three transects throughout the study (Figure 6). Several individual pools consistently harbored mosquito larvae among seasons and years, particularly at middle elevations. We found *Culex* larvae

at some of the highest elevation pools sampled (1800 m asl) in spring and summer seasons. Comparatively few larvae were collected at the lowest elevation pools and these were primarily found in the summer seasons.

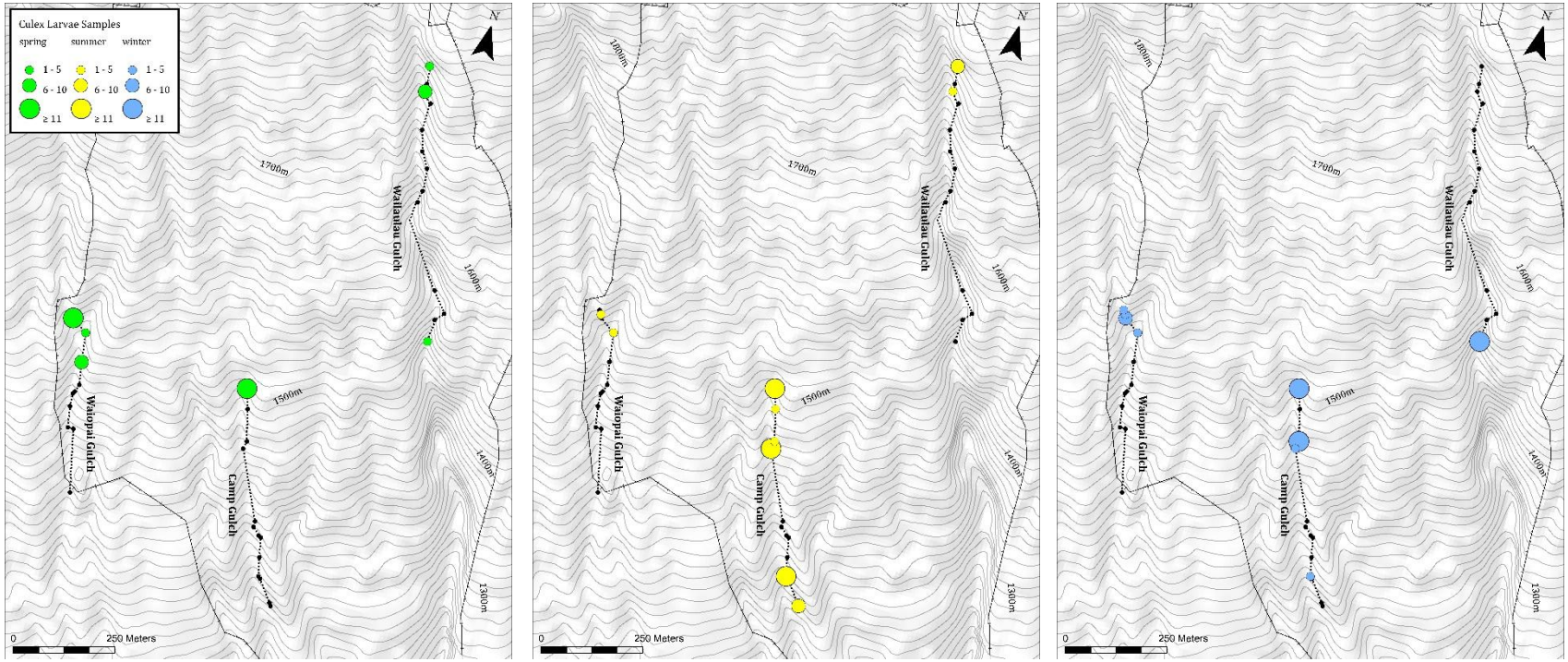

Figure 6. Larval mosquito captures throughout the Wailaulau unit of Nakula NAR by season (2015 and 2016 combined).

Disease Samples

Mosquito samples: All adult mosquitoes captured were tested for the presence of *Plasmodium* genetic material using qPCR. No mosquitoes captured in 2015 (n = 40) tested positive for *Plasmodium* and no *Aedes* (n = 22) tested positive in either year. Out of the 182 *Culex* samples collected in 2016, 10 tested positive for *Plasmodium*, a rate of 5.5% (4.5% of all samples including both years) (Figure 4, Table 2). All positive mosquitoes were captured in CO_2 traps. Of the positive samples, seven were captured in the spring (5% of spring captures), two were captured in summer (5% of summer captures) and one was captured in the winter (10% of winter captures).

Avian samples: Of the 56 blood samples analyzed at the University of New Hampshire (n = 30 for native spp., n = 26 for non-native spp.), one sample from each of the two native species (Hawai‘i ‘Amakihi and ‘Apapane) and a single Red-billed Leiothrix (*Leiothrix lutea*) sample tested positive for *Plasmodium* (Table 4). An additional 121 blood samples of native forest birds from Nakula and Waikamoi were analyzed at the University of South Florida. Of the samples from Nakula, 8.7% (4/46) of the ‘Amakihi and 19.0% (8/42) of the ‘Apapane samples tested positive for *Plasmodium*. Combined, the overall prevalence of *Plasmodium* among all samples was 8.2% (5/61) in ‘Amakihi and 15.8% (9/57) in ‘Apapane in Nakula. Including all bird species (non-native and native), *Plasmodium* prevalence was 10.4% (15/144) in Nakula. The proportion of positive ‘Amakihi blood samples was marginally greater in 2016 compared to 2015 ($x^2 = 3.19$, $p = 0.074$, $df = 1$). However, we found no difference in the proportion of positive ‘Apapane samples between years ($x^2 = 1.55$, $p = 0.213$, $df = 1$). Positive samples were collected from between 1440 and 1755 m (4724–5758 ft) asl, some of the lowest and highest elevations where samples were collected (Figure 7). We collected comparatively few samples from Waikamoi. Of these, one ‘Amakihi (n = 7, 14.3%), two ‘I‘iwi (n = 7, 28.6%), and zero ‘Apapane (n = 3) samples tested positive. All bird samples tested from Waikamoi were collected from the upper portion of the West Honomanu Trail, above 1900 m asl (Figure 8).

Definitive symptoms of active avipox infections were rare at both sites among all MFBRP bird captures with an overall prevalence rate of 0.7% (4/565). Three out of the four active pox infections recorded in Nakula during this study were in ‘Apapane and the other was in a Red-billed Leiothrix. An additional 33 (5.8%) individuals (Nakula = 29, 5.97%; Waikamoi = 4, 5%) were found with missing digits that may indicate past infections but may also be the result of previous injuries unrelated to disease. Of the ‘Apapane and ‘Amakihi that tested positive for *Plasmodium* in Nakula, 42.9% (6/14) were also missing at least one toe and two of the *Plasmodium*-positive ‘Apapane also showed active pox-like lesions. The one ‘Amakihi that tested positive for *Plasmodium* in Waikamoi was also the only bird tested for malaria from that site that was found to be missing a digit.

Although we did not conduct an analysis of annual survivorship, MFBRP's continued presence in Nakula afforded them the opportunity to recapture (both physically recapture and/or resight color-banded individuals in the wild) birds that were tested for *Plasmodium* in this study. Despite the fact that consistent effort was not expended to recapture birds, MFBRP recaptured 3.5% (2/57) of the individual 'Apapane and 24.6% (15/61) Hawai'i 'Amakihi (14.45% of all tested birds) tested in this study at least one year after blood samples were taken. Five of these individuals, 29.4% of reacptures, were recaptured in the spring of 2019. Included in these was a male 'Amakihi that tested positive for malaria in 2015. As such, we know that a minimum of 20% (1/5) 'Amakihi has lived at least four years after testing positive for malaria.

Table 4. Malaria prevalence by species from Nakula Natural Area Reserve (Nakula) and The Nature Conservancy's Waikamoi Preserve (Waikamoi) using qPCR. Species tested included Hawai'i 'Amakihi (HAAM), 'Apapane (APAP), 'I'iwi (IIWI), House Finch (HOFI), Hwamei (HWAM), Japanese Bush Warbler (JABW), Japanese White-eye (JAWE), Red-billed Leiothrix (RBLE), and Scaly-breasted Munia (SBMU). Results are shown from samples tested at the University of South Florida (USF) and the University of New Hampshire (UNH), and both labs combined for HAAM and APAP from Nakula.

