Local prevalence and transmission of avian malaria in the Alakai Plateau of Kauai, Hawaii, U.S.A.

Anouk Glad[1] and Lisa H. Crampton[2]

[1]University of Hawai`i at Manoa, Department of Botany, Kaua`i Forest Bird Recovery Project, Hanapepe, HI 96716, U.S.A., gladanouk@gmail.com
[2]State of Hawai`i, Department of Land and Natural Resources, Kaua`i Forest Bird Recovery Project, Hanapepe, HI 96716, U.S.A.

ABSTRACT: Avian malaria is among the most important threats to native Hawaiian forest birds. It is caused by the parasite *Plasmodium relictum* and is transmitted by the introduced mosquito vector *Culex quinquefasciatus*. Temperature increases and precipitation declines due to climate change over the last decade may be responsible for the observed recent expansion in the range and prevalence of avian malaria on the Alakai Plateau, Kauai Island. To examine the hypothesis that conditions are now favorable for transmission of malaria on the Plateau, mosquitoes were sampled with CO_2 and Reiter oviposition traps at three sites (Kawaikoi, Halepa'akai, and Koke'e) on several occasions between October, 2013 and April, 2014. *P. relictum* infection was assessed by PCR or dissection under a microscope. We also surveyed mosquito larvae along Halepa'akai and Kawaikoi streams. We observed that *Cx. quinquefasciatus* is well established on the Alakai Plateau, as mosquitoes were caught on all field trips, except in April at Halepa'akai, and larvae were found throughout the year. We observed differences in adult abundance among sites and microhabitats (stream vs ridge lines). ***Journal of Vector Ecology* 40 (2): 221-229. 2015.**

Keyword Index: Climate change, mosquito-borne disease, stream flow, honeycreeper.

INTRODUCTION

Introduced diseases, such as avian malaria, are among the major threats to bird populations in the United States, after habitat fragmentation and introduction of predators (Gurevitch and Padilla 2004). Hawaiian native birds suffer greatly from these threats (Wilcove et al. 1998) and are one of most endangered wildlife groups in the United States (Leonard and David 2008). The isolation of the Hawaiian Islands led to a unique evolution and speciation of the avifauna, which flourished in a predator and disease-free environment until human colonization of the islands 1,800 years ago (Atkinson et al. 2013). During their evolution, many native Hawaiian forest birds lost or failed to develop resistance to mosquito-borne diseases and now are highly sensitive to the recently introduced avian malaria. This is manifested by a very high mortality for some native species, 90% for the I'iwi (*Vestiaria coccinea)* or 75% for the Maui 'alauahio *(Paroreomyza montana)* after exposure to a single infected mosquito (Atkinson 2005). The species causing avian malaria on the Hawaiian archipelago is *Plasmodium relictum*, transmitted mostly by the mosquito *Culex quinquefasciatus.* Other common mosquitoes, including *Aedes albopictus* and *Wyeomyia mitchelli,* are refractory to infection (LaPointe et al. 2005, Atkinson et al. 2008).

Culex quinquefasciatus is a nocturnal, widely distributed mosquito that has been established in the Hawaiian archipelago since 1826 (LaPointe et al. 2009). Females occur in warm and temperate areas, feed mostly on birds, and do not hibernate. Thus, *Cx. quinquefasciatus* continue to feed and reproduce in winter, even if the low temperatures slow down larval development and reduce adult survival (Farajollahi et al. 2011). Indeed, cold temperatures reduced female survival to two to six weeks at a constant temperature of 5° C (Almirón and Brewer 1996). The incubation period is between three to five days, and larval development between 10 to 12 days with a developmental threshold of 9.5°-10.4° C, but low temperature increased both incubation and development to up to 28 days at 17° C (Almirón and Brewer 1996, Rueda et al. 1990). This species is known to exist in various habitats: canals, marshes, ornamental ponds, swimming pools, temporary pools, puddles, or ditches (Muturi et al. 2008, Manimegalai and Sukanya 2014). On Hawaii Island, it is principally found in anthropogenic larval habitats when available. On Hawaii Island, Maui, and Kauai, larvae were observed in rock pools in the bed of stream drainages and stream margins. However, larvae were also found in fern tree cavities and ground pools (created by feral game) on Hawaii Island (Reiter and LaPointe 2009, Aruch et al. 2007, Atkinson et al. 2014).

Across the archipelago, many native bird populations have declined dramatically and have almost disappeared from lower and mid-elevations as a result of malaria (Atkinson 2005, Reynolds et al. 2003). Above 1,500 m, ambient temperature historically has been sufficiently cool at any time of year to prevent mosquito and *P. relictum* development, which are both temperature-dependent (Benning et al. 2002). Thus, malaria is less prevalent and some native forest birds species that disappeared from low and mid-elevation are still present in high elevation forests (Atkinson and LaPointe 2009). However, recent findings suggest that temperature increases at high elevations throughout Hawaii may promote increased prevalence of avian malaria and more drastic forest

bird declines (Benning et al. 2002, Atkinson and LaPointe 2009).

