Laying the Groundwork for Landscape-level Mosquito Suppression to Protect Endangered Forest Birds and Human Health from Mosquito Borne Disease in Hawaii

Final Report for Hawaii Invasive Species Council FY19 Funding
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INTRODUCTION

The endangered endemic forest birds in Kauai are at critically low numbers: Akikiki numbers around 468 birds, Akekee numbers 945 birds and Puaiohi numbers 487 birds. Coinciding with their population declines, the prevalence of mosquito-borne avian malaria has increased on the Alakai Plateau, strongly implicating disease-driven population declines. Even the once common Iiwi has seen a severe reduction in range and abundance and is now listed as threatened due to threats from mosquito-borne diseases. Changes in precipitation and surface hydrology due to climate change may have altered density and permanence of larval mosquito habitat, thus increasing distribution and abundance of mosquitos. Concurrently, *Aedes japonicus*, an important potential vector of human diseases, has invaded the Alakai Plateau.

Innovations in landscape-level mosquito suppression and eradication may allow managers to efficiently halt avian species declines and protect human health. Incompatible Insect Technique (IIT) uses naturally-occurring strains of *Wolbachia*, an endosymbiotic bacteria of mosquitoes, to facilitate mosquito incompatibility and infertility through cross matings. Sustained releases of male mosquitoes infected with incompatible *Wolbachia* will suppress wild populations.

To meet regulatory approval for initial releases, and achieve successful deployment and suppression of these mosquitoes, key ecological parameters (population density, seasonality, dispersal, and location of emergent populations or larval mosquito habitat) need to be assessed. A major objective of this research is to determine these parameters and further effective monitoring techniques of *Culex quinquefasciatus* and *Aedes japonicus* populations in the Alakai Plateau to target future control efforts. Efforts made now to document basic ecology of mosquitos in the Alakai and at lower elevations will provide the critical tools and parameters needed for successful IIT control.

OBJECTIVES

(1) support development of Wolbachia-*based IIT at UH*

(2) ascertain key mosquito ecology parameters as follows: a) determine relative abundance, seasonality, and survivorship, using elevational transects, and targeted or opportunistic catches

on the Alakai Plateau; b) continue to assess larval mosquito distribution and habitat use; c) analyze disease prevalence trapped adult mosquitos and larvae

(3) locally eradicate mosquito larvae in core bird breeding habitat using Bti and map mosquito breeding habitat so that future action can be taken to reduce it

(4) assess disease exposure of birds and gain understanding of infection rates of avian malaria.

(5) conduct public outreach on danger of introduced mosquitoes to endemic bird species and human health

METHODS AND RESULTS

1) Support development of Wolbachia-*based IIT at UH*

We provided funds to Dr. Floyd Reed (Biology Department, UH Manoa) to help support ongoing work. HISC funds were used to purchase materials to establish *Culex* mosquito cell lines to support work with *Wolbachia* transfections and to support a student working on maintaining mosquito colonies and conducting microinjections for *Wolbachia* transfection. Hawaiian *Culex* mosquitoes were cleared of their endogenous *Wolbachia* to prepare them for transfection that would result in cytoplasmic incompatibility. Some previously published successful transfections have passed *Wolbachia* through cell cultures before injection into insect embryos to allow the *Wolbachia* to adapt to the new cellular environment. It took longer than we anticipated, but now several independent Culex cell lines have been established and are stably propagating in the lab at this time. More than 7,000 total mosquitoes were injected with *Wolbachia* (before we had cell lines established) and unfortunately none were successfully transfected. However, recent results show that using another strain of *Wolbachia* (AlbA vs. wMel) results in much higher rates of transfection in Culex mosquitoes so that, combined with passage through cell lines, is the next strategy to pursue.

2) Ascertain key mosquito ecology parameters

We sampled one high-elevation site at Halepa'akai approximately once a month from February 2019 through August 2019 and six remote sites once each between March 2019 and July 2019 (Figure 1). At Halepa'akai we trapped mosquitoes using 10 CO^2 and 10 gravid traps for 4-8 nights depending on the month (Table 1). We also quantified and described larval mosquito habitat on stream and upland transects using dip surveys; we divided the stream into 10-m segments and dipped 10x in each segment. At the remote sites, we ran three-four gravid and three-four CO2 traps for three-four nights per site. We collected adult and larval mosquito samples for future isotope (to determine migration) analysis and to establish a breeding mosquito population for *Wolbachia* injection.