		HAAM	APAP	IIWI	HOFI	HWAM	JABW	JAWE	RBLE	SBMU
USF										
Nakula	Total samples	46	42	-	-	-	-	-	-	-
	Malaria +	4	8	-	-	-	-	-	-	-
	rate	8.7%	19.0%	-	-	-	-	-	-	-
Waikamoi	Total samples	7	3	7	-	-	-	-	-	-
	Malaria +	1	0	2	-	-	-	-	-	-
	rate	14.3%	0.0%	28.6%	-	-	-	-	-	-
UNH										
Nakula	Total samples	15	15	-	5	1	5	5	5	5
	Malaria +	1	1	-	0	0	0	0	1	0
	rate	6.7%	6.7%	-	0.0%	0.0%	0.0%	0.0%	20.0%	0.0%
All samples										
Nakula	Total samples	61	57							
	Malaria +	5	9							
	rate	8.2%	15.8%							

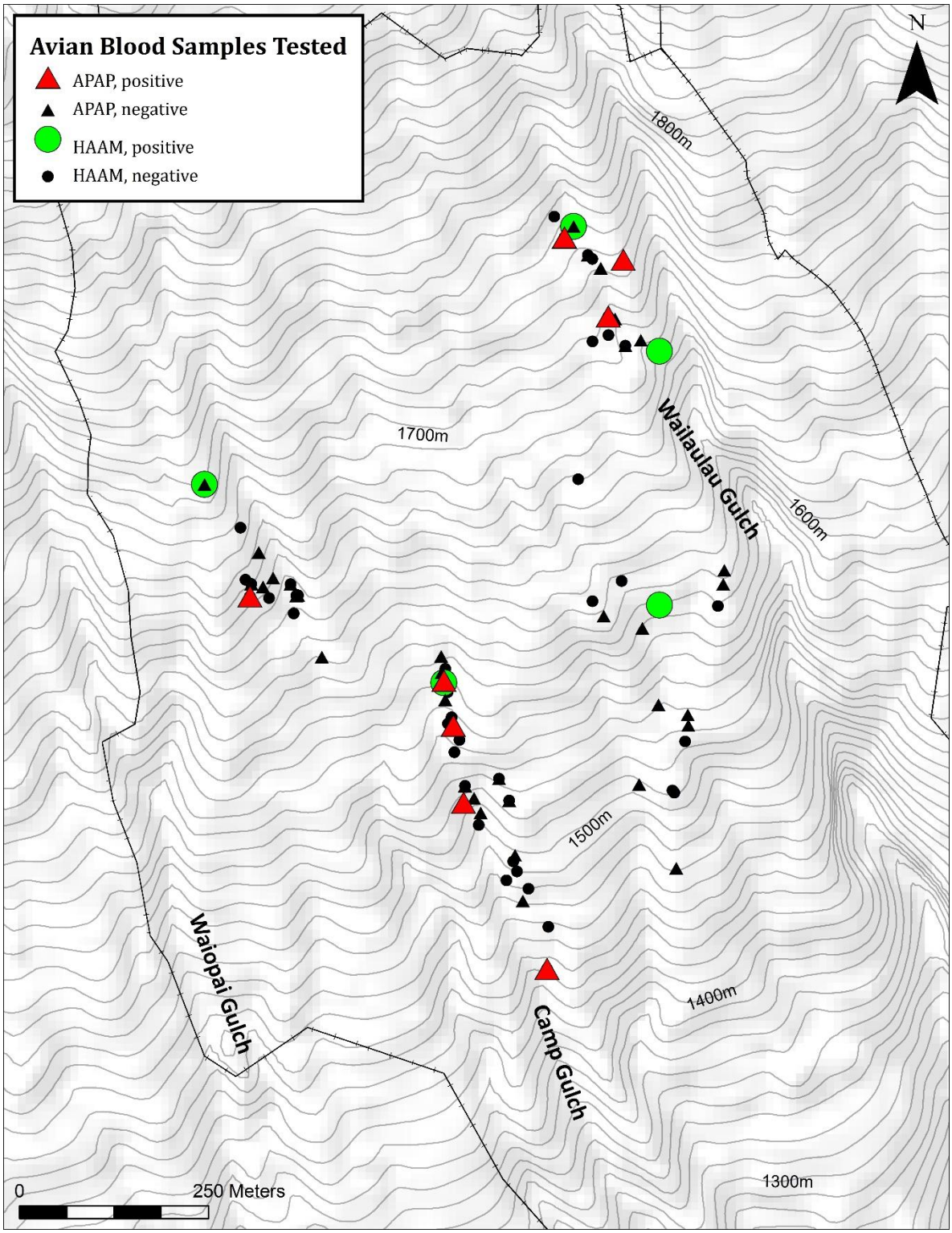

Figure 7. A map of the Nakula NAR study site showing the locations of avian blood samples collected from ʻApapane (APAP) and Hawaiʻi ʻAmakihi (HAAM) and whether samples tested positive or negative for *Plasmodium relictum*. Some individuals were captured at the same locations; number of positive locations ≤ number of positive samples.

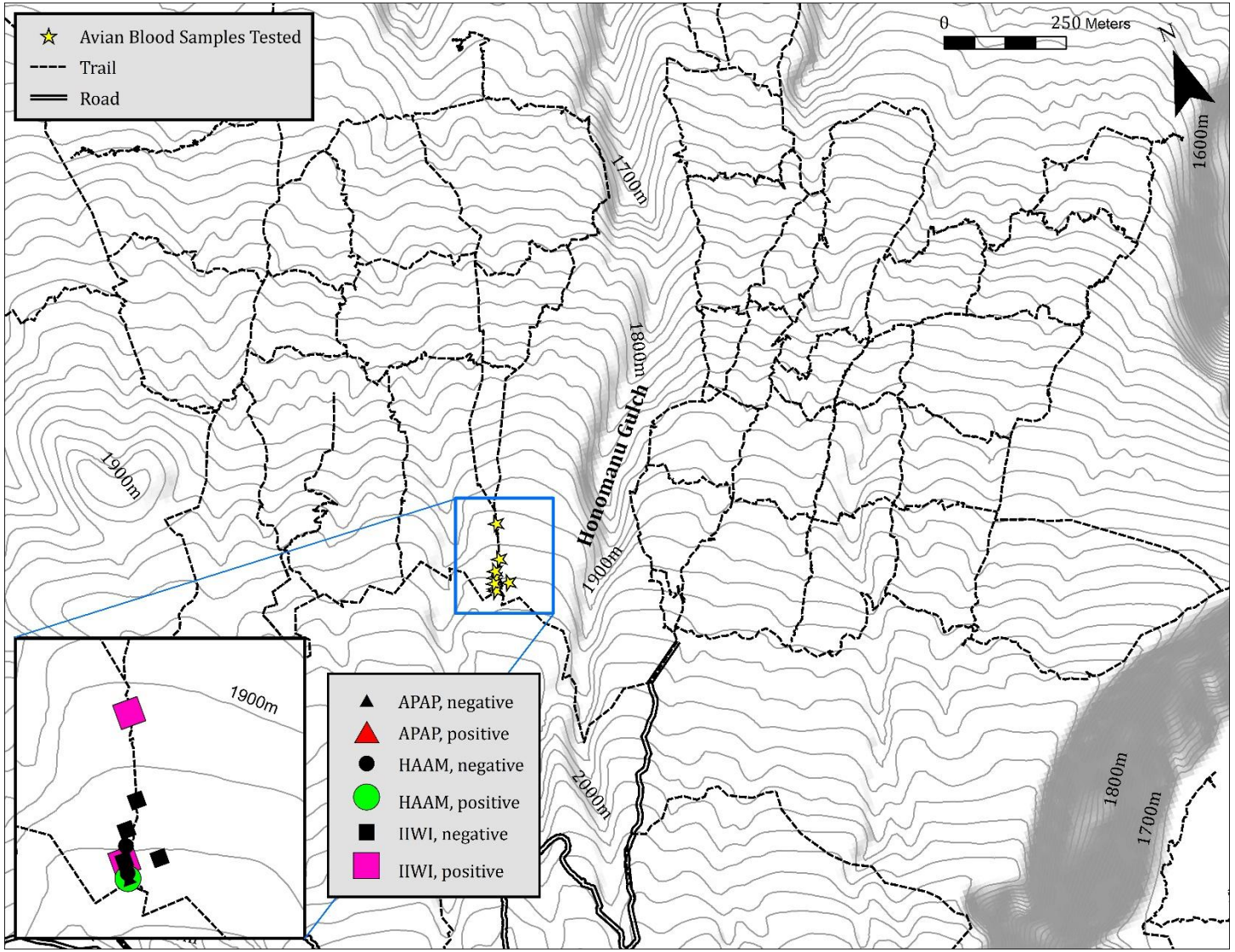

Figure 8. A map of The Nature Conservancy's Waikamoi Preserve study site showing the locations of avian blood samples collected from 'Apapane (APAP), Hawai'i 'Amakihi (HAAM), and 'I'iwi (*Drepanis coccinea*) and whether samples tested positive or negative for *Plasmodium relictum*.

Discussion

Establishing a leeward population of Kiwikiu is paramount to the long-term survival of the species (USFWS 2006, MFBWG 2018). There simply are few other high elevation native forests on Maui that the species does not already occupy. The leeward forest presents the best opportunity to create a second population, increase the species' overall range, and potentially increase overall population size. Further, leeward forests may offer additional benefits to Kiwikiu, such as less frequent storms and large stands of koa that may provide additional foraging sites (MFBWG 2018). The greatest potential obstacle to successful establishment of the species in the Nakula region is the relatively poor condition of the habitat. Forest restoration is currently taking place and natural regeneration of native plant species has been observed throughout areas where ungulates have been excluded. Feral pig eradication may also directly reduce larval mosquito habitat by reducing wallows and hallowed out tern fern (*Cibotium* spp.) trunks (Baker 1975, LaPointe et al. 2009, LaPointe et al. 2012), although this may be less of a concern in mesic-dry forests (Reiter and LaPointe 2009). Over time and with proper management, this habitat should improve.

