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 FY20 Funding

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INTRODUCTION

The endangered endemic forest birds in Kauai are at critically low numbers: according to surveys in 2018, Akikiki numbers around 454 birds and Akekee numbers around 1162 birds (Paxton et al. 2020). Meanwhile the most recent surveys in 2011-2013 estimated that the Puaiohi numbered 487 birds (Crampton et al. 2017). Coinciding with population declines in the honeycreeper species, 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 naturallyoccurring 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) ascertain key mosquito parameters on the Alakai Plateau: a) relative abundance of adult mosquitoes b) larval mosquito distribution and habitat; c) disease prevalence and movement of adult mosquitoes

(2) locally eradicate mosquito larvae in core bird breeding habitat using Bti and mechanical filling
(3) assess infection rates of avian malaria in birds
(4) conduct public outreach on danger of introduced mosquitoes to bird and human health and potential for IIT to mitigate this risk

METHODS AND RESULTS

1) *Ascertain key mosquito ecology parameters*

We sampled six sites representing elevation, temperature and moisture gradients across the Alakai Plateau between 17 August and 2 November 2020 (Figure 1). Each site was surveyed with active traps twice for three nights during this period. During each survey, we trapped mosquitoes using four Biogents CO^2 and four active CDC gravid traps (Figure 2). 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. We collected adult and larval mosquito samples for future isotope and genomic (to determine migration) and genetic (to screen for *Plasmodium* infection) analyses. We also ran three GAT traps at each site for the length of the study, collecting the sticky cards each week. GAT traps are passive gravid traps baited with stinky water; mosquitoes that visit the trap get stuck on the cards.

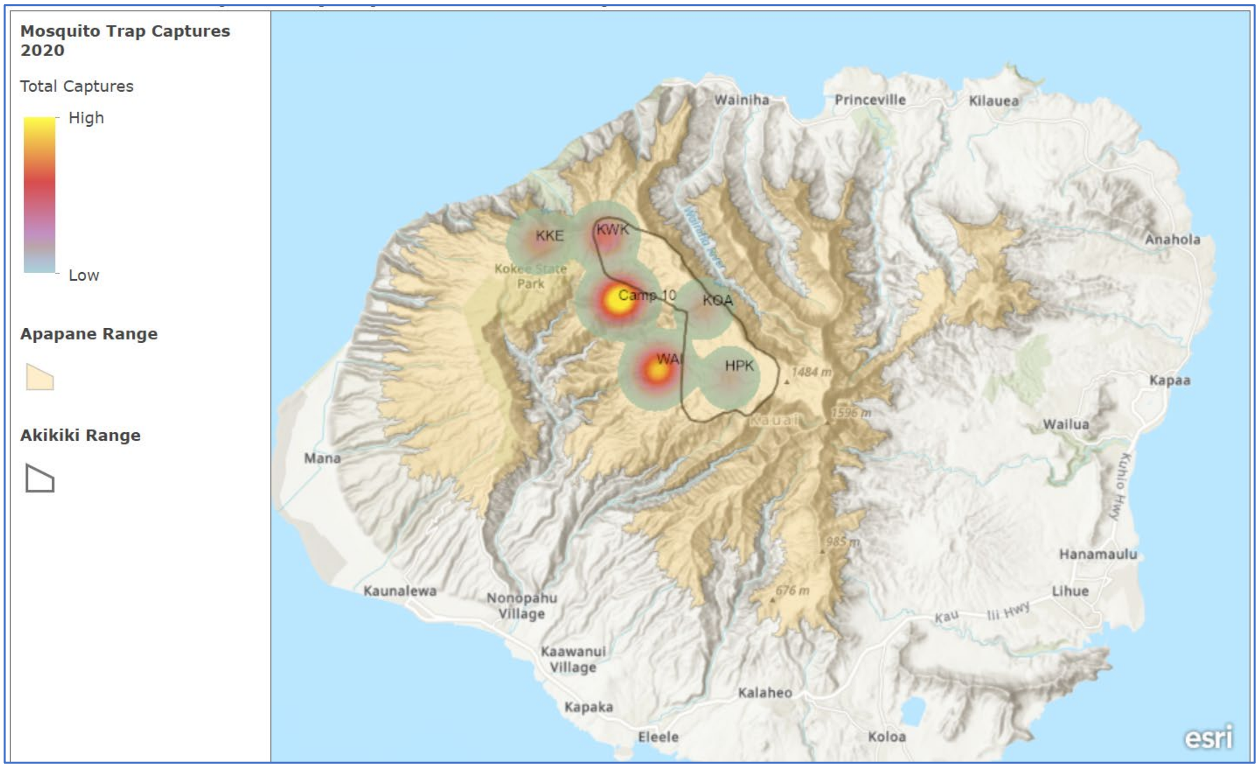

Figure 1. Mosquito survey sites on the Alakai Plateau, Fall 2020. The intensity of the color represents the total number of mosquitoes caught by four CO2 and four gravid traps in two three-night surveys at each site. Larvae were found at the Camp 10, KKE, KOA, and WAI sites.