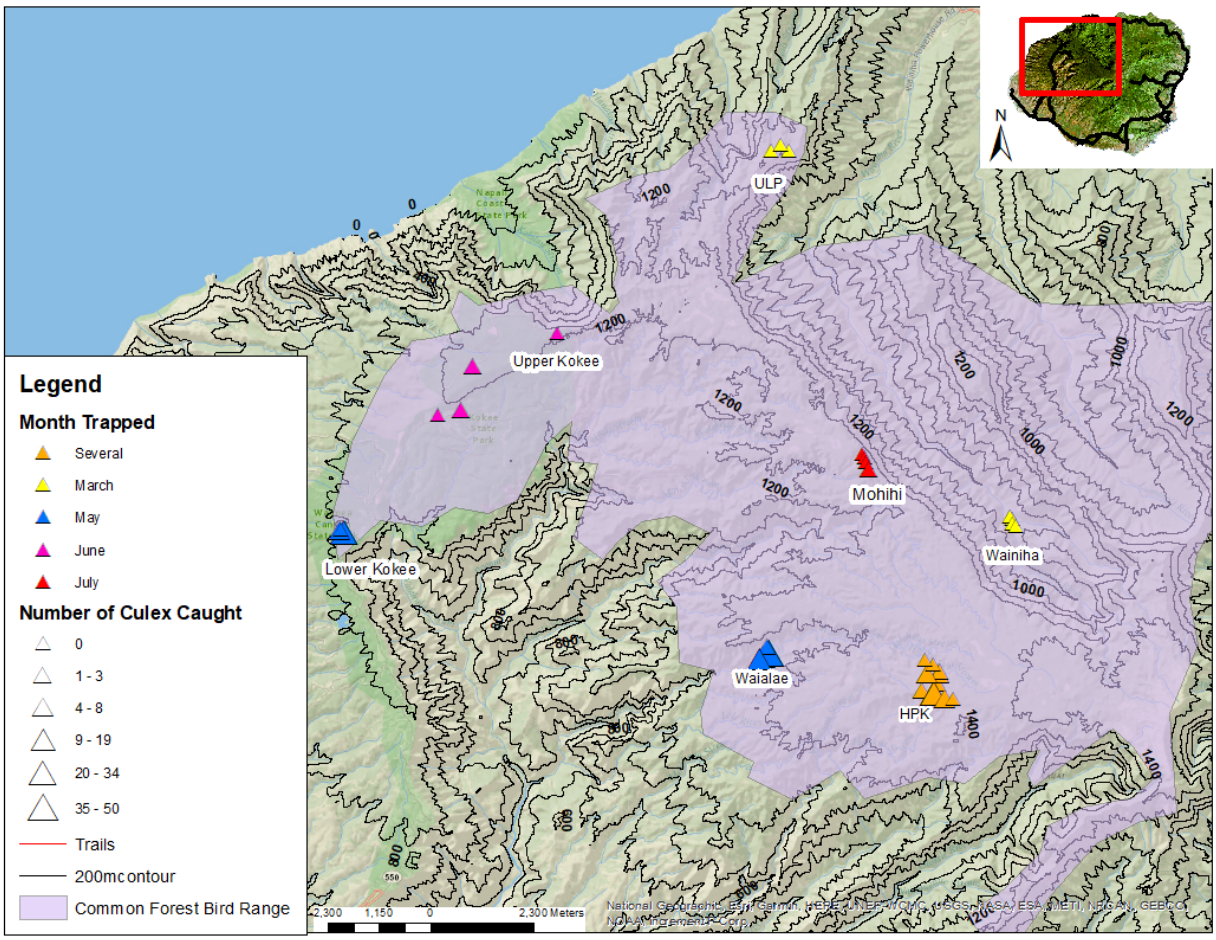

Figure 1. Mosquito sampling locations in 2019 within native forest bird range on Kauai. Every triangle represents a pair of CO2 and gravid traps, color indicates the months trapped at each site and size indicates the number of Culex *mosquitos caught. HPK= Halepa'akai. ULP = Upper Limahuli Preserve*

At Halepa'akai, 49 adult *Culex* mosquitos were caught during this time period in the 10 CO2 traps: 1 in early June and 48 in August (Table 1). Only 1 adult *Culex* mosquito was captured in the 10 gravid traps during that same time period. No *Culex* mosquitos were caught in the months of March, April, and May at Halepa'akai. One pool of *Culex* larvae was discovered in February and a total of five pools were found to have *Aedes* larvae in the months June, July and August.