Avian malaria presents a threat to Kiwikiu throughout its range and both the prevalence of the disease throughout the bird's range and Kiwikiu tolerance to the disease are largely unknown. We infer that Kiwikiu have little natural tolerance to the disease mostly from their elevational distribution, which is above 1400 m asl. The most plausible explanation for this distribution pattern is the prevalence of avian malaria at lower elevations. There are large areas adjacent to and just below the current range of Kiwikiu that show little to no difference in habitat compared to occupied habitats and the species is known to have occurred at low elevations in the past (James and Olson 1991). Climate change is causing a warming trend throughout Hawai'i and the range of *Culex* is predicted to increase, potentially increasing rates of malaria and other diseases in higher elevation habitats that have thus far acted as refugia for honeycreepers like Kiwikiu (Atkinson and LaPointe 2009). This will likely continue to shrink the already small Kiwikiu range (30 km^2) (Fortini et al. 2015). This is particularly concerning for species like Kiwikiu that are naturally low-density and occupy large home ranges (Warren et al. 2015). The low population density, small overall range, and low productivity rates of the species have resulted in low population size, and the loss of any individuals to disease has a large impact on the species as a whole. Increasing the species' overall range by reintroducing the species to the leeward high elevation forests will, hopefully, help offset the predicted range loss on the windward slopes due to climate-induced range expansion of disease.

Prior to the reintroduction of Kiwikiu to Nakula, we sought to estimate the prevalence of avian malaria and its primary vector, *Culex* mosquitoes, within the release site. As a basis of comparison, we estimated vector prevalence and tested avian blood samples from Waikamoi, an area currently occupied by Kiwikiu and other endangered bird species. The most striking finding of this study was the presence of a persistent population of *Culex* mosquitoes in Nakula at higher elevations than previously thought. We documented the presence of larval *Culex* mosquitoes up to 1800 m asl and adults and larvae were captured throughout the year above 1500 m asl. The elevations where these mosquitoes were captured in Nakula are consistent with those where Kiwikiu persist in their current range. Larval *Culex* have been found throughout the year at similar elevations elsewhere in Hawai‘i, e.g. 1500 m asl (Goff and van Riper III 1980), and at times much higher within the current Kiwikiu range, 2100 m (Berlin et al. 2001). We did not detect any *Culex* mosquitoes at similar elevations in Waikamoi in 2016. However, we did detect the presence of *Plasmodium* in avian blood samples collected in Waikamoi, likely indicating the presence of mosquitoes in Waikamoi at some point in the past. However, *Culex* presence and disease transmission may be seasonal and the positive samples we collected may be chronic. It is clear that several factors beyond elevation are driving the prevalence of mosquitoes and parasites in these two sites.

Mosquito prevalence – When selecting the site for a second population of Kiwikiu, one of the perceived benefits to the leeward forest was that the drier weather conditions might provide less mosquito breeding habitat than the windward slopes. The results of this study clearly indicate that this is not the case and that *Culex* can persist in Nakula throughout the year and seemingly in higher numbers than comparable elevations on the windward side. Further, the “mosquito line” (Warner 1968), the point at which mosquitoes cannot persist, may be non-existent on the leeward slope or at such high elevations as to not be relevant to forest bird populations in this region of Maui.

Few studies of mosquito prevalence in Hawai‘i have included elevations as high as those in this study. However, capture methodology was nearly identical and direct comparisons can be made among capture rates. On Hawai‘i Island, LaPointe (2000) captured 0.04 mosquitoes/trap-night (mq/TN) in CO_2 traps at sites above 1500 m asl. This included one site at 1800 m asl where the capture rate was 0.08 mq/TN. Gaudioso-Levita et al. (2015) captured 1.36 mq/TN across all sites and elevations including lower elevations (1178–2194 m). Other studies have primarily focused on lower elevation sites. In Haleakalā National Park, Aruch et al. (2007) had an overall capture rate of 1.46 mq/TN in CO_2 traps but did not detect any mosquitoes at their highest elevation site at 1430 m asl. Woodworth et al. (2005) reported an overall capture rate of 0.34 mq/TN with a maximum of 0.62 mq/TN at sites < 300 m asl on Hawai‘i Island. McClure (2017) had an overall capture rate of

10.5 mq/TN from sites below 350 m asl. Our overall capture rate of 1.02 mq/TN in CO_2 traps was much higher than what has been reported from sites above 1500 m asl (LaPointe 2000, Gaudioso-Levita et al. 2015) and more similar to what others have found at much lower elevations, although not nearly as high as some low elevation sites (Woodworth et al. 2005, Aruch et al. 2007, McClure 2017). However, capture rates in Nakula were variable and the spring 2016 season accounted for a large percentage of all adult mosquitoes captured. During the spring 2016 season, a single night in Nakula accounted for 30% of all adult mosquitoes captured in this study. Our overall capture rate without the spring 2016 season was 0.3 mq/TN, similar to rates seen in other studies.

One possible factor leading to higher rates of mosquito captures in our study compared to others was the placement of traps within each site. In Nakula, we placed traps in the only truly forested areas available, which also corresponded to drainages and thus, mosquito breeding habitat. It is likely that placing the traps near drainages positively biased our capture rates by placing them in areas where mosquitoes concentrated for breeding. However, these forest patches are the best available habitat for native songbirds, particularly with respect to roosting and nesting areas. Thus, the capture rate may also best represent the mosquito concentrations in the areas utilized most by honeycreepers. Additionally, the majority of the adult mosquitoes were captured in the CO_2 traps targeting females seeking out a blood meal. These female mosquitoes may have also been concentrated where the food resources (e.g. birds) were denser. Although, placing the oviposition traps near drainages may have negatively biased capture rates in this trap type given that gravid females had more choices of water sources to lay their eggs. In Waikamoi, traps were also never far from drainages in large part because drainages occur approximately every 50–100 m along the trails where the traps were placed. In this respect, the trap placement was similar between the two sites. The biggest difference between the sites is that in Nakula, there are far fewer drainages and water sources and forest habitat is more concentrated and patchy. As a result, mosquito breeding and mosquito food resources may have been more concentrated in Nakula compared to Waikamoi. Additionally, the Nakula site's proximity to disturbed habitat, i.e. ranchland, and previous grazing history may also increase the suitability of the site for larval mosquito habitat compared to a more intact site like Waikamoi (McClure et al. 2018).

Two main factors are most important to the presence of breeding *Culex* populations, favorable temperatures and standing water for larval development (Reiter and LaPointe 2009). Nakula is generally warmer than Waikamoi with an average annual temperature of 14.3 ˚C (57.8 ˚F) in Nakula compared to 11.9 ˚C (53.4 ˚F) in Waikamoi (Giambelluca et al. 2014). This is, in part, because monthly temperatures fluctuate less in Nakula with average monthly temperatures varying only ~

1 ˚C throughout the year (12.3 ˚C [February] to 13.3 ˚C [August]). In Waikamoi, average monthly temperatures vary >2 ˚C throughout the year (10.3 ˚C [February] to 13.6 ˚C [August]). Summer temperatures at both sites are similar but Nakula winters are typically much warmer (>2 ˚C on average). Cold snaps could provide significant barriers to the persistence of a mosquito population. Almirón and Brewer (1996) estimated a minimum developmental threshold for *Culex* of 9.5 ˚C (49 ˚F). In theory, if a pool were to drop below 9.5 ˚C, any larvae within would fail to develop. Average hourly temperatures in February drop to a low of 8.5 ˚C (47.4 ˚F) in Waikamoi and 9.1 ˚C (48.4 ˚F) in Nakula (Giambelluca et al. 2014). During the sampling intervals, our on-site weather station in Nakula recorded a minimum temperature of 6.7 ˚C (44.1 ˚F) per 30-min interval and a record minimum of 5.4 ˚C (41.7 ˚F) since it was installed in 2014. A minimum monthly temperature below 9.5 ˚C was recorded in Nakula during the winter and spring seasons (December-April). In February 2016, these limiting temperatures for *Culex* were recorded during 7.5% of temperature readings (30-min intervals). However, in January and March, these temperatures were recorded during < 3% of the time and these low temperatures were very rare or not recorded in other months. Although we did not have a weather station on site in Waikamoi, we can presume that the colder average temperatures in the winter months likely translated into lower minimum monthly temperatures at this time scale compared to Nakula. We do not have information on the duration of these cold temperatures in Waikamoi. Thus, the potential exists at both sites for cold snaps to stop larval mosquito development if the water temperatures correspond to the minimum air temperatures recorded. However, the cold temperatures capable of halting *Culex* larvae development that occurred in Nakula were generally rare even during the coldest months of the year.