On Kauai, the Alakai Plateau historically has been a refuge for forest birds due to the low prevalence of malaria, even though the Plateau lies only between 1,100 and 1,569 m. The particular climate occurring on the Alakai Plateau may prevent a high transmission rate of *P. relictum* (LaPointe et al. 2010). The Alakai Plateau constitutes the entire remaining range of three endangered endemics species: Akikiki (*Oreomystis bairdi*), Akeke'e (*Loxops caeruleirostris*) and Puaiohi (*Myadestes palmeri*) (Reed et al. 2012). The three other endemic species on Kauai island (Kauai Amakihi *(Hemignathus kauaiensis),* Kauai Elepaio *(Chasiempis sclateri),* and Anianiau *(Magumma parva)),* and two widely distributed species across the Hawaiian archipelago *(*Apapane *(Himatione sanguine)* and I'iwi *(Vestiaria coccinea)),* are also found only on the Alakai Plateau. At least five of these species were formerly widespread on Kauai Island (Kauai Amakihi, Kauai Elepaio, Anianiau, Puaiohi, and Apapane) (Burney et al. 2001). Since 1973, some species, such as I'iwi, Akeke'e, and Akikiki, have exhibited alarming range contractions and population declines on Kauai, which are thought to be related to increased prevalence of malaria, among other threats (Atkinson et al. 2014, Foster et al. 2004). This hypothesis is supported by the increase in malaria prevalence in Kauai forest birds, observed between two sampling period (1994-1997 and 2007-2013) and by the detection of *Cx. quinquefasciatus* larvae by Atkinson et al. (2014).

The occurrence of malaria is dependent on environmental conditions, such as temperature (Rueda et al. 1990), precipitation (Shaman and Day 2007), wind (influencing vector dispersion), availability of larval habitat influenced by habitat degradation, forest fragmentation (feral ungulates, human activities) (Reiter and LaPointe 2007, 2009, LaPointe et al. 2012), and potential host abundance (Samuel et al. 2011), which impact both parasite and vector life cycles (Atkinson and LaPointe 2009). On Hawaii Island, elevation gradient, temperature, and precipitation were the three major factors influencing *Cx. quinquefasciatus* population demography by affecting larval development and adult mosquito survival (Freed et al. 2005, Ahumada et al. 2004, Samuel et al. 2011). The many streams, pools, and wetlands on both ridge line and stream drainages likely provide ample habitats for mosquitoes, and if temperatures are high enough, *Cx. quinquefasciatus* may be able complete its life cycle. The high frequencies of rainfall (1.84 m in 2012 and 1.57 m in 2013 observed at Waialae rain gauge station, USGS) throughout the year provide constant potential habitat availability, however, winter flash flood events are common and may reduce adult survival and wash away larval habitats. Thus, rainfall frequency and intensity are major factors influencing *Cx. quinquefasciatus* population dynamics. On Kauai, temperature is one of the main factors likely to influence the *P. relictum* life cycle and transmission rates, as it influences development of oocytes and sporozoites in the vector (LaPointe et al. 2010). Those particular conditions explain the historically low malaria prevalence on the Alakai Plateau where the best conditions for *P. relictum* transmission (higher

temperature and lower rainfall frequency) occurred from July to September. Temperature increases and precipitation declines due to climate change over the last decade may have extended the favorable period for *P. relictum* transmission by allowing mosquitoes to better hatch and survive throughout the year, and *P. relictum* to complete its life cycle, in high altitude areas on Kauai (Atkinson et al. 2014). This possibility is one focus of this study. In addition, climate change may have allowed mosquitoes from lower altitudes to migrate to upper elevations throughout the year and to survive longer on the Plateau, increasing the transmission of *P. relictum* to forest birds.

We hypothesized that current conditions on the Alakai Plateau may allow mosquitoes to complete their life cycles on the Plateau even in the cooler winter and spring months because climate change favors both mosquito and larval survival and development. The varied topography of the Alakai Plateau offers diverse microhabitats (lower elevation streams, higher elevation ridgelines) and altitude and precipitation decrease from east to west (Giambelluca et al. 2013). Thus, we hypothesized that mosquito and malaria prevalence is more likely to occur at Koke'e and Kawaikoi than Halepa'akai, since the latter site is located at a higher altitude and experiences a higher rainfall average and fewer anthropogenic disturbances. We also hypothesized that mosquito abundance and *P. relictum* transmission will be greater near streams than on ridge lines, as *Cx. quinquefasciatus* larvae had never been found anywhere other than in stream margins and rock pools along stream drainages on Kauai (Atkinson et al. 2014). To address these hypotheses, samples of both larval and adult *Cx. quinquefasciatus* were collected to assess their relative abundance and distribution and to determine the prevalence of *P. relictum* in different areas of the Alakai Plateau.

MATERIALS AND METHODS

Study sites

Samples were collected at sites representing different altitudes, meteorological conditions, and human perturbation. The three study sites (Halepa'akai, Kawaikoi, and Koke'e State Park) are located on Kauai Island in the Alakai Wilderness Preserve and Na Pali Kona Forest Reserve on the Alakai Plateau (22°05′N 159°30′W; Figure 1). In the east, Halepa'akai (1,280 m) is situated in a wet montane forest dominated by ohia lehua (*Metrosideros polymorpha*), whereas Kawaikoi (1,100 m) lies in a relatively mesic mixed ohia-koa (*Acacia koa*) forest in the middle of the Plateau. Koke'e State Park (1,097 m) is located on the west side of the Alakai Plateau and is characterized by intense human activity in a mesic ohia-koa forest (Figure 1).