Table 1. Dates of mosquito surveys at Halepa'akai in February-August 2019, with number of total trap nights and Culex *mosquitoes caught for each of the two trap types. We also indicated whether or not* Culex *larvae were found.*

Dates	Total CO2 Trap Nights	Number of *Culex* Mosquitos Caught in CO2 Traps	Number of *Culex* Mosquitos Caught in C02 Traps/Trap Nights	Total Gravid Trap Nights	Number of *Culex* Mosquitos Caught in Gravid Traps	Number of *Culex* Mosquitos Caught in Gravid Traps/Trap Nights	*Culex* Larvae Found
February 20-26 2019	12	0	0	12	0	0	Y
March 8-12 2019	16	0	0	16	0	0	N
April 2019	0	0	0	0	0	0	N
May 20-23 2019	34	0	0	27	0	0	N
May 30-June 2 2019	30	1	0.03	30	1	0.03	N
July 2019	0	0	0	0	0	0	N
August 1-11 2019	69	48	0.70	62	1	0.02	N

No mosquitos were caught during our roving trips in March and early April (Table 2). However, several adult *Culex* mosquitoes were caught on private property at ~500m ASL in late April in CO^2 traps, and atWaialae Cabin (~1200m ASL) and lower Koke'e State Park (~1000m ASL) in May in both gravid and CO^2 Traps. Larvae were also found near Waialae Cabin at UTMS 439928/2442353. Observations of adult and larval mosquitoes in gravid traps suggest that *Culex* mosquitos are not only using these sites for blood meals but are also breeding in these areas as early as May. These sites are both slightly lower in elevation than Halepa'akai, but Waialae cabin is only ~2km away as the bird or mosquito flies. More mosquitos were caught in upper

Koke'e than at Halepa'akai in June. In July, few mosquitos were caught at Mohihi, which is the second highest elevation site after Halepa'akai, with only one adult capture in a gravid trap. This site is very windy and most traps were put on ridges (as opposed to along streams as in other areas).

Table 2. Dates and locations of "roving" mosquito surveys at sites on and near the Alakai Plateau, Kauai in 2019, with number of total trap nights and Culex *mosquitoes caught combined for each of the two trap types. We also indicated whether or not* Culex *larvae were found.*

Trip Dates	**Location**	**Total CO2 Trap Nights**	**Number of *Culex* Mosquitos Caught in C02 Traps**	**Number of *Culex*/C02 Trap Nights**	**Total Gravid Trap Nights**	**Number of *Culex* Mosquitos Caught in Gravid Traps**	**Number of *Culex*/Gravid Trap Nights**	***Culex* Larvae Found**
March 11-15 2019	Upper Limahuli Preserve	12	0	0	12	0	0	N
March 25-28 2019	Wainiha	16	0	0	16	0	0	N
April 22-26 2019	Private property (not shown on map)	16	11	0.69	16	0	0	N
May 8-13 2019	Waialae	20	72	3.60	20	10	0.5	Y
May 26 – June 1 2019	Lower Koke'e	15	14	0.93	15	7	0.47	N

June 4-7 2019	Upper Koke'e	12	0	0	12	2	0.17	N
July 22-24 2019	Mohihi	8	0	0	8	1	0.13	N

3) Locally eradicate mosquito larvae in core bird breeding habitat

Unfortunately, due to weather and a strong push to provide living adult mosquitos for *Wolbachia*-based IIT we did not focus on local eradication of mosquito larvae, so no pools were treated using Bti. However, Figure 2 could help focus future eradication action in key forest bird habitat.