Two important factors may affect whether the cold periods recorded at both locations can have an impact on mosquito populations at these sites. One, these temperatures are recorded as ambient air temperature, not water temperature. Given the higher heat capacity of water, the temperature of the stagnant pools may remain greater than the air temperature. This is likely to be the case if the air temperature is warm for long periods during the day and only drops for short periods at night, as the Nakula weather data indicates. In this way, the duration of the cold periods may be a more important factor affecting the survivorship of mosquito larvae in these areas as opposed to whether or not cold snaps occur. Even in winter, average afternoon temperatures are fairly warm at both sites; 14.2 ˚C in Nakula and 13 ˚C in Waikamoi in February. In these conditions, the temperature of the pools may experience less daily variability and may stay closer to the average daily temperature. Thus, even under conditions where temperatures may temporarily drop below the 9.5 ˚C threshold, some pools may maintain higher temperatures. The second factor to consider for

mosquito persistence in an area is adult survivorship. Almirón and Brewer (1996) reared some adult mosquitoes at 5 ˚C, below the coldest temperature recorded in Nakula. Some of those mosquitoes survived for up to seven weeks, provided they were fed. Thus, the temperatures recorded at our sites were unlikely to affect adult survivorship. If adults are capable of surviving several weeks, they are also likely able to wait out the cold periods.

Larval development time for *Culex* is positively affected by temperatures (Rueda et al. 1990, Almirón and Brewer 1996, Ciota et al. 2014). Ciota et al. (2014) found time to emergence was nearly twice as slow for larvae reared at 16 ˚C compared to those reared at ≥ 24 ˚C. As such, the average air temperatures recorded in Nakula and Waikamoi during much of the year were within the temperature tolerance for larval development. However, the rate of larval development may be reduced compared to warmer sites. Ciota et al. (2014) also found that adult survivorship declined rapidly above 20 ˚C, much warmer than the average temperature in the warmest months at both sites. We recorded a maximum daily temperature above 20 ˚C in 14 out of the 20 months during the study period (May 2015–December 2016). However, these high temperatures occurred for short periods; accounting for < 8% of temperature readings per month. When well fed, adult *Culex* mosquitoes are capable of surviving up to six weeks; with females surviving an average of 18 days in a laboratory setting (Almirón and Brewer 1996). Thus, while the conditions found in our study sites may not be ideal for larval development, temperatures were generally cool enough to allow adults to survive for longer periods.

Although not ideal, air temperature in Nakula and Waikamoi throughout the year seems unlikely to significantly restrict mosquito persistence and reproduction. However, the persistence of standing water for larval development likely has a large influence on the mosquito populations. Nakula represents a considerably drier habitat than found in the current Kiwikiu range. However, the lower annual rainfall does not mean that the area is too dry to support mosquitoes. There is often not enough rain to allow the streams to consistently flow in Nakula; streams flow only semi-annually following large rainfall events. The rain that does fall often collects in small pools within the drainages and these pools can persist for several months. Reiter and LaPointe (2009) found rock holes in intermittent streams, precisely like those found in Nakula, to be the primary larval habitat in native mesic-dry forest habitats. Regardless of actual precipitation amounts, there clearly is enough breeding habitat in the form of standing pools in Nakula for the persistence of *Culex* throughout the year. In contrast, while pools existed in Waikamoi during the adult mosquito surveys, the frequency and intensity of rainfall events may have regularly flushed out pools that could have served as habitat for larval development. Some rain fell during part of five of the six

survey periods in Waikamoi. Rain fell constantly for several days during some of these trips and streams were often flowing during the adult mosquito surveys in Waikamoi. Stream gage data from West Wailuaiki Stream that drains a portion of Waikamoi just east of the study area showed a daily average of 10,083 ± 1276 ft^3 of water flowing from the area during the study period (13 March – 21 December, 2016; USGS 2019). Two mosquito sampling periods, one in the spring and one in the winter 2016, coincided with particularly high flow rates (>25,000 ft^3), while the other periods had average daily flow rates < 4300 ft^3 (Figure 9).

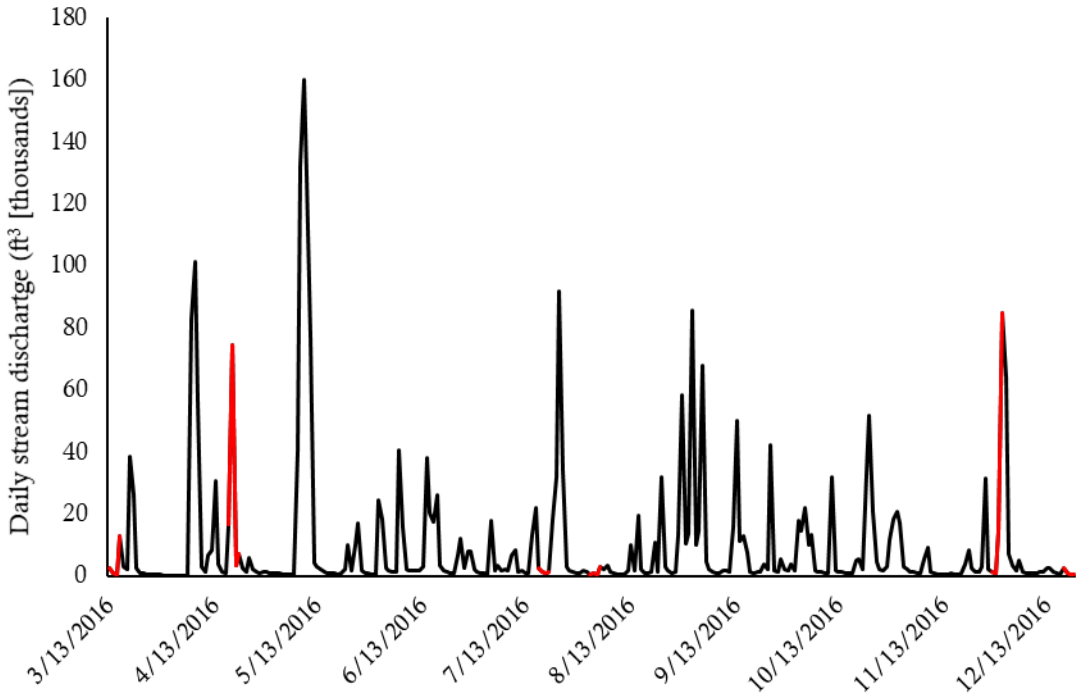

Figure 9. Stream discharge data from West Wailuaiki Stream during the adult mosquito sampling periods in TNC Waikamoi Preserve (USGS 2019). The lines in red indicate the sampling days.

Atkinson et al. (2014) hypothesized that increased temperatures and reduced stream flow could explain an increase in disease prevalence in native bird population at higher elevations on Kaua‘i Island. They found that, although overall flow rates had not declined, the interval between low-flow periods had increased potentially allowing larvae to develop before high-flow periods flushed them out of pools. During the study period in Waikamoi there were on average only 7.4 ± 1.2 days (maximum of 25 days) between high-flow periods (continuous days with daily stream discharge greater than the average; >10,000 ft^3) according to the West Wailuaiki Stream gage data. If it takes 28 days for *Culex* larvae to develop at 17 °C (Kokkinn et al. 2012), there may have been

few opportunities for mosquitoes to develop during the study period, at least in larger streams (Atkinson et al. 2014). Although air temperature in Waikamoi may be favorable enough to support mosquitoes, the frequency and duration of high-flow periods that routinely flush out streams may restrict mosquito reproduction. In Nakula, high-flow periods are rare throughout the year and mosquitoes may be more limited by pools drying up rather than being flushed out. In this way, stream hydrology may have the greatest impact on the persistence of mosquito populations at both sites but the mechanism differs between the sites.

Malaria prevalence – While we found more mosquitoes than predicted in Nakula, whether these mosquitoes represent a significant threat as vectors of disease is a separate issue. Minimum temperatures for the development of *Plasmodium* are generally higher than for mosquitoes. Thus, while mosquito presence is necessary for the presence of *Plasmodium*, the parasite has its own, more restrictive environmental parameters. Ideal temperatures for sporogonic development of *Plasmodium* in *Culex quinquefasciatus* is between 21 and 28 ˚C and complete development can occur as low as 16 ˚C (Chao and Ball 1962, Garnham 1966, Noden et al. 1995, LaPointe et al. 2010). Suboptimal temperatures (16-21 ˚C) generally increase sporogonic development time; e.g. 4 × development time at 17 ˚C compared to 21 ˚C (Ball and Chao 1964, LaPointe et al. 2010). LaPointe et al. (2010) estimated 13 ˚C to be the minimum threshold temperature for oocyst development. Thus, *Plasmodium* development is likely limited to temperatures above 13 ˚C but sporogonic development may only occur above 16 ˚C and optimal conditions are above 20 ˚C. Under these parameters, the temperatures observed in Nakula and Waikamoi are mostly outside optimal conditions for *Plasmodium* reproduction but suboptimal conditions may occur at certain times of the year. Between January and March in Nakula, fewer than 50% of temperature readings were above 13 ˚C and fewer than 15% of readings were above 16 ˚C through June (Figure 10). Thus, while sporogony was possible during these months, optimal temperatures (> 20˚C) were extremely rare and even suboptimal temperatures (> 16˚C) were uncommon. In contrast, the majority of temperature readings between July and October were within the suboptimal temperature range for sporogonic development, although < 10% of readings were within the optimal temperature range. As such, temperatures in Nakula were generally poor for sporogonic development February to May when daily temperatures typically remained below the threshold temperature (16 ˚C) and did not reach ideal temperatures (20 ˚C). During the other months, July-November, conditions were suboptimal for sporogonic development. However, the presence of the parasite in captured mosquitoes in all seasons suggests that the parasite is capable of developing at least to the oocyst stage throughout the year.