Local variations in temperature were measured with remote data loggers during each field trip (Maxim iButtons™), and rain gauge data from the USGS national information system (http://wdr.water.usgs.gov/) were used to approximate local rainfall (Waialae rain gauge station). Stream gauge data were used to estimate the impact of flood events on larval developmental success. The Kawaikoi stream gauge station was used to estimate flood events at Kawaikoi,
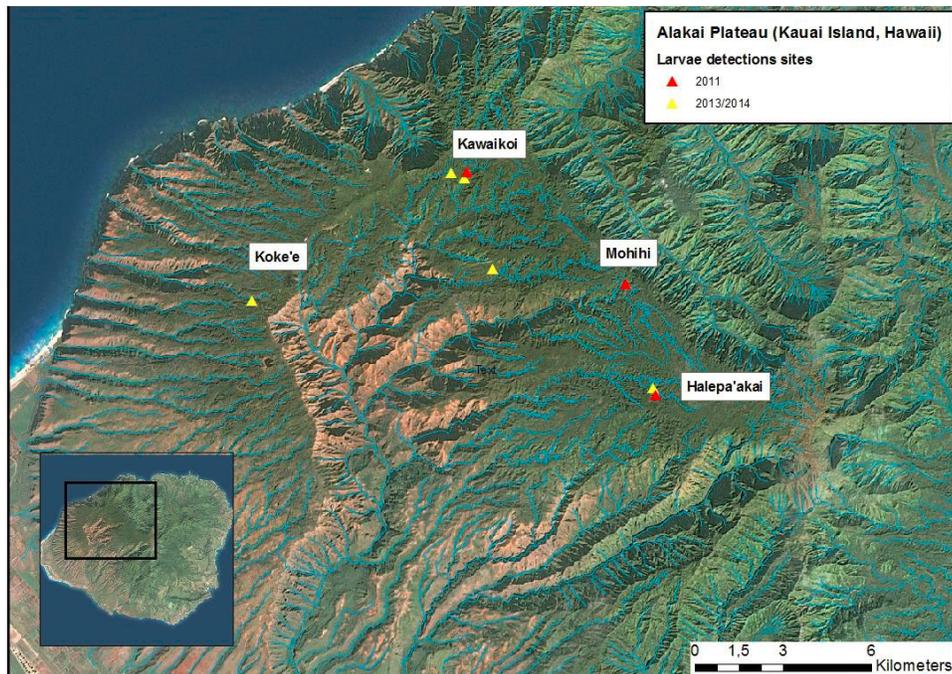
Figure 1. The three study sites on the Alakai Plateau, Kauai Island, Hawaii, at which larval (detections in 2011 represented by ▲ and detection in 2013/2014 represented by △) and adult mosquitoes were sampled from October 2013-April 2014. These sites represent west to east gradients of increasing elevation and rainfall, and decreasing anthropogenic disturbance.

and the Waialae stream gauge station was used to estimate flood events at Halepa'akai. We added data from 2011 to compare with earlier larvae surveys.

Adult mosquito sampling

Adult mosquitoes were sampled with CO_2 traps (designed for trapping females seeking a blood meal during October and November, 2013 field trips only) and Reiter oviposition traps (designed for trapping gravid specimens during all field trips from October, 2013 to April, 2014). Oviposition traps were filled with an aged alfalfa infusion and checked daily. CO_2 traps were baited with dry ice and paired with an oviposition trap at a trapping station and checked daily. One CO_2 trap and one oviposition trap were set at each station in October and November. Trapping stations were located every 100 m along the stream or along the ridge. After January, only oviposition traps were used (one at each station). Due to logistic constraints, traps were not set at Kawaikoi, Halepa'akai, and Koke'e at the same time. Traps were set at Kawaikoi (November 7th to 14th, January 7th to 16th, and March 2nd to 12th, March 27th to 30th), Halepa'akai (October 25th to 30th, January 27th to 30th, and April 15th to 18th 2014), and Koke'e (February 12th to 17th). To assess the effect of local environmental variation, traps were located in two different microhabitats at Kawaikoi (stream vs ridgeline).

Malaria detection in mosquitoes

Mosquitoes were kept in a lysis buffer solution after each autumn field trip. DNA from mosquito tissues were extracted and analyzed by PCR in December using 213F and 372R primers designed to distinguish between *Haemoproteus spp.*, *Plasmodium* spp. and *Leucocytozoon* spp. (Beadell and Fleischer 2005). The *Plasmodium* spp. digestion product is characterized by a size of 160 bp. PCR products were electrophoresed at 100V on a 1.50% agarose gel for 30 min.

After January, mosquitoes caught in the field were kept alive in order to dissect them. *Plasmodium relictum* infection in mosquitoes then was assessed morphologically only by dissection under a microscope. Midgut and salivary glands were observed to assess the presence of both oocysts and sporozoites.

Larval sampling

Larval mosquito sampling was conducted along Kawaikoi (November 8th, January 15th, and March 31st) and Halepa'akai (October 27th, 28th, and April 14th) streams, as larvae had been previously observed on streams but not in tree cavities, pig wallow, and ground pools in the Alakai (Atkinson et al. 2014). For comparison with earlier surveys from 2011, we surveyed 500 m (Kawaikoi Stream) and 1,000 m (Halepa'akai Stream) transects, using the same protocol as the earlier surveys. Due to bad weather, the initial survey transect at Kawaikoi was reduced to 500 m. Transects were divided into 10 m segments. Sampling involved dipping a larval cup (standard larval mosquito dipper 250 ml) into slow-moving or stagnant water along the edges of the stream to assess the presence of mosquito larvae in the habitat. A minimum of ten dips per 10 m section was made while attempting to sample each distinct rock hole or ground pool. The number of dips containing mosquitoes was recorded. Representative specimens were collected for each section.

Statistical analysis

Statistical analyses were performed using SAS version 9.3 (2010). We analyzed differences between sites and microhabitats on mosquito capture rate with a Generalized Linear Model using a negative binomial regression. We chose a negative binomial distribution because it is frequently used for over-dispersed count data (Cameron and Trivedi 2013) and because AIC was smaller than with other distributions

(Poisson or quasi-Poisson). The influence of temperature and rainfall on nightly capture rate was also tested using this modeling approach. We report mean values +/- SE and use α = 0.05 as the level of significance in all tests.