Figure 2. Traps used for mosquito surveys. From left to right: BioGents CO2 traps (targets females seeking blood meals); active CDC gravid trap (targets females seeking oviposition sites); passive GAT gravid trap (also targets females seeking to oviposit).

We caught *Culex* mosquitoes at all six sites on both visits (Figure 3). The number of mosquitoes caught decreased with increasing elevation, with almost 10 times as many mosquitoes caught at C10, the lowest elevation site than at HPK, the highest elevation site. More mosquitoes were caught in surveys run in October/early November (second visit) than in late August/September.

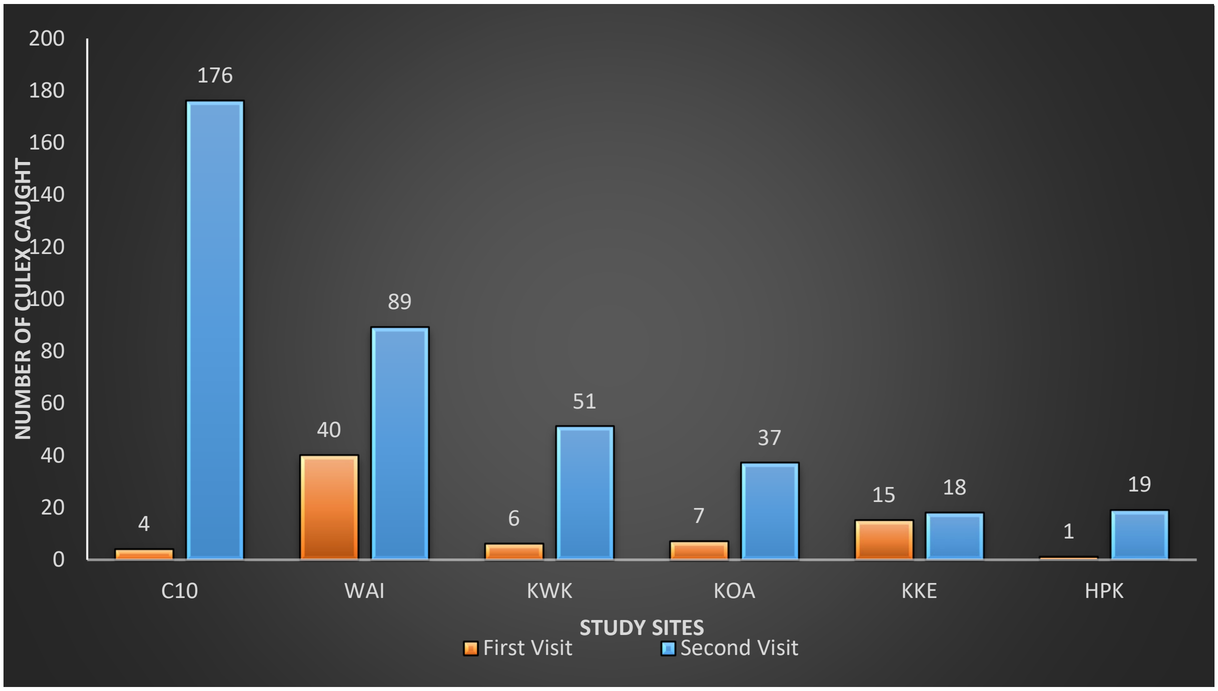

Figure 3. Total number of Culex *mosquitoes caught at sites on the Alakai Plateau between 20Aug and 2Nov 2020. Site elevation increases from left to right.*

We used a hierarchical Bayesian framework in a generalized linear mixed model to model *Culex quinquefasciatus* probability of occurrence in response to variance in topographic classification (stream and ridge), trap type (CO2 trap vs gravid trap) and two continuous variables representing the mean monthly temperature, and the variance in temperature (Δ temperature) of the collection period. As the data is comprised of repeated measures within sites and by observers, we also used these variables assess utility of random effects (Câmara et al. 2020). The formulation of the generalized linear mixed model used in this analysis is below.

Models were developed using the *rstanarm* package (Goodrich et al. 2020) in R vers. 4.0.3 (Team 2020). The *rstanarm* package uses a Hamiltonian Markov Chain Monte Carlo sampling technique as applied to a linear regression framework (Goodrich et al. 2020). A Bayesian framework was applied for two reasons, because the equivalent frequentest mixed model approach (even with transformation) violated the assumption of normally distributed residuals and to allow for a model which characterizes the variance in *C. quinquefasciatus* density across sites with probability estimates, allowing for a more transferable assessment.