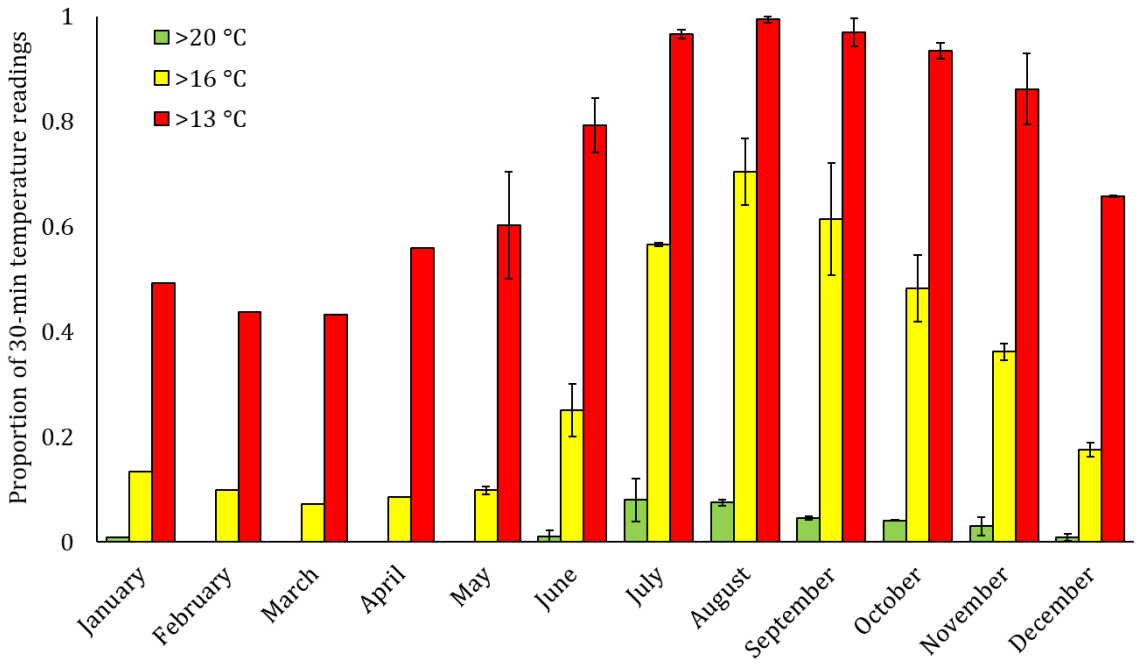

Figure 10. The proportion of temperature readings (30-min intervals) above the minimum threshold for oocyst development (>13˚C) and sporogony (>16˚C) and optimal sporogonic development (>20 ˚C) temperatures in Nakula between May 2015 and December 2016.

Differences in methodology make precise comparisons of malaria prevalence in adult mosquitoes challenging between ours and other studies. In using a whole body sample for mosquito qPCR analysis, we chose the most sensitive technique available. In this way, we were able to determine if *Plasmodium* was present in a given mosquito at any stage of development. A positive sample does not indicate that a mosquito is currently harboring live parasites or that the parasites are in the life stage necessary for transmission to hosts (i.e. sporozoites). In this study, a positive sample may indicate a mosquito that had once been host to live *Plasmodium* parasites, currently contains oocytes in the hindgut, and/or contains sporozoites in the salivary glands ready to be transmitted. Given the limited forest habitat in Nakula (limiting elevational bird movement), if a mosquito tests positive for *Plasmodium* it likely contracted the parasite locally. However, we cannot know if the mosquito was capable of transmitting the parasite at the time it was collected.

In our study, we found 5.5% of captured mosquitoes in 2016 (4.5% in both years) were positive for *Plasmodium*. With a few exceptions, most studies that investigated *Plasmodium* prevalence in adult mosquitoes did not include elevations as high as in the current study. One important caveat is that in most of the studies discussed here, malaria prevalence was detected by dissection of the

midgut and salivary glands, not qPCR as in this study. On Hawai'i Island, LaPointe (2000) captured infected *Culex* up to 1500 m asl and found *Plasmodium* prevalence as high as 26% at their highest elevation site during the warmest months. Also on Hawai'i Island, Gaudioso-Levita et al. (2015) found an overall *Plasmodium* prevalence rate of 24% in mosquitoes captured at sites between 1178-1201 m asl but no positive samples were found at higher elevations, including the few mosquitoes captured at 1500 m. On Maui, Aruch et al. (2007) found 3–4% of mosquitoes captured at sites below 760 m tested positive for *Plasmodium*, but none of the mosquitoes captured at higher elevations tested positive. Woodworth et al. (2005) considered their prevalence rate of 15% to be high among samples collected at sites below 300 m asl. McClure (2017) found rates between 15-30% among sites around 300 asl using qPCR. The fact that most studies sampled lower elevations than Nakula, did not capture mosquitoes at high elevations, and/or used different methodologies, it is somewhat challenging to contextualize the *Plasmodium* prevalence we found in Nakula mosquitoes. Based on the qPCR method used here, we would expect our rates of infection in mosquitoes would be biased high compared to results from studies that using dissection. If true, then the prevalence we found in Nakula mosquitoes (5.5%) may be similar to or lower than comparable locations.

With the caveat that positive results using qPCR does not indicate if an individual mosquito was capable of transmitting the disease, the presence of positive samples in all three sample periods indicates that the potential for transmission existed throughout the year. The lack of positive samples from 2015 may indicate inter-annual variation in *Plasmodium* abundance but does not indicate that the disease was absent from the site in 2015, as the results from the avian blood sample analysis show. The lack of positive samples is possibly related to sample size as we collected 4.5 × fewer samples in 2015 than 2016. However, capture rates in the summer and winter seasons between the years were similar and positive samples were collected in these seasons in 2016. However, the exceptional spring abundance of mosquitoes may have had a lasting effect throughout the rest of 2016 by boosting the number of infected hosts in Nakula, which then translated into an increased the number of infected mosquitoes later in the year.

Malaria prevalence in Hawaiian honeycreeper populations varies greatly among sites and elevations but some general patterns have been observed. Several studies have investigated *Plasmodium* prevalence in 'Apapane and 'Amakihi (*Chlorodrepanis* spp.) at various elevations. In general, the pattern of infection is strongly influenced by elevation wherein prevalence decreases as elevation increases (Krend 2011, Samuel et al. 2015). Low elevation sites can show surprisingly high rates of infection in these species, e.g. 75%-100%, but mortality at these low elevations can

also be quite low (Atkinson et al. 2005, Samuel et al. 2015, McClure 2017). At mid-elevations (1000 – 1300 m asl) *Plasmodium* prevalence can still be quite high in ʻApapane, 30-60% depending on the study, but is often much lower in ʻAmakihi (*C. flava*/*virens*), 11.5-17% (Krend 2011, Samuel et al. 2015). Following the general trend, several studies have found comparatively low *Plasmodium* prevalence (but notably not absence) in these species at high elevations (1500-1850 m) (Atkinson et al. 2005, Samuel et al. 2015). Interpretation of infection rates of ʻApapane, including those at high elevations, should be tempered by the fact that they exhibit regular elevational movements in search of nectar sources and samples may not accurately reflect infection rates at the elevations at which samples were collected (Ralph and Fancy 1995).

Direct comparisons of infection rates between our study, using qPCR, and others using serology or microscopy is difficult as these methods have different sensitivities and limitations. Results from qPCR tests are likely to be much more sensitive than microscopy results but less so than serology (Jarvi et al. 2002). Additionally, these tests all indicate slightly different things. Serology for example, detects antibodies formed after exposure to the disease (Fallon et al. 2003). This method is typically the most sensitive of the three techniques because it does not require the parasite to be present in a sample. Microscopy is typically less sensitive than the other two methods because it requires an experienced observer to physically examine blood samples for the presence of parasites and may miss low parasite loads, particularly in chronic infections (Jarvi et al. 2002, Fallon et al. 2003). In comparison, microscopy diagnoses a currently infected bird, serology indicates an immuno-response to the parasite but not the parasite itself, and qPCR detects the presence of the parasite but not necessarily in a transmissible form. Microscopy is the only technique of the three that can detect currently live parasites in a blood sample. Both serology and qPCR may detect past infections as well as current.