Unfortunately, because no mosquitoes were caught at some sites during some field trips, we could not use a multivariate model combining site, microhabitat, and temperature but had to model the effect of most independent variables separately. Because of differences in timing of deployment, capture rates, and the mosquitoes caught by the two trap types, only oviposition trap captures were considered in site, date, rain, and temperature analyses (we had much more data for this trap type since it was used throughout the study). We examined the effect of microhabitat at Kawaikoi using data from all traps (November), as CO_2 and oviposition traps were running at the same time for both microhabitats, making pooling of data possible. Due to low capture rates for Halepa'akai, nightly temperature and nightly rain were only statistically examined on data collected at Kawaikoi. Koke'e was not included in models because we caught no mosquitoes there.

When trapping effort was high, but capture rates were low, we used the total number of mosquitoes per night summed across all traps as the dependent variable (to avoid left skew, i.e., reduce the number of zeroes in the data). Such was the case in models assessing effect of site, rain, and temperature. The number of traps deployed each night was used as an offset variable in all these models, so all results are portrayed as a capture rate (mean number of mosquitoes caught by trap each night).

When the number of trap nights per model category was low, and there were relatively few zeroes in the dataset, we used the number of mosquitoes per night in each trap as the dependent variable. Such was the case for models evaluating microhabitat.

RESULTS

Effect of temperature and rainfall on nightly mosquito capture rate

Temperature and rainfall can influence mosquito activity for the night of capture, so we examined that possibility before any further analysis. Nightly temperatures were measured for 26 days at Kawaikoi and 13 days at Halepa'akai from October to April. Mean nightly (19:00 to 06:00) temperatures were used to assess the effect on mosquito capture rate. No significant effect of temperature on mosquito capture rate from oviposition traps was found (χ^2_1=0.02 P=0.90). Mean rainfall (in mm) recorded at Waialae rain gauge was used as an indicator of rainfall for both sites. No significant effect of rain on mosquito capture rate in oviposition traps was observed (χ^2_1=0.19 P=0.67).

Effect of site and microsite on mosquito trapping

Culex quinquefasciatus abundance among sites on the Alakai Plateau was the main focus of this study. Mosquitoes were caught in both trap types, and at both Halepa'akai and Kawaikoi (but not at Koke'e), where they were found in both microhabitats, but not on all field trips (Table 1). Differences between sites throughout our study were assessed using oviposition trap data (to avoid an effect of trap type between sites as they do not attract mosquitoes at the same reproductive stage). Capture rates were significantly lower at Halepa'akai (0.067 +/- 0.047 captures per trap) each night than at Kawaikoi (0.53 +/- 0.21 captures per trap) each night (Z_{37}=-2.45 P=0.0141). Throughout the study, the capture rate of mosquitoes was significantly lower on the ridge (0.035 +/-0.020 captures per trap) each night than near the stream (0.33+/-0.074 captures per trap) each night (Z_{345}= -3.72 P=0.0002).

Table 1. The total number of *Culex quinquefasciatus* caught with the total number of traps running during each trip at Kawaikoi (LWK) and Halepa'akai (HPK). NA=traps not deployed.

	Dates	Microsite	CO_2 Traps Mosquitoes	Traps	Oviposition Traps Mosquitoes	Traps
HPK						
Trip 1 (6 days)	October 25-30	Stream	8	30	3	30
Trip 2 (4 Days)	January 27-30	Stream	NA	NA	1	13
Trip 3 (4 Days)	April 15-18	Stream	NA	NA	0	20
Total (14 days)	NA	NA	8	30	4	63
KWK						
Trip 1 (6 days)	November 7-14	Ridge	4	25	0	25
		Stream	12	22	4	22
Trip 2 (7 Days)	January 7-16	Ridge	NA	NA	0	20
		Stream	NA	NA	16	22
Trip 3 (8 Days)	March 2-12	Ridge	NA	NA	0	26
		Stream	NA	NA	0	30
Trip 4 (4 days)	March 27-30	Ridge	NA	NA	0	16
		Stream	NA	NA	29	16
Total (25 Days)	NA	NA	16	47	49	177

Mosquito larval surveys

Mosquito larvae were not found during stream surveys at either Kawaikoi (November, January, and March) or Halepa'akai streams (October and April). However, hundreds of larvae were found along Halepa'akai stream in pools immediately following the April survey as we returned along the survey route to camp. Furthermore, hundreds of larvae were found in pools along trails and roads through the Plateau in October, March, and April (Figure 1). Both *Cx. quinquefasciatus* and *Ae. albopictus* larvae were found in a firepit in Koke'e in February (Figure 1).

Plasmodium relictum infection in *Culex quinquefasciatus*

The infection rate of *Plasmodium relictum* is also essential to understanding the transmission rate to birds on the Alakai Plateau. We screened 17 mosquitoes caught at Halepa'akai and 16 mosquitoes caught at Kawaikoi in October and November for *P. relictum* infection using PCR. One mosquito from Halepa'akai tested positive for infection. We dissected 33 mosquitoes caught at Kawaikoi (winter and spring); none of them tested positive for infection by *P. relictum* (neither oocysts nor sporozoites were observed). Only three mosquitoes caught at Halepa'akai (January) were dissected, and none of them were infected (neither oocysts nor sporozoites were observed). Thus the prevalence rate of *P. relictum* in our study is 1.45% (n=69).

DISCUSSION

The aim of this study was to collect data on *Cx. quinquefasciatus* and the transmission of the parasite *P. relictum* in the last refuge for endemic forest birds on Kauai Island: the Alakai Plateau. The historical low malaria prevalence on the Alakai Plateau was believed to be caused by a seasonal local transmission during the driest and warmest period of the year (July to September). Our observations and results confirm that the Plateau is no longer a mosquito-free refuge even in winter and that there is ongoing local malaria transmission to birds.