In this approach, weekly informative normal (location: 0, scale: 2.5) priors on the intercept were estimated and applied using *rstanarm*. Models were fit assuming a negative binomial distribution with 3,000 iterations across four chains. The first 1,500 iterations were used as warm-up projections. Convergence and fit was assessed by means of the effective sample size (>2000 for all parameters), ensuring that $\hat{R}$ is < 1.05, and inspection of residual plots (Gelman et al. 2013).

Variable selection was conducted using the *projpred* package (Piironen et al. 2020). To select a the most important variables in the assessment a model was developed with all variables and weakly informative horseshoe priors (Piironen and Vehtari 2017). As this method does not work with a negative binomial distribution, a Poisson model was used to infer variables of significance. After model fitting a leave one out cross-validation approach was applied using a forward selection approach. Visualizations and tables were developed using the packages *bayesplot* (Gabry et al. 2019) and *SJplot* (Ldecke 2021).

The results of this modeling effort are shown in Table 1, which shows the output statistics for the developed regression model, including the Bayesian marginal and conditional R^2 and 95% Highest Density Interval (logically equivalent to Confidence Intervals) metrics per variable. The model outputs show that the effects of temperature, trap type and topography are correlated with *C. quinquefasciatus* numbers when controlling for fixed effects. More mosquitoes were collected in BG traps than GT traps, in streams than on ridges, and in warm weather. For every 1 BG Cq trap collection you would get 0.06 GT Cq trap collections (95% CI 0.03 - 0.1) (0.06 GT: 1 BG). For every 1 Cq ridge collection you would get 18.26 Cq stream collections (95% CI 5.56 - 67.68).

Table 1: Output statistics of the Bayesian generalized linear mixed model with fixed effects used in this analysis

	NumCulex	
Predictors	*Incidence Rate Ratios*	*CI (95%)*
(Intercept)	474.59	0.00 – 110066143.64
MeanTemp	0.10	0.03 – 0.32
DeltaTemp	31.57	5.08 – 190.81
StreamMidRidge: Stream	18.26	5.56 – 67.88
TrapType: GT	0.06	0.03 – 0.10
reciprocal_dispersion	1.66	1.43 – 2.07
Random Effects		
σ^2	0.00	
$\tau_{00\ \mathrm{Location}}$	3.06	
$\tau_{00\ \mathrm{Observer}}$	0.39	
ICC	1.00	
N_{Location}	6	
N_{Observer}	5	
Observations	290	
Marginal R^2 / Conditional R^2	0.575 / 1.000	

The longitudinal sampling we conducted with passive gravid (GAT) traps largely paralleled the results from the active gravid traps, with the Kawaikoi (KWK) site catching more females seeking to oviposit (Figure 9).

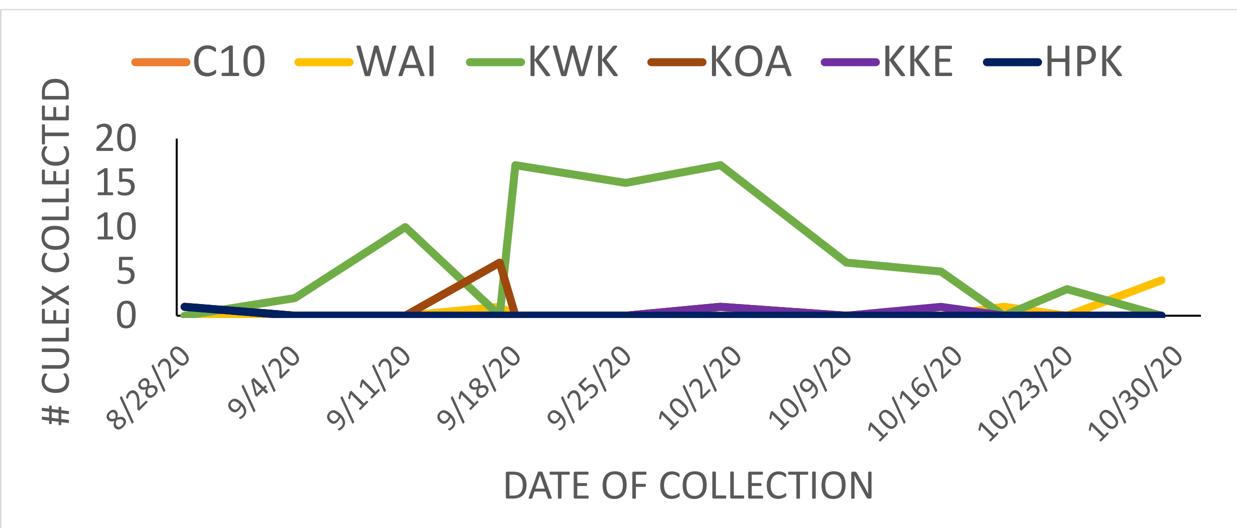

Figure 9. Trends in gravid mosquito captures by GAT traps over time and by site on the Alakai Plateau, Fall 2020. The date represents the day the card was collected. No GAT traps were run the last week of October/early November.