Atkinson et al (2014) estimated prevalence of avian malaria along an elevation gradient of 1040-1350 m asl on Kauaʻi Island using qPCR and found similar rates of infection in ʻApapane (15.2%) compared to Nakula but much higher rates in Kauaʻi ʻAmakihi (*Chlorodrepanis stejnegeri*, 31%, Table 5). Although we captured birds between 1440-1755 m asl in Nakula, higher than the Kauaʻi sites, the habitat in Nakula ranges from 1050-1900 m asl and birds may range within this area in search of food. At a leeward site on Hawaiʻi Island at 1520 m asl Atkinson et al. (2005) found infection rates of 18% and 55% in Hawaiʻi ʻAmakihi and ʻApapane, respectively. Even at their highest elevation site (1830 m asl), they found a greater percentage of infected ʻApapane (30%) than what we recorded in Nakula (15.8%). Samuel et al. (2015) found rates of 1.5% for ʻAmakihi and 7.8% for ʻApapane at sites above 1650 m asl. Both Atkinson et al (2005) and Samuel et al.

(2015) used serology to diagnose malaria infection. Jarvi et al. (2002) estimated that qPCR accurately diagnosed 61% of known infections compared to 97% in serology. If we adjust our infection rates to account for this differences (+37%), malaria prevalence in Nakula in ʻApapane (21.6% adjusted) and ʻAmakihi (11.2% adjusted) were similar to or possibly lower than those reported by Atkinson et al. (2005) but higher than those reported by Samuel et al (2015). Samuel et al. (2015) further estimated that annual survivorship was > 0.5 in infected ʻApapane and > 0.7 in infected ʻAmakihi at mid- and high-elevations. Although they did not measure *Plasmodium* prevalence in these species, many of the sites studied by Atkinson et al. (2005) and Samuel et al. (2015) included areas within the ranges of several endangered honeycreepers including Hawaiʻi ʻAkepa (*Loxops coccineus*), ʻAlawī (*Loxops mana*), and the closest living relative to Kiwikiu, ʻAkiapolaʻau (*Hemignathus wilsoni*).

Table 5. Comparative malaria prevalence in ʻApapane and ʻAmakihi species (*Chlorodrepanis stejnegeri* [Kauaʻi], *C. flava* [Oʻahu], and *C. virens* [Hawaiʻi and Maui]) in selected sources. Values represent the percentage of tested individuals positive for malaria infection using either qPCR or serology. Values in parentheses are adjusted values for comparison between diagnosis methods (+37% qPCR to serology, -37% serology to qPCR) based on Jarvi et al. (2002).

	This study	Atkinson et al. (2014)	Krend (2011)	Atkinson et al. (2004)	Samuel et al. (2015)
	Maui	*Kauaʻi*	*Oʻahu*	*Hawaiʻi*	*Hawaiʻi*
	1442-1766 m	1040-1350 m	125-1160 m	1520 m	> 1650 m
	qPCR	qPCR	qPCR	serology	serology
ʻApapane	15.8 (21.6)	15.2 (20.8)	30.0 (41.1)	55.0 (34.7)	7.8 (4.9)
ʻAmakihi spp.	8.2 (11.23)	31.0 (42.5)	11.5 (15.8)	18.0 (11.3)	1.5 (0.9)

An important caveat is that it is possible that the ʻApapane and ʻAmakihi that tested positive in our study were not infected in the same year that they were captured, as some individuals have tested positive in captivity using qPCR for more than four years after infection and infections may be chronic (Jarvi et al. 2002). If this is the case, there may be annual variation in infection rates and mosquito abundance that were not possible to capture in this two-year study. We collected more positive ʻAmakihi samples in 2016 compared to 2015 suggesting that annual variation in infection rates occurs, but we do not know if this was related to variation in mosquito densities between years given how long a bird may test positive following infection. We also did not see the same pattern in ʻApapane. Further, we do not know if the mosquito capture rates or infection rates that

we saw in Nakula represent a high or low point in the population dynamics of the disease within the site. Given that essentially no habitat exists below the Nakula site (i.e. pastureland) and the forest band that includes Nakula is isolated from other forest habitats, there is little chance that these birds were infected outside of the general area surrounding Nakula. Thus, these malaria infections were very likely endemic to Nakula and Kahikinui.

Although we tested comparatively few avian blood samples from Waikamoi, both the rates of *Plasmodium* in the samples and elevations at which the positive samples were collected are notable. We found positive samples in ʻAmakihi (1/7; 8.5%) and ʻIʻiwi (2/7; 28.6%) collected above 1900 m asl. There is good evidence that ʻApapane and ʻIʻiwi exhibit some elevational movement throughout the year to exploit food resources (Ralph and Fancy 1995, Berlin et al. 2001, Guillaumet et al. 2017). Guillaumet et al. (2017) captured ʻIʻiwi at 1920 m asl (similar to our Waikamoi capture site) and found some individuals moved down as low as 738 m asl, although the average lowest elevation was 1240 m asl. Thus, the individuals that tested positive in Waikamoi may have contracted the parasite at lower elevations than where they were captured. ʻAmakihi are considered far more sedentary (Knowlton et al. 2017) and it is more likely that the individual that tested positive from 1900 m asl in Waikamoi contracted the parasite locally. Additionally, the ʻIʻiwi sampled were captured in February, during the breeding season, a time when the species tends to travel shorter distances than during the non-breeding season (Knowlton et al. 2017). However, a positive qPCR result could include an individual that contracted the parasite much earlier than the season or even the year in which it was captured. Given the low sample size, the rates of *Plasmodium* infection are hard to compare results from Waikamoi to those in Nakula and elsewhere in Hawaiʻi. On the other hand, the fact that we detected positives with so few samples might suggest higher-than-predicted rates in the population. Atkinson et al. (2005) tested only nine ʻIʻiwi samples from their highest elevation site (1830 m asl) and found one positive sample (16%).

One of the assumptions stemming from Wagner's (1968) "mosquito-line" hypothesis is that native birds at high elevations are relatively "safe" from avian malaria. This is often misinterpreted to mean that these upper elevation refugia are "disease-free". In the context of the Kiwikiu reintroduction to Nakula, many have been concerned (and rightfully so) whether the released birds will be at a greater risk of contracting malaria in Nakula compared to their current range. Results from this and other studies (e.g. Atkinson et al. 2005, Samuel et al. 2015) demonstrate that mosquitoes and avian malaria are rarely absent from high elevation sites. Rather, there is a general trend of lower disease prevalence with increasing elevation. The persistent mosquito population and evidence of local malaria transmission demonstrate that the risk of contracting disease

(without mosquito management) is higher in Nakula than Waikamoi. This does not mean that Kiwikiu in Waikamoi or other parts of their current range are free from disease (as evidenced by positive blood samples) but the lack of mosquito captures at the very least indicates that the risk of infection is lower and possibly seasonal. However, given the dire projections for the species the question for Kiwikiu conservation is less whether malaria presents *any* risk to Kiwikiu in the release site but rather, is this risk acceptable? This study also highlights the need for mosquito management during the reintroduction and possibly beyond. The results from Waikamoi invite additional study into malaria prevalence in honeycreeper populations in high elevation forests on Maui, including in endangered species like Kiwikiu. These results are consistent with other high elevation sites tested and if rates of malaria are increasing in these areas, this could help explain continued population declines in many of the endangered honeycreepers even in high elevation protected habitat (Judge et al. 2019). It is clear that the biology and distribution of *Plasmodium* and its vector, *Culex quinquefasciatus*, are complex. The precise interplay between seasonal temperature changes, rainfall, hydrology, topographic features, and a host of other factors are likely far more important to whether disease rates are low enough to allow sensitive species like Kiwikiu to persist in an area than elevation alone.

Conclusion – In Nakula, *Culex quinquefasciatus*, the primary vector of the avian malaria parasite, is able to maintain a persistent population year-round. Although no adult *Culex* mosquitoes were captured in Waikamoi, several honeycreepers captured in Waikamoi above 1900 m asl tested positive for the parasite, indicating that at least some local transmission is occurring at the site even at fairly high elevations. In general, temperatures were not ideal for larval mosquito development in either location. Both sites experience temporary temperature conditions that likely reduce larval mosquito development. However, these periods may be too short in duration to affect pool temperature, and adults are capable of surviving these conditions. The frequency of high-flow periods in Waikamoi may restrict mosquito reproduction within the site by periodically flushing pools and may help explain the apparent lower densities of *Culex* in Waikamoi above 1550 m asl. Larval habitat in Nakula may be more limited by pools drying up in periods with insufficient rainfall. This may be reflected in the spotty distribution of larvae found in Nakula. Nakula may be right on the edge of acceptable temperature conditions for the development of *Plasmodium* in vectors and this may explain the comparatively low levels of the disease found in the bird populations despite relatively high capture rates of mosquitoes. However, given that conditions are marginal now may also mean that little change in climate would be required to increase the suitability of the Nakula site for disease transmission and the current rates of disease may be

temporary. On the other hand, if local transmission is occurring at the highest elevations within the current Kiwikiu range, disease limitations that have allowed these bird species to persist may be tenuous throughout the Kiwikiu range. Beyond the use of more broad-scale methods to control mosquito populations, such as the sterile insect technique or gene drive, there may be methods for local control of vector populations within Nakula. There are only a few large drainages that hold persistent rocky pools within Nakula and even fewer of these pools consistently harbored mosquito larvae in this study. It could be logistically feasible to treat pools with biopesticides, like *Bacillus thuringiensis* bacteria, at regular intervals to reduce or eliminate larval mosquitoes. Additional research is ongoing to determine the prevalence of mosquitoes and the parasite within the bird populations in a number of locations around Maui including areas within and outside the current Kiwikiu range. This work will greatly enhance our understanding of disease distribution on Maui and potentially target areas for management.