Environmental conditions such as temperature during our collections, stream flooding events, and rainfall frequency are essential to understanding the actual status of malaria prevalence and transmission on the Alakai Plateau. The two lowest average temperatures were 13.1° C (November

at Kawaikoi) and 13.2° C (January at Halepa'akai), but those temperatures were averaged over only ten and five days, respectively (Table 2). The lowest daily temperature recorded was 8.6° C (14th of November at Kawaikoi) and the highest was 17.3° C (27th of October at Halepa'akai). *Cx. quinquefasciatus* larvae can have a slow but complete development at 14.2° C annual average temperature, and populations can persist if seasonal average is above 13.2° C (Ahumada et al. 2004). Thus, temperatures in the Alakai may not be a main limiting factor for mosquito populations as the developmental threshold temperature is between 9.5° and 10.4° C (Almirón and Brewer 1996). However, those temperatures may be a limiting factor for *P. relictum* development in the vector. Indeed, below a daily average of 12.6° C, the development of oocysts is very slow or even non-existent (Samuel et al. 2011). The optimal range for oocyst and sporozoite development is between 17° and 25° C, leading to increased transmission rates at the highest temperatures (LaPointe et al. 2010) but to incomplete or delayed development at the lowest temperatures. Flash flood events can have an impact on both adults and larvae. Thus, in 2013/2014, the month with the greatest frequency of flood events was February, with six days above 100 cubic feet per second (cfs) discharge and 14 days above the stream discharge average (18 cfs) at Halepa'akai. A similar pattern was observed at Kawaikoi with nine days above 100 cfs discharge and 21 days above the stream discharge average (25 cfs) in February. However, the two highest flood events in 2013/2014 occurred in November and December. For our study period, the driest months of the year were October, 2013, and March and April, 2014. The rainiest month was February with a total of 336.8 mm. Furthermore, November, December, and January had a total precipitation higher than 200 mm.

Although specific environmental pressures that do not seem to meet all the conditions for a high transmission rate of *P. relictum*, our field observations were to the contrary. We found adult *Cx. quinquefaciatus* at two different sites on the Alakai Plateau where they were never previously studied and observed. We caught mosquitoes in both CO_2 and oviposition traps, indicating that females are actively seeking a blood meal on the Alakai Plateau and successfully finding hosts. Adult mosquitoes were caught from November to March at Kawaikoi and from October to January at Halepa'akai. Capture rate, an indication of mosquito abundance, and defined as the mean number of mosquitoes caught by each

Table 2. Temperature average measured for each season at both sites, Kawaikoi (KWK) and Halepa'akai (HPK), with the lowest and the highest temperature measured for each season.

Date	Site	Number of days	Mean	Std Dev	Std Err	Minimum	Maximum
November 8th to 14th	KWK	7	13.1	2.5	0.3	7.5	17.0
March 3nd to 12th	KWK	9	14.2	4.3	0.3	6.2	32.3
January 7th to 16th	KWK	10	14.1	2.6	0.2	7.7	21.0
October 24th to 31st	HPK	8	15.3	3.1	0.3	8.4	21.4
January 27th to 31st	HPK	5	13.2	2.6	0.2	7.7	21.0

trap in one night, showed variable population dynamics among sites and microhabitats. Across the overall study period, mosquito abundance was lower at Halepa'akai (oviposition trap data) than Kawaikoi. Those results indicate that, as expected, mosquito abundance is higher at the lower altitude and more western site (Kawaikoi). Surprisingly, we caught *Cx. quinquefasciatus* mosquitoes even in January at Kawaikoi, despite the fact that winter was the rainiest season overall in the Alakai, confirming the ongoing establishment of the vector (Giambelluca et al. 2013). Indeed, the Kawaikoi high capture rate (0.53 mosquitoes per trap each night) is similar to results observed at low altitudes in the Hawaii Island forest (0.622 mosquitoes per trap/night at 85 m ASL and higher than 0.25 mosquitoes per trap each night at 270 m ASL) (Woodworth et al. 2005) or in a disturbed habitat at Volcano Village (Hawaii Island) (0.27 mosquitoes per trap each night 1,400 m ASL) (Reiter and LaPointe 2007). This capture rate is also much higher than observed on coastal towns in the Galapagos Islands (0.02 mosquitoes per trap each night), where *Cx. quinquefasciatus* was first recorded in 1989 and is now considered established (Whiteman et al. 2005). More troubling, one of the rainiest days of 2013-2014 was November 9th, at the beginning of the first trip to Kawaikoi (204 mm in three days recorded at Waialae USGS station; i.e., average 1.65 mm per day in November, 2012 vs 11.68 mm per day in November, 2013, and the Kawaikoi stream gauge reached 3,270 cfs). This may have led to an underestimation of mosquito abundance at Kawaikoi for this first trip, as major rainfall events can dramatically reduce adult mosquito survival (Samuel et al. 2011).