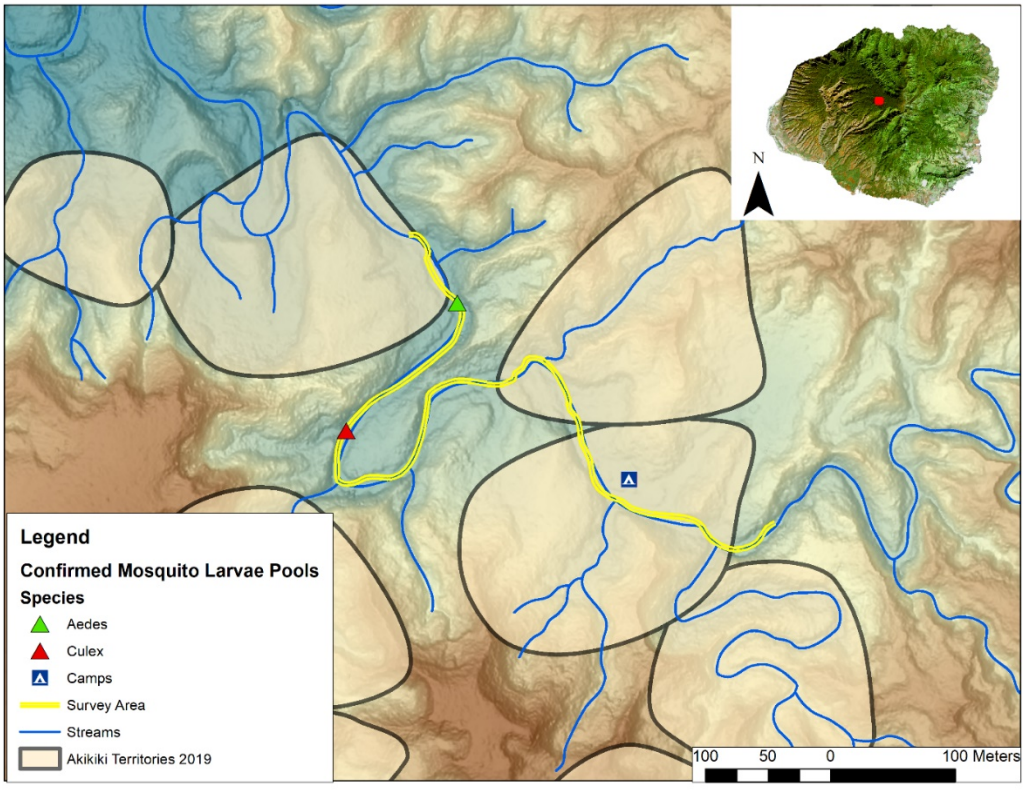

Figure 2. Confirmed mosquito larvae pools in Halepa'akai stream survey area overlaid on Akikiki 2019 territories.

4) *Assess disease exposure of birds to gain understanding of infection rates*

In 2019, we conducted 11 trips into the Alakai Plateau to capture birds using mist nets in order to take blood samples and band the birds with unique combinations of color bands to further understand the population and survivorship, particularly as a function of disease. During those 11 trips, 56 blood samples were taken from different individual birds and sent to the University of Arizona for avian malaria testing. Of these samples, 12 birds representing a range of species and sites tested positive for avian malaria (Table 3).

Table 3. Numbers of whole blood samples taken per species and site on the Alakai Plateau in 2019. Number of positive samples is in parentheses.

Site	**Alakai Swamp Trail (Koke'e)**	**Halepa'akai**	**Mohihi**	**Total**
Akikiki		4(1)		**4**
Anianiau		8(1)	4	**12**
Apapane		7		**7**
Iiwi		1		**1**
Warbling White-eye	1	9(3)	1(1)	**11**
Kauai Amakihi	3(1)	3	3(2)	**9**
Kauai Elepaio		5(1)	5(1)	**10**
Scaly-breasted Munia		1(1)		**1**
Puaiohi		1		**1**
Total	4	39	13	**56**

5) *Conduct public outreach on danger of introduced mosquitoes to endemic bird species and human health*

We promoted understanding among local people of the threats of mosquito-borne diseases to endangered forest birds and human health, and the importance of this new tool for the survival and recovery of these species and disease reduction throughout the year. We presented at the Chiefess Kamakaheli Middle School Career Day. We attended four environmental festivals in the local community. We were filmed for a national television production to be released in fall 2020. We developed a forest bird curriculum for grades 4-12 with a focus on Kaua'i forest birds and their threats, which was disseminated on our website and via social media. We issued three press releases and were featured in three newspaper articles. We submitted an abstract based on this work to the Hawaii Conservation Conference; it was accepted for oral presentation.

DELIVERABLES (IN ADDITION TO FINAL REPORT):

1) Documentation of key ecological parameters for *Culex quinquefaciastus* to support EPA Experimental Use Permit application for future CI-SIT release

This report contains information showing that mosquitoes do not occur or breed year-round in the highest elevations of the Alakai Plateau. It is not clear when breeding starts at lower elevations, but certainly by May.

2) Map of confirmed larval habitats within key Akikiki and Akekee breeding areas

See Figure 2.

3) Collection of larval and adult mosquito samples for future disease analysis and future isotopic analysis and 4) Development of locally sourced CI *Culex quinquefasciatus* for experimental control releases

Fewer than 10 samples were shipped to Dr. Dennis LaPointe at USGS for isotope and disease analysis. The rest of the adult mosquitos captured were sent to Dr. Adam Vorsino to develop a locally-sourced population of *Culex* for *Wolbachia* injections.

4) Screening of native forest birds for future disease analysis

Five species (12 individuals), including three native (Akikiki, Akekee, and Kauai Elepaio) tested positive for avian malaria (Table 3).

5) Outreach via one media appearance, one press releases, two festival events, one website update and regular social media updates.

See above.

Appendix A. Expenditures from HISC funds (N.B. Some additional helicopter flights trips were paid via a DOFAW PO provided by Cynthia King, not HISC funds).

Helicopter flights (HISC funded): $5,000

Funding to University of Hawaii for staff time, back country per diem, supplies, and UH student stipend: $41,818

CO2 cylinder refills: $1647

Field, camping and laboratory supplies: $3,094

Total expenditures: $51,559