Seven of 173 mosquito samples screened for avian malaria tested positive. Four samples were from the Camp 10 traps (n=40 samples total), and one each were from the Waialae (n=31), Koaie (n=25) and Kokee (n=22) sites.

Larval surveys along streams resulted in few detections; larvae were only found on Waialae and Koaie streams in October and were *Aedes japonicus*. *Culex* larvae were found in ruts along Camp 10 road in both September and October, and along the Alakai Swamp Trail boardwalk in September (Figure 10) A total of 712 *Culex* larvae were found (707 of which were on Camp 10).

Figure 10. Larval habitat along boardwalk (left) and Camp 10 road (right).

2) *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. After trapping was completed we filled several puddles on Camp 10 road by hand but the rest must be filled with machinery. Camp 10 road should be a major focus of larval control efforts.

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

From 22 March – 9 December 2020, we captured 138 birds using mist nets or in the nests 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. When possible, blood samples were taken from individual birds and sent to the University of Arizona for avian malaria testing. We are awaiting results from those samples.

Table 3. Numbers of birds captured on the Alakai Plateau in 2020.

Site	Total
Akekee	**2**
Akikiki	**3**
Anianiau	**22**
Apapane	**19**
Iiwi	**1**
Kauai Amakihi	**9**
Kauai Elepaio	**28**
Puaiohi	**48**
Scaly-breasted Munia	**1**
Warbling White-eye	**16**
Total	**138**

4) *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 the use of Incompatible Insect Technology (IIT) for the survival and recovery of these species and disease reduction throughout the year, as possible on social media and via Zoom given the Covid-19 pandemic. We participated in a national Zoom panel "Sustainachella", the theme of which was bird conservation, hosted by the City of Miami Beach in May. The episode of Jeff Corwin "Ocean Adventures" that we filmed in fall 2019 was released on ABC television in fall 2020. This episode discussed the threats to Kauai's birds and the steps being taken to address those threats, with a particular focus on avian malaria. We hosted the "Symphony of the Hawaiian Forest Birds, Kauai Edition" in February 2020, which featured numerous references to mosquito-borne diseases; we issued a press release in conjunction with this event. Our annual newsletter featured an article on our mosquito research and an update by Teya Penniman on IIT developments (see https://2pvyu91ewlp51s010ap8cr11-wpengine.netdna-ssl.com/wp-content/uploads/2020/12/KFBRP-Newsletter-2020-Final-1.pdf). We presented our results in the HISC brownbag series in December 2020 and to Kauai DOFAW in January 2021. We released a short film with American Bird Conservancy and Corolis Flims in December 2020 documenting Kauai's forest birds and their declines: https://kauaiforestbirds.org/video-release-the-forest-birds-of-kaua'i-a-declining-population/

DELIVERABLES (IN ADDITION TO FINAL REPORT):

1) 8 active traps run at each of 4-6 sites twice, and 4 passive traps run at each site for 10 weeks to determine relative abundance of adults by breeding status

Done, see above

2) 500 m of larval habitat sampled per site (two times) to determine relative abundance of larvae

Done, see above

3) Larval and adult mosquitoes collected for disease and isotope analysis

We sent 173 *Culex* mosquitoes that we captured or hatched out to Renee Bellinger at USGS in December, who dissected them and extracted DNA. She is running genomic analysis on some parts of the samples; the rest were sent to Jeff Foster at NAU for malaria analysis. More samples that we collected in 2020 but did not send to USGS until 2021 have not yet been dissected.

4) Disease analysis conducted to determine prevalence of *Plasmodium* in ~ 500 mosquitoes

Seven of 173 mosquito samples tested positive for avian malaria over four sites. See above.

5) Map of sites sampled and presence of adult and larval mosquitoes across the Alakai and lower elevations

See Figure 1 and Table 1.

6) Predictive map of mosquito and *Plasmodium* hotspots across the Alakai

In development.

7) Biological and mechanical control of larval habitat where found

We filled several of the ruts on Camp 10 road where larvae were found in November 2020. We are coordinating with Kauai DOFAW to fill other ruts in conjunction with road work in 2021.

8) One press release, two social media posts, four outreach events, one public speaking engagement.

See above.

9) One presentation to the Hawaii conservation community

We gave a presentation to the HISC brown bag series in December 2020. We also participated in the mosquito and disease symposium at HCC in September 2020.

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