We also confirmed that larval mosquitoes are now present on the Alakai Plateau throughout the year. Contrary to 2011 stream surveys, mosquito larvae were not found on stream transects this year. However, previous larval surveys were conducted in August, September, and October, 2011, whereas we conducted surveys in October, November, January, and March 2013-2014. Differences in survey timing, especially because of the highest occurrence of rainfall in winter, might explain this difference in larval results. Furthermore, at Kawaikoi, major flooding events occurred a few days before each survey in 2013-2014 and may explain the absence of larvae that year. However, in our study larvae were opportunistically found in puddles on trails and roads for the first time on Kauai. Larvae were also found once in pools near Halepa'akai stream (on the way back from the stream survey). Those observations indicate that *Cx. quinquefasciatus* eggs are able to hatch at least from early spring to autumn in the heart of the Plateau. The fact that no larvae were observed in winter does not mean that larvae were not present at that time, as most our detections of larvae were opportunistic. Moreover, we found larvae (*Cx. quinquefasciatus* and *Ae. albopictus*) in February on the outskirts of the Plateau at Koke'e (Figure 1). *Cx. quinquefasciatus* is known to preferentially use smaller volume habitats (Reiter and LaPointe 2009, Aruch et al. 2007, Muturi et al. 2008) and seems to use a variety of habitats from large pools on roads to very small puddles near streams and anthropogenic firepits. Unfortunately, the presence of larvae on hiking trails and roads also confirms that human

disturbances create appropriate habitat for larval development and thus, as it was observed on Hawaii Island, participate in the expansion of *Cx. quinquefaciatus* and *P. relictum* distribution range (Reiter and LaPointe 2007). Furthermore, on other islands, the presence of pigs and feral ungulates are also responsible for creating habitat for larvae (Baker 1975), although we do not have any evidence of this on Kauai. New surveys should be conducted in the forest and along trails to better understand alien species and human disturbance on potential breeding habitat abundance.

Although we have documented the presence of the vector, the occurrence of malaria transmission on the Plateau is more difficult to establish. The prevalence of infected mosquitoes was low, as only one infected mosquito was detected (1.45% prevalence), whereas in two other studies on Hawaii Island, 15% (n=279) and 12% (n=847) of mosquitoes, respectively, were infected (Woodworth et al. 2005, Reiter and LaPointe 2007). The low prevalence might be the result of the small sample size (only 69 mosquitoes were examined). However, the occurrence of malaria transmission on the Plateau is supported by the prevalence of malaria in birds. By comparing data collected from 1994-1997 and 2007-2013, Atkinson et al. (2014) examined the possibility of increasing malaria prevalence in Kauai forest birds. They observed that the number of infected birds increased between the two sampling periods in both native and non-native species. One of the most worrisome observations of this study was the highest malaria prevalence in more sedentary species such as Kauai Elepaio (27% prevalence) and Kauai Amakihi (31.0% prevalence). For comparison, a prevalence of 22% in the great tits (*Parus major*) population and 22.6% in the blue tits (*Cyanistes caeruleus)* populations was observed in the United Kingdom (Isaksson et al. 2013, Knowles et al. 2011). However, in some populations and species it could be even higher, as it was observed in Arles, France, with prevalence of 78.6% in a house sparrow (*Passer domesticus*) population (Loiseau et al. 2013) or on Hawaii Island where a prevalence between 55% to 83% was observed in a Hawaii Amakihi (*Hemignathus virens*) population (Woodworth et al. 2005). Furthermore, Atkinson et al. (2014) observed an increase in malaria prevalence in two sites, Kawaikoi (1,100 m) and Halepa'akai (1,280 m), but not in Mohihi despite its lower elevation. Indeed, local environmental variation seems to be an important factor influencing avian malaria dynamics, as differences in malaria transmission rate can occur on a very small spatial scale (Lachish et al. 2011, Knowles et al. 2011).

As a result of these observations, two hypotheses regarding *Cx. quinquefasciatus* population sustainability and *P. relictum* transmission on the Plateau can be proposed. First, mosquito populations may now be established at least on the west part of the Plateau as the population at Kawaikoi did not show a dramatic decrease over the study, even with the high frequency of rainfall events. Furthermore, the various places where larvae have been observed since 2011 suggest that on-site breeding might occur on the Plateau and favor local transmission. However, due to temperatures measured on the Alakai Plateau, the development of the parasite in the vector *Cx. quinquefasciatus* may still be restricted to the warmest

months of the year. Thus, if the transmission of *P. relictum* from an infected bird to a mosquito occurs throughout the year on the Plateau, the successful development and transmission of *P. relictum* in a mosquito living on the Alakai Plateau to a bird is still to be proven. Secondly, mosquitoes found on the Plateau may also originate from lower altitudes, especially from the west side (gradual elevation from the ocean to Koke'e), and be blown by wind to the Plateau carrying *P. relictum* and then be able to transmit the parasites to forest Hawaiian birds. This idea is supported by the work of Freed and Cann (2013) that shows, using an enhanced mosquito- movement model, that climate warming led to an increase of malaria transmission at upper elevations where the temperature did not allow parasite sporogony in the vector because of their migration from a lower altitude. Moreover, *Cx. quinquefasciatus* are capable of flying up to 3 km from their emergence sites and are probably able to seek a host at high elevation (LaPointe 2008), expanding the transmission area. In addition, human activities in Koke'e State Park and multiple roads and trails at lower altitudes might provide permanent habitats for mosquitoes near the edge of Alakai Swamp (Reiter and LaPointe 2009). The very low capture rate observed on the ridge line at Kawaikoi indicates that the specific topography of the Plateau (different from the gradual elevation gradient on Hawaii Island) is an important factor for *Cx. quinquefasciatus* dispersion across the Plateau. This specific environment may promote a heterogenic migration and distribution of *Cx. quinquefasciatus* limiting *P. relictum* transmission range expansion. This might also explain a part of the lower prevalence of infected birds at Mohihi observed by (Atkinson et al. 2014), as this site is located at the top of the Alakai Plateau very steep edge with a dramatic elevation change. Intensive searches for larvae may be the best way to confirm one of those hypotheses, although biomarkers like stable isotope signatures may also be able to confirm hatching sites of adult mosquitoes on the Plateau.