Acknowledgments

We would like to thank Katy Parise at Northern Arizona University and Jeanette Calarco at the University of South Florida for their excellent laboratory work on qPCR. We would also like to thank Kristina Paxton for sharing her unpublished qPCR probe and technique. Numerous staff and volunteers with Maui Forest Bird Recovery Project helped gather samples for this project. We would like to specifically acknowledge Heather Mackey and Robert Taylor for writing an early proposal and methods for mosquito sampling. This work would not have been possible without the access permission and support from The State of Hawai‘i Department of Land and Natural Resources – Division of Forestry and Wildlife: Native Ecosystem Protection and Management and The Nature Conservancy.

Literature Cited

Almirón W.R. and M.E. Brewer. 1996. Winter biology of *Culex pipiens quinquefasciatus* Say, (Diptera: Culicidae) from Córdoba, Argentina. Memórias do Instituto Oswaldo Cruz 91:649-654.

Armstrong, D.P. and J.G. Ewen. 2002. Dynamics and viability of a New Zealand robin population reintroduced to regenerating fragmented habitat. Conservation Biology 16:1074-1085.

Aruch, S., C.T. Atkinson, A.F. Savage, and D.A. LaPointe. 2007. Prevalence and distribution of pox-like lesions, avian malaria, and mosquito vectors in Kīpahulu Valley, Haleakala National Park, Hawai‘i, USA. Journal of Wildlife Diseases 43:567-575.

Atkinson, C.T., J.K. Lease, R.J. Dusek, and M.D. Samuel. 2005. Prevalence of pox-like lesions and malaria in forest bird communities on leeward Mauna Loa Volcano, Hawaii. Condor 107:537-546.

Atkinson, C.T. and D.A. LaPointe. 2009. Introduced avian diseases, climate change, and the future of Hawaiian honeycreepers. Journal of Avian Medicine and Surgery 23:53-63.

Atkinson, C.T., R.B. Utzurrum, D.A. LaPointe, R.J. Camp, L.H. Crampton, J.T. Foster, and T.W. Giambelluca. 2014. Changing climate and altitudinal range of avian malaria in the Hawaiian Islands – an ongoing conservation crisis on the island of Kaua'i. Global Climate Change 20:2426-2436.

Baker, J.K. 1975. The feral pig in Hawai'i Volcanoes National Park. California-Nevada Wildlife Society Transactions 11:74-80.

Ball, G.H. and J. Chao. 1964. Temperature stresses on the mosquito phase of *Plasmodium relictum*. Journal of Parasitology 50:748-752.

Beadell, J.S., E. Gering, J. Austin, J.P. Dumbacher, M.A. Peirce, T.K. Pratt, C.T. Atkinson, and R.C. Fleischer. 2004. Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. Molecular Ecology 13:3829-3844.

Becker, C.D., H.L. Mounce, T.A. Rassmussen, A. Rauch-Sasseen, K.J. Swinnerton, D.L. Leonard. 2010. Nest success and parental investment in the critically endangered Maui parrotbill *Pseudonestor xanthophrys* with implications for recovery. Endangered Species Research 11:189-194.

Benning, T.L., D. LaPointe, C.T. Atkinson, and P. Vitousek. 2002. Interactions of climate change with biological invasions and land use in the Hawaiian Islands: Modeling the fate of endemic birds using a geographic information system. PNAS 99:14246-14249.

Berlin, K.E., J.C. Simon, T.K. Pratt, J.R. Kowalsky, and J.S. Hatfield. 2001. 'Ākohekohe response to flower availability: Seasonal abundance, foraging, breeding, and molt. Studies in Avian Biology 22:202-212.

Camp, R.J., P.M. Gorresen, T.K. Pratt, and B.L. Woodworth. 2009. Population trends of native Hawaiian forest birds, 1976-2008: The data and statistical analyses. Technical Report HCSU-012. Hawaii Cooperative Studies Unit, Pacific Aquaculture and Coastal Resources Center, University of Hawaii, Hilo, HI.

Chao, J. and G.H. Ball. 1962. The effect of low temperature on *Plasmodium relictum* in *Culex tarsalis*. Journal of Parasitology 48:252-254.

Ciota, A.T., A.C. Matacchiero, A.M. Kilpatrick, and L.D. Kramer. 2014. The effect of temperature on life history traits of *Culex* mosquitoes. Journal of Medical Entomology 51:55-62.

(DLNR) Department of Land and Natural Resources, State of Hawaii. 2017. New research my help protect people and birds from mosquitoes. Press release http://dlnr.hawaii.gov/hisc/news/new-research-may-help-protect-people-and-birds-from-mosquitoes. Downloaded 6 October, 2017.

Fallon, S.M., R.E. Ricklefs, B.L. Swanson, and E. Bermingham. 2003. Detecting avian malaria: An improved polymerase chain reaction diagnostic. Journal of Parasitology 89:1044-1047.

Fortini, L. B., A. E. Vorsino, F. A. Amidon, E. H. Paxton, and J. D. Jacobi. 2015. Large-scale range collapse of Hawaiian forest birds under climate change and the need for 21st century conservation options. PLoS One 10: e0140389. doi:10.1371/journal.pone.0140389.

Foster, J.T., B.L. Woodworth, L.E. Eggert, P.J. Hart, D. Palmer, D.C. Duffy, and R.C. Fleischer. 2007. Genetic structure and evolved malaria resistance in Hawaiian honeycreepers. Molecular Ecology 16:4738-4746.

Fox, J. and S. Weisberg. 2011. An R companion to applied regression, 2nd ed. Sage, Thousand Oaks, CA.

Garnham, P.C. 1966. Malaria parasites and other Haemosporidia. Blackwell Scientific Publications, Oxford, UK.

Gaudioso-Levita, J.M., D.A. LaPointe, C. Atkinson, and A.N. Egan. 2015. Avian disease and mosquito vectors in the Kahuku Unit of Hawai‘i Volcanoes National Park and Ka‘u Forest Reserve. Hawai‘i Cooperative Studies Unit, University of Hawai‘i at Hilo, Technical Report HCSU-070.

Giambelluca, T.W., X. Shuai, M.L. Barnes, R.J. Alliss, R.J. Longman, T. Miura, Q. Chen, A.G. Frazier, R.G. Mudd, L. Cuo, and A.D. Businger. 2014. Evapotranspiration of Hawai‘i. Final report submitted to the U.S. Army Corps of Engineers—Honolulu District, and the Commission on Water Resource Management, State of Hawai‘i.

Goff, M.L., and C. van Riper III. 1980. Distribution of mosquitoes (Diptera: Culicidae) on the east flank of Mauna Loa Volcano, Hawai‘i. Pacific Insects 22:178-188.

Guillaumet, A., W.A. Kuntz, M.D. Samuel, and E.H. Paxton. 2017. Altitudinal migration and the future of an iconic Hawaiian honeycreeper in response to climate change and management. Ecological Monographs 87:410-428.

Henshaw, H. W. 1902. Birds of the Hawaiian Islands, being a complete list of the birds of Hawaiian possessions with notes on their habits. Thrum, Honolulu, HI.

Hobbelen, P.H.F., M.D. Samuel, D.A. LaPointe, and C.T. Atkinson. 2012. Modeling future conservation of Hawaiian honeycreepers by mosquito management and translocation of disease-tolerant Amakihi. PLoS ONE 7: e49594. https://doi.org/10.1371/journal.pone.0049594.

IUCN. 2017. The IUCN Red List of Threatened Species. Version 2017-2. <http://www.iucnredlist.org>. Downloaded on 4 October 2017.

James, H.F and S.L. Olson. 1991. Descriptions of thirty-two new species of birds from the Hawaiian Islands: Part II. Passeriformes. Ornithological Monographs 46:3-88.

Jarvi, S.I., J.J. Schultz, and C.T. Atkinson. 2002. PCR diagnostics underestimate the prevalence of avian malaria (*Plasmodium relictum*) in experimentally infected passerines. Journal of Parasitology 88:153-158.

Judge, S.W., R.J. Camp, C.C. Warren, L.K. Berthold, H.L. Mounce, P.J. Hart, and R.J. Monello. 2019. Pacific island landbird monitoring annual report, Haleakalā National Park and East Maui Island, 2017. Natural Resource Report NPS/PACN/NRR—2019/1949. National Park Service, Fort Collins, Colorado.

Kilpatrick. A.M. 2006. Facilitating the evolution of resistance of avian malaria in Hawaiian birds. Biological Conservation 128:475-485.

Kokkinn, M.J, D.M. Roberts, and C.R. Williams. 2012. Larval development rate of the mosquitoes *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae) varies between clutches: implications for population ecology. Australian Journal of Entomology 51:22-27.

Knowlton, J.L., D.J. Flaspohler, E.H. Paxton, T. Fukami, C.P. Giardina, D.S. Gruner, and E.E. Wilson Rankin. 2017. Movements of four native Hawaiian birds across a naturally fragmented landscape. Journal of Avian Biology 48:921-931.