This study shows that there are important implications for the conservation of Kauai forest birds. As the Plateau is the highest area on Kauai, there is already an ongoing selection pressure on all Kauai forest birds. With the permanent presence of *Cx. quinquefasciatus* in some parts of the Plateau, the selection pressure may lead some species to develop resistance to *P. relictum,* as Apapane or Hawaii Amakihi already did on other islands (Atkinson and Samuel 2010, Woodworth et al. 2005). Also, the high prevalence observed for many species in 2007-2013 shows that it might already be an ongoing process, as birds that remain alive after an infection can be caught and thus identified as positive for infection. However, with six endemic species (three of them have an estimated population between 500 and 1,000 individuals only) the hope for sufficiently rapid resistance development to maintain the population is low. Furthermore, those species such as Akeke'e, Akikiki, and Puaiohi are not well studied and only a very few individuals are caught each year (L.H.C unpublished data). Thus, the effect of malaria on their survival, their breeding success, or their capacity to respond to other environmental pressures is unknown.

Culex quinquefasciatus was believed to occur in the Alakai Plateau only in late summer and early fall, when temperatures were higher and rainfall and stream flooding frequency lower (Atkinson et al. 2014). Yet, mosquitoes were caught from November to March at Kawaikoi and from October to January at Halepa'akai. Furthermore, larvae were found in August, September, and October of 2011, October of 2013, and April and March of 2014 (all sites and habitats), and even in February at Koke'e, indicating that *Cx. quinquefasciatus* is found even during the coldest months of the year in some areas of the Alakai. However, we do not have any evidence that larvae found on the Plateau successfully developed to adults. A larger effort to trap adults and survey larval habitat is needed to better understand the dynamics of *Cx. quinquefasciatus* on the Alakai Plateau, especially in winter, as we still do not know if most adult mosquitoes present originated on the Plateau or if they came from lower altitude sources. An isotope study on mosquitoes living at different altitudes on Kauai may allow us to answer the question of *Cx. quinquefasciatus* dispersal across altitudes. The topography, the meteorologically small-scale differences, and anthropogenic disturbances made it difficult to predict mosquito distribution and population dynamics.

Since the vector apparently is already well established on the Plateau, a rise of temperature due to climate change may cause a quick increase in transmission rate and allow transmission to occur year-round. Controlling larval habitat on the Plateau and at low altitude might be the easiest way to reduce mosquito abundance that is created by anthropogenic activities. Furthermore, new techniques of vector control may also be good management tools to reduce transmission, for example the release of sterile mosquitoes (Benedict and Robinson 2003). Still, developing resistance is the best hope for Kauai forest birds in the absence of current management tools to directly control mosquitoes and malaria.

REFERENCES CITED

Ahumada, J.A., D.A. Lapointe, and M.D. Samuel. 2004. Modeling the population dynamics of *Culex quinquefasciatus* (*Diptera: Culicidae*), along an elevational gradient in Hawaii. J. Med. Entomol. 41: 1157–1170.

Almirón, W.R. and M.E. Brewer. 1996. Winter biology of *Culex pipiens quinquefasciatus say*,(*Diptera: Culicidae*) from Córdoba, Argentina. Mem. Inst. Oswaldo Cruz 91: 649–654.

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 Kipahulu valley, Haleakala National Park, Hawaii, USA. J. Wildl. Dis. 43: 567–575.

Atkinson, C.T. 2005. Ecology and diagnosis of introduced avian malaria in Hawaiian forest birds (No. 3151), USGS FS 2005.

Atkinson, C.T. and D.A. LaPointe. 2009. Introduced avian diseases, climate change, and the future of Hawaiian honeycreepers. J. Avian Med. Surg. 23: 53–63.

Atkinson, C.T., T.K. Pratt, P.C. Banko, J.D. Jacobi, and B.L. Woodworth. 2013. When worlds collide: challenges and opportunities for conservation of biodiversity in the Hawaiian islands. Conserv. Biol. Voices Trop. 188–196.

Atkinson, C.T. and M.D. Samuel. 2010. Avian malaria *Plasmodium relictum* in native Hawaiian forest birds: epizootiology and demographic impacts on 'apapane *Himatione sanguinea*. J. Avian Biol. 41: 357–366.

Atkinson, C.T., N. Thomas, and D. Hunter. 2008. *Avian Malaria*. Wiley-Blackwell, IA.

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 the altitudinal range of avian malaria in the Hawaiian islands - an ongoing conservation crisis on the island of Kaua'i. Glob. Change Biol. 20: 2426-2436.

Baker, J.K. 1975. The feral pig in Hawaii Volcanoes National Park. Trans. Calif.-Nev. Sect. Wildl. Soc. 22: 74–80.

Beadell, J.S. and R.C. Fleischer. 2005. A restriction enzyme-based assay to distinguish between avian hemosporidians. J. Parasitol. 91: 683–685.

Beadell, J.S., F. Ishtiaq, R. Covas, M. Melo, B.H. Warren, C.T. Atkinson, S. Bensch, G.R. Graves, Y.V. Jhala, and M.A. Peirce. 2006. Global phylogeographic limits of Hawaii's avian malaria. Proc. R. Soc. B 273: 2935–2944.

Benedict, M.Q. and A.S. Robinson. 2003. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. Trends Parasitol. 19: 349–355.

Benning, T.L., D.A. LaPointe, C.T. Atkinson, and P.M. 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. Proc. Natl. Acad. Sci. 99: 14246–14249.

Burney, D.A., H.F. James, L.P. Burney, S.L. Olson, W. Kikuchi, W.L. Wagner, M. Burney, D. McCloskey, D. Kikuchi, and F.V. Grady. 2001. Fossil evidence for a diverse biota from Kaua'i and its transformation since human arrival. Ecol. Monogr. 71: 615–641.

Cameron, A.C. and P.K. Trivedi. 2013. *Regression Analysis of Count Data*. Cambridge University Press.

Farajollahi, A., D.M. Fonseca, L.D. Kramer, and A. Marm Kilpatrick. 2011. "Bird biting" mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infect. Genet. Evol. 11: 1577–1585.

Foster, J.T., E.J. Tweed, R.J. Camp, B.L. Woodworth, C.D. Adler, and T. Telfer. 2004. Long term population changes of native and introduced birds in the Alaka 'i Swamp, Kaua 'i. Conserv. Biol. 18: 716–725.

Freed, L.A. and R.L. Cann. 2013. Vector movement underlies avian malaria at upper elevation in Hawaii: implications for transmission of human malaria. Parasitol. Res. 112: 3887–3895.

Freed, L.A., R.L. Cann, M.L. Goff, W.A. Kuntz, and G.R. Bodner. 2005. Increase in avian malaria at upper elevation in Hawai'i. Condor 107: 753–764.

Giambelluca, T.W., Q. Chen, A.G. Frazier, J.P. Price, Y.-L. Chen, P.-S. Chu, J.K. Eischeid, and D.M. Delparte. 2013. Online rainfall atlas of Hawai'i. Bull. Am. Meteorol. Soc. 94: 313–316.

Gurevitch, J. and D.K. Padilla. 2004. Are invasive species a major cause of extinctions? Trends Ecol. Evol. 19: 470–474.

Isaksson, C., I. Sepil, V. Baramidze, and B.C. Sheldon. 2013. Explaining variance of avian malaria infection in the wild: the importance of host density, habitat, individual life-history and oxidative stress. BMC Ecol. 13: 15.

Knowles, S.C., M.J. Wood, R. Alves, T.A. Wilkin, S. Bensch, and B.C. Sheldon. 2011. Molecular epidemiology of malaria prevalence and parasitaemia in a wild bird population. Mol. Ecol. 20: 1062–1076.

Lachish, S., S.C. Knowles, R. Alves, M.J. Wood, and B.C. Sheldon. 2011. Infection dynamics of endemic malaria in a wild bird population: parasite species dependent drivers of spatial and temporal variation in transmission rates. J. Anim. Ecol. 80: 1207–1216.

LaPointe, D. 2008. Dispersal of *Culex quinquefasciatus* (*Diptera: Culicidae*) in a Hawaiian rain forest. J. Med. Entomol. 45: 600–609.

LaPointe, D.A., C.T. Atkinson, S. Jarvi, T. Pratt, C. Atkinson, P. Banko, J. Jacobi, and B. Woodworth. 2009. Managing disease. In: *Conservation Biology of Hawaiian Forest Birds*. pp. 405–424. Yale Univ. Press New Haven.

LaPointe, D.A., C.T. Atkinson, and M.D. Samuel. 2012. Ecology and conservation biology of avian malaria. Ann. N. Y. Acad. Sci. 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*. J. Parasitol. 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. J. Parasitol. 96: 318–324.

Leonard, J. and L. David. 2008. Recovery expenditures for birds listed under the US Endangered Species Act: The disparity between mainland and Hawaiian taxa. Biol. Conserv. 141: 2054–2061.

Loiseau, C., R. Harrigan, C. Bichet, R. Julliard, S. Garnier, A. Lendvai, O. Chastel, and G. Sorci. 2013. Predictions of avian *Plasmodium* expansion under climate change. Sci. Rep. 3: 1126.

Manimegalai, K. and S. Sukanya. 2014. Biology of the filarial vector, *Culex quinquefasciatus* (*Diptera: Culicidae*). Int. J. Curr. Microbiol. App. Sci. 3: 718–724.

Muturi, E.J., J. Mwangangi, J. Shililu, B.G. Jacob, C. Mbogo, J. Githure, and R.J. Novak. 2008. Environmental factors associated with the distribution of *Anopheles arabiensis* and *Culex quinquefasciatus* in a rice agro-ecosystem in Mwea, Kenya. J. Vector Ecol. 33: 56–63.

Reed, J.M., D.W. Desrochers, E.A. Vanderwerf, and J.M. Scott. 2012. Long-term persistence of Hawaii's endangered avifauna through conservation-reliant management. BioScience 62: 881–892.

Reiter, M.E. and D.A. LaPointe. 2007. Landscape factors influencing the spatial distribution and abundance of mosquito vector *Culex quinquefasciatus* (*Diptera: Culicidae*) in a mixed residential-agricultural community in Hawai'i. J. Med. Entomol. 44: 861–868.

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 Hawai'i. J. Vector Ecol. 34: 208–216.

Reynolds, M.H., R.J. Camp, B.M. Nielson, and J.D. Jacobi. 2003. Evidence of change in a low-elevation forest bird community of Hawai'i since 1979. Bird Conserv. Int. 13: 175–187.

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

Samuel, M.D., P.H. 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 modeling approach. Ecol. Appl. 21: 2960–2973.

Shaman, J. and J.F. Day. 2007. Reproductive phase locking of mosquito populations in response to rainfall frequency. PLoS One. 2: e331.

Whiteman, N.K., S.J. Goodman, B.J. Sinclair, T. Walsh, A.A. Cunningham, L.D. Kramer, and P. G. Parker. 2005. Establishment of the avian disease vector *Culex quinquefasciatus Say*, 1823 (*Diptera: Culicidae*) on the Galápagos Islands, Ecuador. Ibis 147: 844–847.

Wilcove, D.S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience 48: 607–615.

Woodworth, B.L., C.T. Atkinson, D.A. LaPointe, P.J. Hart, C.S. Spiegel, E.J. Tweed, C. Henneman, J. LeBrun, T. Denette, and R. DeMots. 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. Proc. Natl. Acad. Sci. U.S.A. 102: 1531–1536.