Krend, K.L. 2011. Avian malaria on Oahu: Disease ecology, population genetics, and the evolution of resistance in Oahu Amakihi. PhD Dissertation, University of Hawai'i – Manoa.

LaPointe, D.A. 2000. Avian malaria in Hawai'i: Distribution, ecology, and vector potential of forest-dwelling mosquitoes. PhD Dissertation, University of Hawai'i – Manoa.

LaPointe, D.A. 2007. Current and potential impacts of mosquitoes and the pathogens they vector in the Pacific region. Proceedings of the Hawaiian Entomological Society, 39:75-81.

LaPointe, D.A., C.T. Atkinson, and S.I. Jarvi. 2009. Managing disease. In: Conservation Biology of Hawaiian Forest Birds (T.K. Pratt, C.T. Atkinson, P.C. Banko, J. Jacobi, and B.L. Woodworth, eds.). Yale University Press, New Haven, CT.

LaPointe, D.A., C.T. Atkinson, and M.D. Samuel. 2012. Ecology and conservation biology of avian malaria. Annals of the New York Academy of Sciences 1249:211-226.

LaPointe, D.A., M.L. Goff, and C.T. Atkinson. 2005. Comparative susceptibility of introduced forest-dwelling mosquitoes in Hawai'i to avian malaria, *Plasmodium relictum*. Journal of Parasitology 91:843-849.

LaPointe, D.A., M.L. Goff, and C.T. Atkinson. 2010. Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria *Plasmodium relictum* in Hawai'i. Journal of Parasitology 96:318-324.

Laven H. 1967. Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. Nature 216: 383-384.

Liao, W., O.E. Timm, C. Zhang, C.T. Atkinson, D.A.LaPointe, and M.D. Samuel. 2015. Will a warmer and wetter future cause extinction of native Hawaiian forest birds? Global Change Biology 21:4342-4352.

Lounibos, L.P. 2002 Invasions by insect vectors of human disease. Annual Review of Entomology 47: 233–266.

McClure, K.M. 2017. Disease ecology of avian malaria in native and introduced birds in lowland Hawai'i. PhD Dissertation. University of California, Santa Cruz. 126 pages.

McClure, K.M., C. Lawrence, and A.M. Kilpatrick. 2018. Land use and larval habitat increase *Aedes albopictus* (Diptera: Culicidae) and *Culex quinquefasciatus* (Diptera: Culicidae) abundance in lowland Hawai'i. Journal of Medical Entomology 55:1509-1516.

(MFBWG) Maui Forest Bird Working Group. 2018. Kiwikiu (*Pseudonestor xanthophrys*) reintroduction plan. Available at: https://mauiforestbirds.org/wp-content/uploads/2018/09/20180813_MAPA-Reintro-Plan-2.pdf.

Mogi M. 1992. Temperature and photoperiod effects on larval and ovarian development of New Zealand strains of *Culex quinquefasciatus* (Diptera, Culicidae). Annals of the Entomological Society of America 85:58-66.

Mounce, H.L., K.J. Iknayan, D.L. Leonard, K.J. Swinnerton, and J.J. Groombridge. 2014. Management implications derived from long term re-sight data: Annual survival of the Maui Parrotbill *Pseudonestor xanthophrys*. Bird Conservation International 24:316-326.

Mounce H.L., C. Raisin, D.L. Leonard, H. Wickenden, K.J. Swinnerton, and J.J. Groombridge. 2015. Spatial genetic architecture of the critically-endangered Maui Parrotbill (*Pseudonestor xanthophrys*): management considerations for reintroduction strategies. Conservation Genetics 16:71-84.

Mountainspring, S. 1987. Ecology, behavior, and conservation of the Maui Parrotbill. Condor 89:24-39.

Noden, B.H., M.D. Kent, and J.C. Beier. 1995. The impact of variations in temperature on the early *Plasmodium falciparum* development in *Anopheles stephensi.* Parasitology 111:539-545.

Olson, S.L. and H.F. James. 1982. Fossil birds from the Hawaiian Islands: Evidence for wholesale extinction by man before western contact. Science, New Series 217:633-635.

Pratt, H.D. 1994. Avifaunal change in the Hawaiian Islands, 1893-1993. Studies in Avian Biology 15:103-118.

Pratt, T.K., J.C. Simon, B.P. Farm, K.E. Berlin, and J.R. Kowlasky. 2001. Home range and territoriality of two Hawaiian forest birds the ʻĀkohekohe and Maui Parrotbill. Condor 103:746-755.

Ralph, C.J, and S.G. Fancy. 1995. Demography and movements of ʻApapane and ʻIʻiwi in Hawaiʻi. Condor 97:729-742.

Reiter, M.E. and D.A. LaPointe. 2009. Larval habitat for the avian malaria vector *Culex quinquefasciatus* (Diptera: Culicidae) in altered mid-elevation mesic-dry forests in Hawaii. Journal of Vector Ecology 31:208-216.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rueda, L.M., K.J. Patel, R.C. Axtell, and R.E. Stinner. 1990. Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). Journal of Entomology 27:892-898.

Samuel, M.D., P.H.F. Hobbelen, F. DeCastro, J.A. Ahumada, D.A. LaPointe, C.T. Atkinson, B.L. Woodworth, P.J. Hart, and D.C. Duffy. 2011 The dynamics, transmission, and population impacts of avian malaria in native Hawaiian birds: A modelling approach. Ecological Applications 21:2960-2973.

Samuel, M.D., B.L. Woodworth, C.T. Atkinson, P.J. Hart, and D.A. LaPointe. 2015. Avian malaria in Hawaiian forest birds: Infection and population impacts across species and elevations. Ecosphere 6:2-21.

Samuel, M.D., B.L. Woodworth, C.T. Atkinson, P.J. Hart, and D.A. LaPointe. 2018. The epizoology of avian pox and interaction with avian malaria in Hawaiian forest birds. Ecological Monographs 88:621-637.

Scott, J.M., S. Mountainspring, F.L. Ramsey, and C.B. Kepler. 1986. Forest bird communities of the Hawaiian Islands: Their dynamics, ecology and conservation. Studies in Avian Biology 9: i-xii.

Simon, J.C., P.E. Baker, and H Baker. 1997. Maui Parrotbill (*Pseudonestor xanthophrys*). In: The Birds of North America Online (A. Poole, ed.). Cornell Lab of Ornithology, Ithaca, NY.

Simon, J.C, T.K. Pratt, K.E. Berlin, and J.R. Kowalsky. 2000. Reproductive ecology of the Maui Parrotbill. The Wilson Bulletin 112:482-490.

Stein, V. 2007. Critical assessment of habitat for release of Maui Parrotbill. Honolulu (HI): Pacific Cooperative Studies Unit, University of Hawaii at Mānoa, Department of Botany. PCSU Technical Report 146. 45 pages

(USFWS) U.S. Fish and Wildlife Service. 1967. Office of the secretary; native fish and wildlife; endangered species. Federal Register 32:4001.

(USFWS) U.S. Fish and Wildlife Service. 2006. Revised recovery plan for the Hawaiian forest birds Region 1. U.S. Fish and Wildlife Service, Portland, OR

(USGS) U.S. Geological Society. 2019. USGS Current Conditions for Hawaii. Available at: https://nwis.waterdata.usgs.gov/hi/nwis/uv? (Accessed 3 July 2019).

van Riper III, C., and D.J. Forrester. 2007. Avian pox. In: Infectious diseases of wild birds. (N.J. Thomas, D.B. Hunter, and C. T. Atkinson, eds.). Blackwell, New York, NY.

van Riper, C. III, S.G. van Riper, M.L. Goff, and M. Laird. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs 56:327-344.

van Riper, C. III, S.G. van Riper, and W.R. Hansen. 2002. Epizootiology and effect of avian pox on Hawaiian forest birds. Auk 119:929-942.

Vreysen, M. J. B., A.S. Robinson, and J. Hendrichs. 2007. Area-wide control of insect pests In Research to Field Implementation. pp. 789 Springer, Dordrecht, The Netherlands

Warner, R.E. 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. Condor 70:101-120.

Warren, C.C., P.J. Motyka, and H.L. Mounce. 2015. Home range sizes of two Hawaiian honeycreepers: implications for proposed translocation efforts. Journal of Field Ornithologists 86:305-316.

Woodworth, B.L., C.T. Atkinson, D.A. LaPointe, P.J. Hart, C.S. Spiegel, E.J. Tweed, C. Henneman, J. LeBrun, T. Denette, R. DeMots, K.L. Kozar, D. Triglia, D. Lease, A. Gregor, T. Smith, and D. Duffy. 2005. Host population persistence in the face of introduced vector borne disease: Hawai‘i ‘Amakihi and avian malaria. PNAS 102:1531-1536.

Zehtindijev, P., M. Ilieva, H. Westerdahl, B. Hansson, G. Valkiūnas, and S. Bensch. 2008. Dynamics of parasitemia of malaria parasites in a naturally and experimentally infected migratory songbird, the great reed warbler *Acrocephalus arundinaceus*. Experimental Parasitology 1198:99-110.